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SARDI

SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

Grape and Wine Research and Development Corporation

Development of methods for the control of vine vigour and water use optimisation based on the concept of Partial Rootzone Drying



GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

APPENDIX 1

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RESPONSE OF GRAPEVINES TO PARTIAL DRYING OF THE ROOT SYSTEM

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List of Abbreviations

ABA	abscisic acid
°C	degrees Celsius
DAW	number of days after water turned off
DAS	number of days after September 1
FWT	fruit weight (kg/vine)
FW/PW	ratio of fruit weight/pruning weight
G-G	glycosyl-glucose (μmol/g fresh wt. or μmol/berry)
g_S	stomatal conductance of leaves (mmol/m^2/s)
LA	leaf area (m^2)
LA/FW	ratio of leaf area/fruit weight (g/cm^2)
LLA	lateral leaf area (m^2)
MLA	main leaf area (m^2)
ns	not significant
Pn	photosynthetic rate (μmol CO_2/m^2/s)
PWT	pruning weight (kg/vine)
RF G-G	red-free glycosyl-glucose (μmol/g fresh wt. or μmol/berry)
signif. diff.	significantly different
SGR	shoot growth rate (cm/d)
se	standard error of the mean
SWC	soil water content (gravimetric: g/g; volumetric: % or mm)
TA	titratable acidity (g/L as tartaric acid)
TDR	time domain reflectometry
TSS	total soluble solids (°Brix)
VPD	vapour pressure deficit (kPa)
[X-ABA]	xylem sap ABA concentration
Ψ_L	leaf water potential (MPa)
Ψ_m	soil matric potential (MPa)

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SUMMARY

An excess of vegetative growth ('excess vigour') is detrimental in most vineyards. For grapevines in general, excess vigour has negative implications for fruit yield, disease control, costs of production and efficient water-use. For winegrapes in particular, the association between vigour and wine quality is important: excessively-vigorous vines tend to have dense, shaded canopies and grapes from shaded canopies tend to produce wines of lower quality. Therefore, control of excess vigour should result in a more open canopy with a concomitant improvement in the quality of fruit for winemaking. Withholding irrigation can form the basis of a useful management tool for improving water use efficiency and reducing vine vigour. However, difficulties may be experienced in obtaining the required degree of water stress, and yield may suffer in comparison with fully irrigated vines. The general hypothesis tested in this study was that: '*partial drying of the root system of grapevines will result in reduced vegetative growth with beneficial effects for fruit yield, fruit composition and water-use efficiency.*' The ideas which allowed the formulation of this hypothesis came from the previously published work of Loveys and Düring (1984) and Gowing *et al.* (1990) which suggested that partial drying of the root system may give rise to non-hydraulic root-derived signals capable of changing leaf development and transpiration.

Experiments were initially conducted with container-grown grapevines (*Vitis vinifera* cultivars Chardonnay, Shiraz; *Vitis champini* cv. Ramsey; *Vitis berlandieri* x *Vitis riparia* cv. Kober 5BB; *Vitis berlandieri* x *Vitis rupestris* cv. Richter 110) with the root system divided between two containers (= 'split-root plants'). When half of the root system was slowly dried while the remaining half was kept well-watered (='half-drying'), both shoot growth rate (SGR), stomatal conductance (g_s) and photosynthetic rate (Pn) were significantly reduced relative to control plants with both halves of the root system maintained in a well-watered state. The initial decrease in both SGR and gas exchange in response to half-drying coincided with the decrease in soil water content of the dried half of the root system. For half-dried plants, there was a relative increase in root development in moist soil layers, both in the 'wet' container as a whole or in the lower part of the 'dry' container. All species of grapevine appear to respond to half-drying of the root system, but genotype may affect the degree of response. The effect of half-drying was transient and both gas exchange and SGR returned to pre-treatment levels within one or two weeks, despite the fact that one half of the root system remained dry. Shoot function of half-dried, non-fruiting vines in the field also recovered in the absence of rewatering of the dried half of the root system, and as with pot experiments, recovery

coincided with a slowing in the rate of soil drying on the non-irrigated side. This appears to be the first report of this phenomenon for any species.

During the course of this study, it became apparent that the benefits of a single brief period of shoot growth depression during the season were likely to be marginal for field vines: significant and long-term reduction in shoot growth would only be possible if the recovery of shoot growth was minimised. Therefore, the strategy of alternating the drying from one half of the root system to the other was tested on both non-fruiting potted vines and fruiting field vines with split-root systems. The aim was to minimise or prevent recovery of shoot function by timing the switch so that it occurred at, or just before, the start of recovery. This strategy was successful in both situations: for container-grown vines, alternation of drying of half of the root system every 11 days on average maintained shoot growth at a substantially lower rate than the well-watered controls and reduced components of shoot growth by up to 40% while average g_s was reduced by ca. 30% over a period of 66 days. There was no significant effect on total root weight per plant in response to treatment but the ratio of shoot weight to root weight was increased.

Field experiments were conducted with *Vitis vinifera* cv. Cabernet Sauvignon grafted to Ramsey at two sites near Adelaide, South Australia. One-year old rootlings with split-root systems were planted with each half of the root system on either side of a plastic membrane, buried to a depth of about 1.6m, which separated the root systems. Drip irrigation to each side of the plastic membrane was controlled independently. The 'treated' vines were maintained with one half of their root system, or the other, without irrigation at any one time: in practice this meant that one side was watered for 1-2 weeks while the other side was dried. The situation was then reversed so that the previously dried part of the root system was irrigated and the previously wetted part was dried. By comparison, both sides of the control vines were well-watered at all times. Soil water content, measured with capacitance probes, was used to determine both the timing of irrigation events and the alternation of the irrigation on 'treated' vines. The irrigation strategy, termed 'partial root-zone drying' (PRD), was evaluated for three seasons at one site (Waite) and for one season at the other (Blewitt Springs) on fruiting vines, commencing in their third growing season. For the last season at the Waite site, PRD was applied over the whole of the irrigation period; in all other cases, it was applied from fruit set to harvest. The timing of the alternation was successful in limiting the recovery of shoot growth: most indices of vegetative growth were reduced by up to 30 to 35% relative to the control. Lateral shoot growth was particularly responsive to PRD treatment: total

lateral shoot length per main shoot was reduced by 50 to 66% relative to the control in the Waite experiment, due to a combination of fewer and shorter lateral shoots. Average stomatal conductance over the whole experimental period was reduced by 12 to 30%. There was a substantial reduction (up to 50%) in the amount of irrigation applied to PRD vines at both sites, without any detrimental effect on fruit yield. The reduction in vegetative growth in response to PRD significantly reduced canopy density and increased bunch exposure. Fruit quality was significantly improved by PRD, with concentration of secondary metabolites (anthocyanins, phenolics, glycosyl-glucose) increased by up to 45% relative to the control: this was not associated with any significant change in berry weight, or any other yield component.

As for container-grown vines, both shoot growth and stomatal conductance were reduced in response to PRD without any significant effect on shoot water status (as indicated by leaf water potential Ψ_L), suggesting that a non-hydraulic signal is produced by roots in drying soil which affects shoot function. There was a positive linear relationship between stomatal conductance and shoot growth rate: this suggests that either the two physiological processes are influenced by the same non-hydraulic signal, or that SGR is influenced by changes in g_S which is affected directly by the signal. The substantial reduction in vegetative growth in response to PRD was not associated with many of the characteristic symptoms of grapevine water stress. For example, shoot diameter, berry size and internode elongation were not greatly affected, nor was there any significant effect on Ψ_L. On the other hand, some of the responses to PRD were characteristic of water-stressed vines; however, the magnitude of the response and/or degree of expression of the symptoms were relatively less than with moderate to severe water stress. Furthermore, the reduction in SGR in response to partial drying was mainly due to a termination of node initiation rather than a reduction in the rate of internode elongation, whereas the latter component of SGR is more typically affected in response to water stress.

The amount of irrigation water applied to PRD-treated vines was substantially reduced relative to the control vines in all field experiments. For example, during the 1995/96 season in the Waite experiment, the control vines received 1.34 ML/ha while the PRD-treated vines received 0.7 ML/ha, ie. the amount of irrigation was effectively halved. The actual amount of irrigation water applied in all seasons to control vines in the Waite experiment was less than that applied to commercial vineyards in a nearby region with similar growing season rainfall and comparable yield per hectare. This study was commenced with the main aim of achieving vine balance through control of shoot

vigour. However, the water conservation potential of the partial root-zone drying technique may prove to be more important from an environmental perspective.

The results from these experiments suggested the possibility of a non-hydraulic signal produced by roots in drying soil. For example, shoot function of half-dried plants recovered at the time when there were no more roots dried in the 'dry' container. Recovery may be best explained in terms of a positive signal, ie. when there is no further decline in water content and/or matric potential of the soil around the drying roots, there is a decrease in flux of the signal from roots to shoots and thus shoot function rapidly recovers. Also, both shoot growth and gas exchange were affected without any associated effect on shoot water status. Consequently, the role of ABA as a putative signal mediating this response was investigated. Stomatal conductance of partially-dried grapevines was negatively correlated with concentration of ABA in xylem sap ([X-ABA]). For grapevines grown in the field, g_S decreased coincidentally with the reduction in soil water content of the dried part of the root system and the increase in [X-ABA]; recovery of g_S coincided with no further soil drying and a decrease in [X-ABA]. Changes in [X-ABA] were not associated with any significant effect of partial drying on shoot water status as indicated by Ψ_L.

The results of this study allowed the original hypothesis to be accepted. The major findings were that:

a. The effect of half-drying was transient and both gas exchange and SGR returned to pre-treatment levels within one or two weeks, despite the fact that one half of the root system remained dry;
b. Prolonged inhibition of shoot growth was achieved by alternation of root-zone drying from one half of the root system to the other;
c. Vegetative growth and water-use of the grapevine were substantially reduced by partial root-zone drying without any detrimental effect on yield; at the same time, fruit quality was significantly improved.

STATEMENT

This thesis contains no material which has been accepted for an award of any degree or diploma in any University and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text.

I give my consent to this copy of my thesis, when deposited in the University library, being available for loan and photocopying.

P. R. Dry
1/10/97

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Chapter One - General Introduction

An excess of vegetative growth ('excess vigour') is detrimental in most vineyards. In the past 30 years or so the problem of excessive vine vigour has become more acute in many vineyards in Australia for several reasons: the proportion of irrigated vineyards, the use of vigour-inducing rootstocks and the proportion of vineyards in cool regions have all increased. Furthermore, changes in management practices such as the increased use of fertilisers and virus-free planting material, and improved soil management can all be said to have increased shoot vigour. For grapevines in general, excess vigour has negative implications for fruit yield, disease control, costs of production and efficient water-use. For winegrapes in particular, the association between vigour and wine quality is important: excessively-vigorous vines tend to have dense, shaded canopies and grapes from shaded canopies tend to produce wines of lower quality. Therefore, control of excess vigour should result in a more open canopy with a concomitant improvement in the quality of fruit for winemaking.

Shoot vigour may be controlled naturally in non-irrigated vineyards in Mediterranean-type climates where vine growth is largely dependent on winter rainfall stored as soil water and water shortages commonly develop during the growing season. This leads to water stress, resulting in reduced shoot growth and generally open canopies, particularly if the vines are stressed in the first half of the growing season. In Mediterranean-type climates (which supply much of the world's winegrapes), non-irrigated vineyards tend to have low fruit yields and consequent poor economic returns. For this reason, most vineyards in Australia are irrigated. Irrigation management strategies have been developed for some vineyards to induce water stress and reduce shoot vigour to desirable levels to improve fruit quality; however, yield also tends to be reduced and the improvement in wine quality may not be sufficient to increase, or even maintain, economic returns.

Many vineyards in Australia, western USA, Israel and South Africa have been converted, in recent times, from systems where the whole surface is irrigated (flood, furrow, high

output sprinklers) to micro-irrigation systems (eg. drip), where only a relatively small area of soil is wet. In regions with Mediterranean-type climates, shoot growth in drip-irrigated vineyards is often observed to slow down earlier in the season in comparison with vineyards irrigated by sprinklers where the total surface is wetted. Also, conversion of mature trees and vines from sprinkler irrigation to drip irrigation on deep soils has often been associated with a decrease in shoot vigour (Mitchell and Goodwin 1996). It may be possible to explain these observations in terms of a partial drying of the root system.

Drying of part of the root system may significantly reduce shoot growth, even when satisfactory water relations are maintained by supply of water from the hydrated parts of the root system. This has been demonstrated for a range of plant species, particularly through the use of split-root systems where part of the root system is allowed to dry while the other part is well-watered (Gowing *et al.* 1990, Davies and Zhang 1991). There is good evidence that plants have some mechanism for sensing the drying of the soil in contact with the roots. This information is communicated to the shoot in the form of a chemical signal which regulates both growth and physiology of shoots. Abscisic acid (ABA) satisfies many of the criteria for a chemical signal, ie. it is sourced in the roots, able to move from the roots to the shoots and able to affect shoot physiology in the field (Davies and Zhang 1991).

The application of water to a relatively small proportion of the soil surface with a micro-irrigation system may satisfy the water requirements of the whole vine and may be thought of as analogous to the well-watered part of the root system of the split-root experiments described above. The remainder of the root system, which may have developed in response to earlier irrigation practice, or to water available in the soil profile after winter rains, will not receive water and may be analogous to the dry roots of the split-root experiment; it is these roots which may be able to exert significant inhibitory effects on vegetative growth. Similarly, grapevines grown on clay soils with limited water availability in hot, intensively-irrigated regions do not appear to have the same vigour problems as those grown on the deep sands (Loveys 1991). It is possible that the vines on the clay soils undergo wetting

and drying cycles such that they accumulate shoot growth-inhibiting signals from their roots as the soil dries and this contributes to their lack of vigour.

There has been some limited work with split-root grapevines grown in containers under controlled conditions to investigate physiology and water relations (Düring 1990,1992; Poni *et al.* 1992). However, there do not appear to have been any field studies where grapevines have been grown with split-root systems to control shoot vigour. Furthermore, there do not appear to have been any field experiments with grapevines where spatially-separated parts of the root system have been deliberately allowed to dry for this purpose; the experiments with regulated deficit irrigation are perhaps the closest in this respect. Loveys (1991) proposed that vigour control of grapevines may be achieved by modifying the irrigation system so that a water deficit develops in part of the root system, thereby producing a shoot-growth inhibiting signal.

Drying of half of the root system of split-root plants, while the other half was well-watered, reduced total plant transpiration in a pot experiment (Poni *et al.* 1992): it is possible that partial drying of field vines may also increase water-use efficiency. Irrigation efficiency is low in many vineyards in Australia and elsewhere. Also, over-irrigation not only wastes water but also causes environmental problems such as depletion of ground-water reserves, rising water tables, salinity and nutrient leaching.

There is little information on the influence of root signals in general, or ABA in particular, on vegetative growth and yield components of grapevines, particularly under field conditions, and none on their possible effects on fruit composition.

The general hypothesis to be tested in this study is that:

'*partial drying of the root system of grapevines will result in reduced vegetative growth with beneficial effects for fruit yield, fruit composition and water-use efficiency.*'

Chapter Two - Literature review

2.1 Grapevine growth and development

The cycle of growth commences with budburst in spring; this is followed by a period of rapid shoot elongation (Figure 2.1). The period from budburst to flowering is ca. 60 days; therefore, unlike many deciduous, temperate fruit trees, much of the vegetative growth occurs before the start of fruit development. The rate of shoot elongation is greatest early in the season and then steadily decreases: by flowering, the rate is 50% of that achieved earlier. Accumulation of both leaf and stem dry matter is linear from just after budburst until the start of fruit growth when expressed as a function of growing degree days (Williams and Matthews 1990); the increase in leaf area has a similar relationship with temperature. Approximately two-thirds of final leaf area may have been formed by fruit set. Shoot growth typically slows in mid-summer and is terminated by abscission of the shoot tip.

'Lateral' shoots, which are borne in the axils of leaves on 'main' shoots (= 'primary' shoots which are derived from latent buds), may continue to grow after the time when main shoots have slowed or ceased to grow, particularly in vigorous situations. On weak main shoots, laterals develop little and abscise early. The degree of lateral shoot development, ie. both the number and the mean length per lateral, are directly proportional to shoot vigour. If the tip of the main shoot is damaged or deliberately removed (= 'topping', 'tipping', 'trimming' etc.), the growth rate of laterals will increase, particularly the most distal ones. A late-season improvement in water status may induce growth of lateral shoots after termination of growth of main shoots (Smart and Coombe 1983). The area of leaves on lateral shoots ('lateral leaf area') may be up to 25% of total leaf area per vine (Williams and Matthews 1990).

The inflorescence primordia (which will provide the bunches of the next season) are initiated in latent buds from about the time of flowering; development of inflorescence

primordia will continue until the buds enter endo-dormancy in mid- to late-summer. Grapevine 'buds' are compound buds comprising three or more latent buds; a 'fruitful' bud is a compound bud with one or more inflorescence primordia. A single flower may develop into a single berry as a result of fruit-set. Berry setting begins the fruit development period which takes place over 10 to 20 weeks. Fruit development has three distinct stages and the increase in berry volume or fresh weight is said to be a double sigmoid. The first stage, coinciding with the latter part of shoot growth, is characterised by an increase in berry size due to division and enlargement of pericarp cells. During the second stage, the increase in berry size slows and the end of this stage is signalled by a softening and colour change of the berries termed 'veraison'. During the third stage, berry size again increases, but only due to cell expansion. The ripening processes occur during the last stage: glucose and fructose accumulate, titratable acidity (TA) decreases, pH increases and the berry continues to soften and change colour. The accumulation of phenolic compounds (including the anthocyanin pigments) and aroma/flavour compounds mainly takes place during this last stage: collectively, these are often termed 'secondary metabolites'. The duration of each stage depends on both variety and locality. While berries ripen, shoot stems lignify and buds become dormant. In autumn in temperate climates, leaves abscise and the vine becomes dormant.

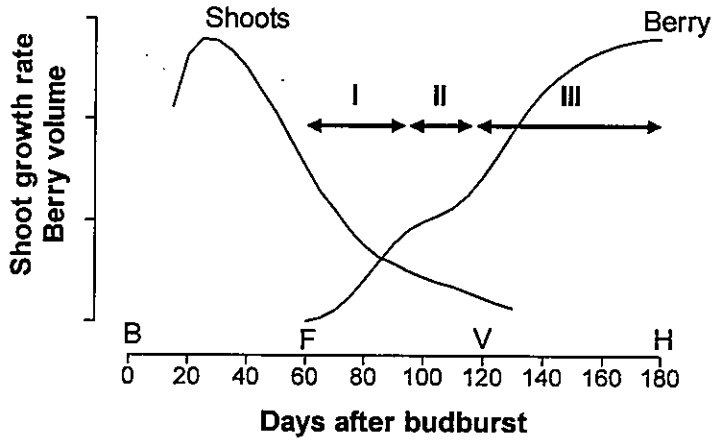


Figure 2.1 Schematic representation of shoot growth rate ('main' shoots) and berry volume of the grapevine (B, F, V, H indicate times of budburst, flowering, veraison and harvest; I, II, III indicate stages of berry development).

Mature shoots of the growing season just completed (known as 'canes') are leafless and woody; they bear the buds which give rise to next season's fruit. Vines are normally pruned in winter to short or long bearers ('spurs' and 'canes' respectively); the level of pruning is usually described in terms of the number of buds or nodes retained per vine. In some Australian regions, vines may be left completely unpruned or just skirted below the cordon (to achieve some degree of crop control or to facilitate cultural operations): this practice is termed 'minimal pruning' (Clingeleffer 1983).

2.2 Definition of vigour

Winkler et al. (1974) defined 'vigour' as "... the quality or condition that is expressed in rapid growth of the parts of the vine. It refers essentially to the rate of growth." Further reading of that chapter reveals that these authors were mainly concerned with the 'vigour' of shoots, particularly in relation to pruning: for example, one of the principles of grapevine pruning elucidated in that chapter is that "... the vigour of shoots of a vine varies inversely with the number of shoots and with the amount of crop." They also defined the term 'capacity' as "...the quantity of action with respect to the total growth and total crop of which the vine or a part of it is capable". 'Capacity' refers to the ability for total production rather than to rate of activity, ie. 'vigour'. At the single shoot level, 'vigour' and 'capacity' are linked, ie. shoots of high 'vigour' tend to have a large 'capacity' for the production of fruit and shoot mass. However, this is not necessarily the case at the whole vine level because a vine with high average shoot vigour may have a relatively low capacity, eg. a young, non-fruiting vine or a severely-pruned, mature vine. At the other extreme, a vine with high capacity may have a low average shoot vigour, eg. a non-pruned, mature vine.

On a single shoot basis, 'vigour' is a relatively easy concept to understand. Thin and short shoots with few and small leaves are considered to have 'low vigour'. On the other hand, 'high vigour' shoots tend to have rapid shoot growth in spring which may be prolonged, well into the growing season, often post-veraison. As a consequence, such shoots are long

with relatively long internodes and thick stems; they have large leaves. As they may also have many lateral (axillary) shoots, a relatively high proportion of their leaf area is contributed by leaves on these lateral shoots. Various measurements have been used to quantify shoot vigour (Table 2.1). There is a general recognition of an optimum vigour of shoots associated with the best combination of quantity and quality of production. The specifications for optimum vigour will lie somewhere between the limits for 'low' and 'high' in Table 2.1. and will vary according to grape cultivar. Varieties differ in their potential shoot vigour: Muscat Gordo Blanco and Shiraz (both *Vitis vinifera*) have 'low' and 'high' potential shoot vigour respectively. However, the potentially-high shoot vigour of a cultivar such as Shiraz will only be expressed when pruning is relatively severe and environmental factors (eg. water supply) necessary for growth are not limiting.

Table 2.1 Some measurements used to characterise shoot vigour (adapted from Smart and Robinson, 1991; Smart 1992).

Variable	'Low' vigour	'High' vigour
Length of 5th internode mm	<60	>180
Diameter of 5th internode mm	<4	>12
Mean main leaf area cm^2/leaf	<100	>170
Mean lateral leaf area cm^2/leaf	<25	>50
Mean cane weight in winter g	<25	>60
Shoot length (untrimmed) cm	<50	>150

The degree of lateral shoot development, ie. both the number and the mean length per lateral shoot, are directly proportional to 'main' shoot vigour. Lateral shoots may continue to grow after the time when main shoots have slowed or ceased to grow, particularly in vigorous situations. A late-season improvement in water status may induce growth of lateral shoots after termination of growth of main shoots (Smart and Coombe 1983). Therefore, measurements such as the ratio of lateral leaf area to total leaf area or the ratio of total length of lateral shoots to main shoot length could be used more frequently as an index of shoot vigour. For example, Smart and Robinson (1991) found that the ratio of lateral leaf area to total leaf area for high vigour Sauvignon Blanc shoots was 0.37. Neither

'low' nor 'high' shoot vigour are necessarily undesirable: it is only when shoots have either insufficient vigour on the one hand, or excessive vigour on the other, that there may be undesirable consequences. It is the latter situation, that of excessive shoot vigour, which is much more of a problem in modern viticulture than the former.

The term 'vine vigour' is widely used in viticulture, both in the literature and in practice, but it is less readily defined than 'shoot vigour'. There are many reports in the literature on the problem of excessive 'vine vigour' and its control where there has been no attempt to define 'vine vigour'. It is almost as if the authors have assumed that the concept of 'vine vigour' is so well-understood in viticultural circles that it does not require definition. Unfortunately, at the whole vine level, the term 'vigour' has been often used when, according to the definitions of Winkler et al. (1974), those authors should have used the term 'capacity'. There are many instances where 'pruning weight' (= the mass of the mature, one-year old canes removed at the time of winter pruning) has been used or recommended as a measure of 'vine vigour' (eg. Shaulis *et al.*1966, Huglin 1986) when it is more appropriately used as a measure of vine 'capacity', because neither high nor low absolute values of the mass of prunings per vine are necessarily indicative of a high average shoot vigour per vine. Also, the term 'vigorous' is often used in a pejorative sense when a term such as 'excessively-vigorous' would be more appropriate. There is nothing inherently wrong with a 'vigorous' vine so long as it produces a high yield of fruit of desirable quality, ie. so long as it is 'balanced'.

For whole vines, or even vineyards, the term 'vigour' has been used in different ways. On the one hand, 'vigour' has been used to convey a sense of potential vine productivity. Although Winkler et al. (1974) have used 'capacity' in this context, perhaps 'vine size' or 'vine strength' would be more appropriate. On the other hand, 'vigour' has been used in relation to whole vines to describe the relationship between the extent of vegetative growth of a vine together with its fruit production, ie. an 'excessively-vigorous' vine could be defined as "...one with an excessive amount of vegetative growth relative to fruit growth". The term 'balance' has been used to describe the amount of vegetative growth relative to

fruit growth (Smart and Robinson 1991; Gladstones 1992) and may be more appropriate than 'vigour' in this context. Gladstones (1992) defined vine 'balance' as "...(the situation) when vegetative vigour and fruiting load are in equilibrium and consistent with high fruit quality." 'Vigorous vines'(ie. excessively-vigorous vines) are said to be 'imbalanced' (Smart 1992). If shoot vigour is optimal, then a vine is likely to be 'balanced'. The ratio of fruit weight to pruning weight (FW/PW), both on a per vine and per area of production basis, may be used to quantify the degree of 'balance': Smart and Robinson (1991) recommended a ratio between 5 and 10 as optimal. Leaf area to fruit weight (cm^2/g), which is strongly correlated with FW/PW, could also be used as an index of balance: ~12 cm^2/g is said to be optimal (Smart and Robinson 1991) with a range of 6 to 15 cm^2/g depending on variety.

In this thesis, the term 'vigour' is used in relation to whole vines to describe both the average shoot vigour of the vine and the relative balance of vegetative growth to fruit production, ie. a 'vigorous' vine is one for which the majority of shoots are vigorous and which also has a relatively low FW/PW ratio.

2.3 The consequences of excess shoot vigour

An excess of shoot vigour is detrimental, particularly if it is occurring on vines trained to a restrictive trellis system. Such vines tend to have dense canopies with a high degree of within-canopy shading (Smart 1992). Therefore, control of excess vigour is desirable because it should not only lead to a more open canopy (with important indirect consequences for yield and fruit composition) but it is also important in producing a balanced vine.

2.3.1 Fruit yield

Shading within the canopy influences each of the yield-affecting processes of budburst, inflorescence initiation and development (= 'fruitfulness'), fruit set and berry growth. While there has been most emphasis in the past on the effects of shade on bud fruitfulness

and particularly the importance of light interception by the renewal zone (May 1965), more recent work has shown that shade reduces other yield components, eg. shade contributes to the development of Early Bunchstem Necrosis (EBSN), a cause of poor fruit set (Jackson 1991). An excessively-vigorous vine may enter a 'vegetative cycle' (Smart and Robinson 1991) whereby the reduced fruit weight per shoot (caused by shading) results in a stimulation of shoot vigour which in turn leads to more shading; this cycle can only be broken if shade in the canopy is reduced and fruit weight per shoot is increased. Shoot vigour may also be directly correlated with yield, independently of the light microclimate: highly-vigorous shoots have a high incidence of both EBSN and primary bud necrosis (Dry and Coombe 1994).

Large yield increases in response to changes in canopy management practices (up to 200-300%; Smart 1992) are usually the result of a change in training system and have often been attributed to the change in the light environment of the canopy. This is appropriate where there has been an overall increase in vine capacity. However, if capacity has not changed and there has been merely an increase in the ratio of fruit weight to pruning weight, ie. the vine has become more balanced, then one could argue that the control of shoot vigour is the most important effect. A decrease in shoot vigour is a common feature of many training systems, particularly where shoots are positioned downwards, eg. Geneva Double Curtain (GDC; Smart 1992).

2.3.2 Fruit composition

The influence of the canopy microclimate on fruit composition has been well documented for a range of cultivars and macroclimates (Smart *et al.* 1990). Fruit from shaded canopies has relatively lower sugar and tartrate concentration, higher malate and K concentration and pH); shading also reduces anthocyanins and phenolics (both concentration and absolute amounts per berry (Smart 1992) and the level of flavour compounds eg. monoterpenes (Reynolds and Wardle 1989). Concentration of flavonols in skin of Pinot Noir berries was correlated with degree of bunch exposure (Price *et al.* 1996). In a controlled environment, visible radiation enhanced sugar accumulation and colour

development of Cabernet Sauvignon; these responses were saturated at very low intensities, ca. 1-10% of full sunlight (Dokoozlian and Kliewer 1995a,b). Such low light intensities occur under dense canopies. Canopy shading has been linked with undesirable 'vegetative' characters in wines made from Cabernet Sauvignon and Sauvignon Blanc grapes (Allen *et al.* 1996). All thus may lead to lower wine quality (Smart *et al.* 1990).

In the past, the association between excess shoot vigour and fruit composition has focussed on the indirect effects via the canopy microclimate with less emphasis on possible direct links. In field experiments it is difficult to separate these effects. Working with vines in a controlled environment, Jackson (1986) found a direct correlation between shoot vigour and fruit composition (acid and pH) which was independent of shading. Total K uptake by actively growing vines is closely related to shoot vigour (Freeman and Kliewer 1983): much of this K will end up in the fruit (resulting in high must pH and reduced wine quality), particularly if there is a high proportion of shaded leaves within the canopy (Smart 1992).

The decrease in rate of shoot growth in grapevines coincides with the onset of ripening. Prolongation of shoot growth may be associated with delayed ripening. Anecdotal evidence suggests that natural cessation of vegetative growth before veraison is desirable for wine quality. This may be a direct effect, ie. diversion of the maximal amount of assimilates to the fruit: Gladstones (1992) suggests that a surplus of sugars will only be available for secondary metabolite production in the fruit when the requirements for vegetative growth (and respiration) have been met. On the other hand, this may be due to an indirect effect via the canopy microclimate. Whatever the explanation, environmental factors and/or management practices which switch-off or limit shoot growth post-veraison, and perhaps during the whole period of fruit development, will often lead to optimal fruit quality.

2.3.3 Disease incidence

The incidence of fungal diseases is higher in dense, shaded canopies because the microclimate is favourable to infection, development and spread. Furthermore, disease control is facilitated with open canopies because penetration of chemical sprays into the canopy is more efficient. Diseases such as oidium [*Uncinula necator* (Schw.) Burr] and Botytis bunch rot (*Botrytis cinerea* Pers.) can reduce yield and detrimentally affect wine quality. The tissues of vigorous shoots are often more susceptible to infection by diseases than those of shoots which are not actively growing.

2.3.4 Costs of production

Vigorous vines are more expensive to manage because larger, more elaborate trellis systems are required to support the weight of the foliage and to avoid canopy shading. Also, costs of pruning and harvesting are higher. Summer pruning of vigorous vines is often required to permit machinery movement along rows and to facilitate other cultural operations such as weed control, harvesting and foliage spraying. Up to six trimmings may be required in very vigorous situations. Trimming of vigorous shoots stimulates lateral growth and may increase shoot crowding and thus shading in the remaining canopy.

2.3.5 Water use

With relatively open canopies, vine water use is largely a function of leaf area (Williams and Matthews 1990). Therefore, reduction in leaf area may reduce vine water use but the relationship is not linear for several reasons, eg. in a dense canopy, the proportion of shaded leaves (with relatively low stomatal conductance) is much higher than in an open canopy. Winkel and Rambal (1993) have suggested that cultivars of temperate origin (eg. Shiraz) tend to regulate their water status under conditions of declining soil water availability by limiting leaf area because they lack efficient stomatal control. Even a relatively modest reduction in leaf area, e.g of the order of 10 to 15%, will significantly reduce plant water use (Saab and Sharp 1989).

2.4 Why is excess vigour more of a problem now than in the past?

The proportion of vineyards in Australia which are irrigated has increased significantly over the last 30 years as irrigation technology has improved and become more widely used; for example, only 12% of vineyards in the Barossa region of South Australia were irrigated in 1978 (Dry and Smart 1988) compared with more than 90% today (M.G. McCarthy, SARDI, South Australia, pers. comm.). Increased amount of water available to the vine has lead to an increase in vegetative growth, which in itself may not be a problem so long as the vines remain balanced. However, very often, installation of irrigation in vineyards has not been accompanied by a change in trellis system; as a result, canopies may become dense and shaded, vines become unbalanced and wine quality declines. Also, poor irrigation management will result in prolongation of shoot growth into the ripening period, particularly in those situations where shoot growth is not switched off by environmental conditions. Furthermore, it is not simply a matter of increase in total water as a result of irrigation: more strategic application, particularly during periods of potential soil water deficit, will result in less inhibition of shoot growth.

The use of rootstocks in Australian vineyards, particularly for nematode control, has increased significantly since the 1970s. This practice has exacerbated the vigour problem due to irrigation because the rootstocks used most commonly, eg. Ramsey, are high in vigour (Hardie and Cirami 1988) and *Vitis vinifera* cultivars grafted to them are more vigorous than when grown on their own roots (Sommer and Clingeleffer 1996). Also, the use of nematode-resistant rootstocks is greatest on light-textured soils which make up a large proportion of intensively-irrigated vineyards (Dry and Smart 1988). Practices such as minimal pruning, which may be successful for shoot vigour control in arid climates with own-rooted *Vitis vinifera,* do not necessarily work with some scion/rootstock combinations.

The area of vineyards in cool regions has increased substantially in Australia, and other 'New World' countries, during the past 20 years or so. Cool climates tend to encourage vegetative growth at the expense of fruit growth (Gladstones 1992). Also, bud fruitfulness

is relatively low due to a combination of low air and soil temperatures and low radiation levels; unless appropriate canopy management practices are employed, fruit yields can decline and vines can readily become unbalanced. This problem is more common in cool, maritime climates where shoot growth can be prolonged, well into the ripening period. Other factors such as increased use of fertilizers (particularly those high in N), clonal selection, virus-free planting material and improved soil management can be said to have increased vine vigour.

2.5 Vigour control

Vigour may be controlled by chemical methods, rootstocks, root pruning or root volume restriction, planting density, pruning level and trellis system or water supply. Whereas all of these treatments may successfully reduce shoot vigour, there is often a penalty associated with their use, either in terms of yield or fruit quality, if one of these treatments is used alone. For example, one of the pruning methods developed in part for this reason, minimal pruning (Clingeleffer 1983), may reduce shoot vigour but the fruit may not be appropriate for all styles of wine (in the absence of bunch thinning). To optimise the gains from the use of some of these techniques it may be necessary to change other practices at the same time. For example, if shoot number per vine is increased in order to reduce shoot vigour, it may be necessary to increase cordon length per vine to maintain a desirable shoot spacing, and this may require conversion of the trellis system.

2.5.1 Chemical methods

Several growth regulators have been shown to be effective in reducing shoot vigour: these include maleic hydrazide, CCC, ethrel and paclobutrazol (Lavee 1987; Reynolds *et al.* 1992). However, the response is difficult to regulate and it may vary according to environmental factors. Also, their use may be associated with undesirable side-effects, eg.

ethephon reduces photosynthetic rate (Smart 1992). There is the possibility that use of such chemicals may be restricted in the future.

2.5.2 Rootstocks

This use of devigorating rootstocks has been successful with other fruit crops, particularly apples, where dwarfing rootstocks are commonly used in high density orchards. For grapevines, no attention has been paid to this characteristic in the past, as the selection and breeding of rootstocks for grapevines has been primarily to provide resistance to soil-borne pests, phylloxera (*Daktulosphaira vitifoliae*) and nematodes. While there are some differences between grapevine rootstocks with respect to the amount of vigour conferred to the scion, the effect is relatively minor in comparison with other fruit crops.

2.5.3 Root pruning and root volume restriction

Root pruning of pome fruits is an effective means of reducing shoot growth (Schupp and Ferree 1989) but experience with grapevines is limited (Dry et al. 1995). Use of permanent mown sward can significantly reduce vegetative growth but this is usually at the expense of fruit production: the effect is due to a combination of root volume restriction and competition for water and nutrients (McCarthy *et al.* 1992).

For as long as grapes have been grown, vigour has been controlled to some extent by site selection, ie. by the choice of soils with limited soil depth and relatively low fertility. Although the devigorating effect of shallow soils has often been attributed to limitation of water supply, research by Rowe (1993) and others has demonstrated that, even in situations where water and nutrient availability is non-limiting, root volume has a direct effect on the size of the root system and therefore on shoot vigour. The overall effect of reduced root volume is to produce smaller plants which produce more fruit per unit of leaf area with less water than non-restricted plants (Rowe 1993). In pot experiments, shoot growth was found to be correlated with root volume without being affected by water or nutritional status (Carmi and Shalhevet 1983). It is, however, not always possible to choose a soil which produces the desirable root volume characteristics and there are also practical problems in

successfully limiting the root volume. Experimentally, an artificial barrier which did not impede drainage has increased fruit weight to vegetative growth in apple and peach (Williamson *et al.* 1992). In arid regions, regulating the size of the soil zone wetted by drip irrigation can be used to limit the root volume because root growth is limited to that zone; the reduction in shoot growth observed in response to 'regulated deficit irrigation' (RDI) may be explained in part by the decrease in wetted soil volume (Mitchell and Goodwin 1996).

2.5.4 Planting density

Traditionally, vineyards in Australia and California (USA) were established with much wider between-row spacings (3.3-3.6m) and within-row spacings (1.5-2.1m) than vineyards elswhere. While the wide row-spacing was largely a reflection of the wide machinery used in vineyards in the past, it was also found to lead to economic benefits with respect to the yield/cost ratio. In more recent times, there has been a tendency to use higher planting densities in new vineyards, particularly in the cooler regions. To a large degree, this practice has been encouraged by the supposition that this will lead to desirable devigoration. European experience, however, indicates that this is only likely to occur on sites of low fertility. Furthermore, the narrow rows of high density vineyards result in high establishment and operating costs. An alternative viewpoint is to combine low vine density with trellises which allow high shoot numbers per vine for devigoration, a system favoured by Smart (1992).

2.5.5 Pruning level and trellis system

Shoot vigour is inversely proportional to shoot number per vine, which is a function of number of nodes retained at pruning (Tassie and Freeman 1992). The technique of 'minimal pruning' (Clingeleffer 1983) which has been widely used in vineyards in hot, intensively irrigated regions of Australia, successfully reduces shoot vigour. Shoots of vines under minimal pruning are shorter than shoots of conventionally pruned vines due to fewer nodes and shorter internodes. The number of mature nodes is also reduced because the distal part of the shoots fails to lignify during autumn (Possingham *et al.* 1990).

However, minimal pruning is not always favoured for quality winegrape production because fruit yield may be difficult to control, leading to delayed and uneven ripening (D.G. Botting, University of Adelaide, pers. comm.). Leaf area in spring may be twice that of conventional vines (Sommer and Clingeleffer 1996) and, as a result, vine water use tends to be higher; therefore, this technique may not be suitable where water supply is limited. In cool or humid regions where environmental conditions do not induce growth cessation by the time of veraison, uncontrolled growth will lead to canopy crowding associated with excessive shading (Smart and Robinson 1991).

A combination of adequate trellising and light pruning, for instance 30 nodes per m^2 (ie. intermediate to the pruning level that is typical of severe, conventional hand pruning and that of minimal pruning), may produce vines with open canopies which have sufficiently devigorated and fruitful shoots to produce high yields (Smart 1992). However, in order to maintain a desirable shoot spacing, this will usually require a divided canopy with low planting density. Conversion of existing vineyards to trellis systems with divided canopies is expensive and not all trellis systems are compatible with complete mechanisation of pruning and harvesting. In addition, the operation of shoot positioning, which is an integral part of many systems, is labour-intensive and requires a high degree of management expertise to optimise the timing of the operation. Furthermore, the beneficial effects of these trellis systems on fruit composition and wine quality, which have been demonstrated in cool regions (Smart and Robinson 1991), have been more difficult to demonstrate in hot regions (Botting *et al.* 1996). Shoots growing upward tend to be more vigorous than those growing downward which, in Cortese grapevines, were shown to have less leaf area, lower shoot diameter and lower stomatal conductance than upward or horizontal shoots (Schubert *et al.* 1995). This phenomenon is employed in some trellis systems, eg. Geneva Double Curtain (Smart and Robinson 1991), designed to reduce vigour and shoot density. With Sultana, where fruit bud initiation is low in comparison to other *Vitis vinifera* cultivars, vertical shoots were shown to be not only more vigorous but also more fruitful than horizontal or normally-growing shoots, at least in seasons of low

fruitfulness (May 1966) and therefore downward training may be not only devigorating but also yield-restricting.

At least part of the effect of shoot number per vine on shoot vigour must be attributed to the competition between vegetative and reproductive growth: fruit weight per vine is a function of bunch number which in turn is directly proportional to shoot number. An inverse relationship between crop load and shoot vigour has been well-documented (Winkler *et al.* 1974). However, 'crop loading' as a method of vigour control, in isolation, has inherent problems because of the relationship between yield (per vine or per unit area) and grape composition. Although high yields will reduce vigour, the associated reduction in ratio leaf area/fruit weight may result in grapes whose composition is undesirable for their end use, be it for wine, dried fruit or tablegrapes. At the other extreme, low crop load per vine (and high leaf area/fruit weight ratio) may stimulate vegetative growth such that shading problems are created, leading to deterioration in grape quality.

2.5.6 Water supply

The shoot growth of grapevines is known to be sensitive to water stress (Smart and Coombe 1983; Williams and Matthews 1990): internode elongation and duration of shoot growth are particularly sensitive, especially when stress occurs early in the season, but production of nodes can also be affected (Williams and Grimes 1987). Duration of shoot growth has a direct influence on node production, as has the rate at which new nodes are formed at the apex. A decrease in the rate of shoot growth in response to water stress has been attributed to a direct effect of shoot water potential on cell turgor (Smart and Coombe 1983; Hardie and Martin 1990) but turgor often does not change in response to soil drying due to osmotic adjustment (Sharp and Davies 1989) which is known to occur in grapevine leaves (Düring 1985) and roots (Düring and Dry 1995). Furthermore, Nagarajah (1989) noted that a decrease in the rate of shoot extension in grapevines may occur well before the onset of any measurable decrease in pre-dawn leaf water potential.

In regions with low rainfall during the growing season, eg. in those with Mediterranean-type climate, vigour may be successfully controlled by irrigation management. Water deficits were shown to inhibit the rate and duration of shoot elongation when drip irrigation was scheduled to maintain various fractions of plant available water (van Zyl 1984) or vineyard evapotranspiration [ET] (Williams and Grimes 1987). Reduction in shoot length resulted in reduced leaf area per vine, with greater effect on lateral than main leaf area (Williams and Matthews 1990). Root mass is increased by moderate water stress but decreased under very dry or wet conditions (van Zyl 1988).

However, in most experiments where shoot growth was significantly reduced, there was also a reduction in yield, associated with a water deficit. The magnitude of deficit in water supply that needs to be imposed to achieve a significant reduction in vegetative growth, eg. a decrease in shoot length or pruning weight of at least the order of 15%, were typically associated with yield decreases of at least that order (McCarthy and Staniford 1984; Matthews and Anderson 1988,1989; Goodwin and Jerie 1992; Poni *et al.* 1993; McCarthy, 1997). The main yield component affected in these experiments was berry weight. In those cases where yield was not significantly reduced, there was little or no effect on vegetative growth (Goodwin and Jerie 1992).

The possibility in Mediterranean climates of controlling vigour by irrigation management through imposing water stress is largely restricted to the period from flowering to veraison: between budburst and flowering it is difficult to induce sufficient soil water deficits to inhibit growth, due to rainfall during that period and storage of winter rainfall in the soil, while post-veraison stress has little or no effect on shoot growth (Matthews and Anderson 1989; Poni *et al.* 1994; Naor *et al.* 1993) because canopy development is largely complete by veraison (Sommer and Clingeleffer 1996). Excessive stress imposed after veraison may lead to reduced sugar accumulation and increased pH (Williams and Matthews 1990) as well as decreased yield (Naor *et al.* 1993).

On the grounds that vegetative growth is more sensitive to water stress than fruit growth during the first stage of berry development, Hardie and Martin (1990) proposed an irrigation strategy which gradually increases soil water tension from flowering onwards; they claimed that yield was not reduced on the basis of limited data from field trials. This, and strategies such as regulated deficit irrigation [RDI] (= replacement of less than the full irrigation requirement; Goodwin 1995) rely on a precise control of water application in order to apply a 'mild' stress at critical stages during the season; accurate monitoring of both vine and soil moisture status is required and the strategy may be difficult to apply across a whole vineyard because of lack of soil uniformity and deficiencies in irrigation system design. RDI has been used successfully with pome and stone fruit to reduce vegetative growth, increase fruit yield and decrease the amount of irrigation applied (Chalmers et al. 1981; Mitchell et al. 1988; Mitchell and Goodwin 1996). However, the results from RDI experiments with grapevines indicate that any substantial reduction in vegetative growth is usually associated with a significant yield penalty, as a result of an effect on berry size (Goodwin and Macrae 1990; Goodwin and Jerie 1992; Mitchell and Goodwin 1996). Furthermore, maintenance of a mild stress during summer may not be practicable, even though vines hardened by mild stress will be buffered to some extent against the effects of such conditions. Nevertheless, a sudden onset of high temperature and low humidity may rapidly push plants from mild to severe stress.

2.6 Effect of partial drying of the root system

The following is a review of the effect of drying of a portion of the root system on shoot and root growth and gas exchange, with particular emphasis on experiments which have used split-root systems.

2.6.1 Shoot growth

For many years, plant physiologists believed that soil water deficit reduced the growth rate of whole shoots, or shoot components such as leaves, by reducing the water potential, and hence the turgor, of elongating cells (Hsiao 1973). However, it has now been

demonstrated that the elongation rate of shoots and leaves of plants in drying soil can vary in the absence of changes in water potential of leaves, shoots or the whole plant. Several types of experimental systems have been used for this purpose, including the system of split-roots (Davies and Zhang 1991). For example, Gowing *et al.* (1990) divided the roots of small apple (*Malus* x *domestica*) trees between two separate rooting containers (= 'split-root' plants): cessation of watering of one container reduced the rate of leaf area development by nearly 50% over a three-week period, compared to plants with both containers watered, without any apparent effect on shoot water relations. Both the expansion of individual leaves and the initiation of new leaves were reduced by soil drying. Rewatering restored the rates of leaf expansion and initiation to the rates shown by plants which had both containers watered.

Experiments with other species, using various types of split-root systems, have shown similar reductions in shoot growth. The response to drying part of the rot system was measured by the rate of leaf expansion or elongation, the leaf area, the rate of shoot extension or the shoot dry weight). Responses were examined on non-woody plant species such as tomato (Tan *et al.*1981), maize (Saab and Sharp 1989), barley (Claxton and Seel 1995), castor oil (Jokhan and Jackson 1995) and sorghum (Ludlow *et al.* 1990, Ebel *et al.* 1994) and with woody species such as peach (Tan and Buttery 1982; Poni *et al.* 1992), pear (Poni *et al.* 1992), citrus (Kosola and Eisenstatt 1994) and passionfruit (Turner *et al.* 1996). In all cases, reductions in shoot growth occurred in the absence of any change in shoot water potential.

With woody species, drying half of the root system typically resulted in reductions in components of shoot growth of the order of 10 to 25%, relative to control plants (with both pots watered) after several weeks of treatment. (Tan and Buttery 1982; Poni *et al.* 1992; Turner *et al.* 1996). The magnitude of the response of both woody and non-woody species was found to be strongly correlated with the amount of roots in the drying soil (Tan and Buttery 1982; Ebel *et al.* 1994). None of the undesirable symptoms of water stress such as

wilting or abscission of leaves were observed in response to the treatment (Poni *et al.* 1992).

2.6.2 Root growth

It is known that drought may cause more inhibition of shoot growth than that of root growth and in some cases the absolute root biomass of plants in drying soil may increase relative to that of plants in well-watered soil (Sharp and Davies 1989). With split-root plants, increased root growth in the 'dry' pots relative to the 'wet' pots was observed with peach (Tan and Buttery 1982) and with apple, pear and grapevine (Poni *et al.* 1992) but not with tomato (Tan *et al.* 1981) or citrus (Kosola and Eisenstatt 1994). Nevertheless, 40% of the total root dry weight of peach seedlings was maintained in the dried half (Tan and Buttery 1982). There was no change in the ratio of shoot to root weight for the whole plant relative to the control in any case. Roots maintained in dry soil obviously preserve their water-absorbing capacity until rewatered when they resume growth.

2.6.3 Stomatal conductance

Stomatal closure during soil drying has often been studied in conjunction with measurements of leaf and shoot growth. It was found that, as for shoot growth, gas exchange may also decrease in response to drying of part of the root system, in the absence of any change in shoot water status. This observation is important in furthering the understanding of factors controlling stomatal opening. Over the last decade, analyses of stomatal control have served mainly to invalidate the theory of stomatal control by leaf water status alone and to demonstrate that changes in leaf physiology are more closely linked to changes in soil water status than to the leaf water status (Davies and Zhang 1991). Proof of this hypothesis has been achieved in different ways: for example, high Ψ_L of plants with roots in drying soil was maintained artificially by either pressurizing the whole root system of droughted plants (Gollan *et al.* 1986; Schurr *et al.* 1992) or by drying half the root system of split-root plants (Zhang and Davies 1990a; Khalil and Grace 1993). Also, there have been several reports which have shown higher Ψ_L values in droughted plants than well-watered, associated with lower g_S (Loveys and Düring 1984; Jones 1985;

Davies *et al.* 1994), suggesting that stomata controlled leaf water status rather than the reverse (which was generally assumed to be the case up to the late 1980's).

With woody plant species, drying half of the root system typically results in reductions in stomatal conductance of the order of 15 to 70% after several weeks of treatment, relative to control plants with both pots watered (Tan and Buttery 1982; Poni *et al.* 1992; Khalil and Grace 1993) while the magnitude of the response in shoot growth is strongly correlated with the amount of roots in the drying soil (Tan and Buttery 1982). Decreases in total plant transpiration in response to the half-drying treatment may (Poni *et al.* 1992) or may not (Turner *et al.* 1996) be associated with changes in stomatal conductance. Gowing *et al.* (1990) observed that the transpiration rate of half-dried plants declined by up to 30% in comparison to controls. While this was largely a function of the smaller leaf area of the half-dried plants, there was also a stomatal component as indicated by the decrease in transpiration rate per unit leaf area.

2.6.4 What is the control mechanism?

For the responses described above to occur, plants need to have some mechanism for sensing the drying of the soil around the root and communicating this information to the shoot, resulting in restriction of both leaf growth rate and gas exchange. Various authors have suggested that this involves transfer of chemical information from roots to shoots via the xylem (Davies and Zhang 1991; Gowing *et al.* 1993; Davies *et al.* 1994). Such control has been termed 'non-hydraulic' or 'chemical' signalling to distinguish it from 'hydraulic' signalling, which represents transmission of reduced soil water availability via changes in xylem sap tension (Dodd *et al.* 1996). Key evidence in favour of a chemical signal was provided by the experiment of Gowing *et al.* (1990): when roots in contact with drying soil were severed from the plant, leaf growth rate recovered to that shown by well-watered plants, even though no more water was made available to the remaining roots. This result indicates that root excision removed the source of an inhibitor of leaf growth which was generated as a result of the association between roots and drying soil.

2.7 Chemical signals

Much evidence has been obtained from many different plant species under both controlled environment and field conditions which suggests that plants can use chemical signals to regulate shoot growth and physiology as the soil dries. This regulation may occur under conditions where shoot water relations are not changed, but may also occur in conjunction with hydraulic regulation (Dodd *et al.*1996). Chemical signals may move from roots to shoots, or from one leaf to another via the roots, and they may be defined as 'positive' or 'negative' (Davies and Zhang 1991).

2.7.1 Positive and negative signals

A *negative* signal was defined as '... the reduced supply of something normally transmitted from roots to shoot' (Davies and Zhang 1991). Negative signals are supplied by turgid roots. As these signals promote stomatal opening and shoot growth, their production and transport would decrease as the soil dries. Cytokinins are an example of a possible negative signal: reduced xylem transport of cytokinins to shoots can be detected in droughted plants. Evidence for cytokinins as root signals was provided by Meinzer *et al.* (1991) for sugarcane and Fußeder *et al.* (1992) for almond respectively. However, Gowing *et al.* (1993) doubted a central role for these compounds in the signalling process. They argued that, unless the roots dry out significantly, the reduction in the flux of cytokinins will be very small and it is unlikely that shoots could detect and respond to anything but a substantial change in cytokinin supply. Furthermore, the effect of root excision in the split-root experiment described above (Gowing *et al.* 1990) cannot be explained in terms of variation in cytokinin supply: the removal of drying roots could not be expected to cause an increase in transport of cytokinins to the shoot and hence could not explain the increase in leaf growth. Rather, these data indicate that leaf growth of plants with roots in contact with drying soil is limited by the transport of an inhibitor from dehydrating roots ie. by a *positive* signal.

Positive signals, whose production increases as the soil dries, can be inhibitors such as ABA. Changes in mineral composition or in the pH of the xylem sap (Gollan *et al.* 1992; Schurr *et al.* 1992) may act alone as signals or in conjunction with other signals (Dodd *et al.* 1996). The possibility of an interaction between positive and negative signals cannot be dismissed: cytokinins are known to modify stomatal response to ABA and decreased absorption of nutrients under drought conditions may influence stomatal response to ABA (Schurr *et al.* 1992).

2.7.2 Evidence in favour of ABA as a positive signal

In the last 15 years or so, a large body of evidence has been accumulated to link stomatal function with ABA eg. decreased stomatal conductance of leaves is correlated with increased endogenous ABA (Jones 1990) and exogenous application of ABA to leaves causes a rapid reduction in g_S (Loveys 1984a). Also, ABA has been shown to have an inhibitory effect on leaf growth (van Volkenburgh and Davies 1983) and leaf growth rate is negatively correlated with endogenous ABA accumulation in leaves (Puliga *et al.* 1996). ABA also plays an important regulatory role in maintaining root growth in drying soil (Sharp *et al.* 1994) and appears to increase hydraulic conductivity of roots in drying soil (Zhang *et al.* 1995).

Many reports contain evidence that ABA synthesis is stimulated as cells dehydrate (Dodd *et al.* 1996). When all of this evidence is combined, it suggests that ABA produced by dehydrating cells can move to the guard cells of leaves to restrict water loss and slow further dehydration. At the same time, ABA limits the increase in leaf area of the whole plant by inhibiting production of new leaves and leaf expansion while it sustains root growth. It was first thought that ABA was synthesized in the leaf mesophyll and moved to the guard cells and to growing points to exert its effect (Mansfield and Davies 1981). However, Loveys (1984a) and Loveys and Düring (1984) suggested that ABA influx from xylem may have a major influence on foliar accumulation of ABA and more recent results have provided evidence that increased xylem ABA concentration [X-ABA] was root-sourced and quantitatively sufficient to account for the physiological changes observed in

the shoots of plants droughted under controlled conditions, ie. reduction of stomatal conductance and leaf growth rate (Zhang *et al.*1987; Zhang and Davies 1989a, 1990a,b; Tardieu *et al.* 1992a). Similar evidence has come from field experiments with almond (Wartinger *et al.* 1990), maize (Tardieu *et al.* 1992b) and *Ceanothus* (Tenhunen *et al.* 1994) showing an apparent effect of xylem ABA on leaf conductance. Many reports have shown that there is a good relationship between [X-ABA] and soil water status (Zhang and Davies 1990b; Khalil and Grace 1993).

ABA can be synthesized in roots in many species (Davies and Zhang 1991). In a split-root experiment with *Commelina communis* grown under controlled conditions, the half of the root system in drying soil contained significantly increased concentrations of ABA compared to the other half of the root system in watered soil (Zhang *et al.* 1987). Concentration of ABA in roots increased with decreasing soil water content; roots in lower layers of the soil profile contained increasingly more ABA as the soil dried (Zhang and Davies 1989a). Since these increases in root ABA took place in the absence of decreased leaf hydration, it is unlikely that the roots were the recipients of ABA transported from water-stressed leaves (Jackson 1993). It appears that as soil water potential around the roots falls towards -0.2 to -0.3 MPa, partial dehydration of roots stimulates ABA production (Zhang and Davies 1989a).

Despite the evidence in favour of ABA, there are reports for several species that g_S frequently correlates with Ψ_L (Sadras *et al.* 1993) or that stomata start to close before changes in xylem ABA are detected (Davies *et al.* 1994), indicating that chemical signals may not be involved in all cases. However, Tardieu *et al.* (1996) found, with sunflower, that stomatal control depended only on [X-ABA] and that Ψ_L was controlled by water flux through the plant (itself controlled by g_S). Furthermore, they proposed that statistical relationships between Ψ_L and g are only observed when Ψ_L has no controlling action on stomatal behaviour. Nevertheless, sensitivity of stomata to xylem ABA may depend on Ψ_L (Tardieu *et al.* 1993): stomatal sensitivity to the ABA signal may increase as Ψ_L decreases (Tardieu and Davies 1992; Trejo and Davies 1994). This indicates that high values of g_S

in droughted plants early in the day can be explained by low stomatal sensitivity to a significant ABA signal; as Ψ_L decreases to its minimum value at midday (which may be no lower than that of well-watered plants), stomata are sensitized to the ABA signal (Dodd *et al.* 1996). [X-ABA] rather than bulk leaf [L-ABA] was correlated with stomatal conductance; this indicates rapid turnover of ABA arriving at leaves and that stomata respond to [X-ABA] rather than the total amount of ABA delivered (Liang *et al.* 1996)

Application of exogenous ABA by root-feeding plants with different concentrations of synthetic ABA (Zhang and Davies 1990a) or stem injection of ABA (Tardieu *et al.* 1993) also demonstrated relationships between stomatal conductance, shoot growth and [X-ABA] that were closely comparable to those caused by roots in drying soil. This result seems to indicate that the extra ABA synthesized as a result of soil drying can account for most of the anti-transpirant activity of xylem sap. A similar conclusion was reached by Zhang and Davies (1991) who removed most of the ABA from xylem sap of laboratory-grown maize plants and found that most of the anti-transpirant activity disappeared.

These results seem to confirm that ABA satisfies the criteria for a chemical signal sourced in the roots, able to move from the roots to the shoots and able to affect shoot physiology in the field. This applies not only for container-grown plants, but also in the field. There is evidence, however, that in some species substantial anti-transpirant activity in the xylem stream cannot be attributed to ABA (Munns and King 1988; Trejo and Davies 1991). Several possible explanations for the anti-transpirant activity of 'ABA-stripped' sap have been proposed including redistribution of existing ABA and high sensitivity of stomata to small changes in ABA concentration (Dodd *et al.* 1996).

2.7.3 Does the same signal affect both stomatal conductance and shoot growth?

In most cases, decreased shoot growth in split-root experiments has been associated with a decrease in g_S but there have been some examples, both in woody and non-woody plant species, where this was not the case (Saab and Sharp 1989; Ebel *et al.* 1994; Claxton and Seel 1995; Turner *et al.* 1996). This indicates that shoot growth as a whole, or at least

some components of shoot growth, may be more sensitive than stomatal closure to non-hydraulic signals such as ABA from drying roots (Gowing *et al.* 1990). On the other hand, it may be that one signal does not control both shoot growth and g_s. If ABA does interfere with growth processes, does it do so directly or does it indirectly inhibit supply of photosynthate to growing points via an effect on g_s?

2.8 Practical implications of this research

Attempts to manipulate shoot vigour and vine balance by the rate and timing of irrigation is not a new concept: such strategies have been developed in vineyards in Mediterranean climates with the aim of improving grape quality by deliberately inducing various degrees of water stress (Williams and Matthews 1990). As outlined earlier, this approach is not entirely successful as fruit yield tends to be significantly reduced and the improvement in fruit quality may not be sufficient to increase, or even maintain, economic returns. Furthermore, a 'mild' stress is difficult to impose uniformly on all plants in a vineyard. By comparison, partial drying of the root system of each vine is potentially less dangerous and easier to manage. As one half of the root system is always well watered, there should be some buffering against severe stress conditions. Experience with orchard trees has shown that only a fraction of the tree roots need to be in well-watered soil for the trees to survive a period of severe stress (Layne *et al.* 1981). Partial drying of field vines may allow shoot vigour to be reduced without the yield-depressing effects, both short-term and long-term, of severe water stress.

A desirable outcome of the manipulation of shoot vigour by partial drying may be an improvement in the quality of fruit for winemaking. This may occur as a consequence of improving the microclimate of the canopy. However, there is also the possibility of a direct effect of a root signal on fruit composition, particularly if that signal turns out to be ABA. There is good evidence in favour of ABA as an hormonal trigger of ripening in grapes: endogenous ABA concentration rises coincidentally with sugar increase and berry softening, and treatment of berries with ABA hastens the onset of ripening (Coombe 1989).

As grapevine yield is closely related to the amount of water applied (Williams and Matthews 1990), and excessive amounts of water are often applied to maximise the crop, the system of partial drying may also help to reduce water consumption in irrigated vineyards. Most vineyards in Australia are irrigated to some extent, and more than 80% of grape production (for all end-uses) is intensively irrigated, ie. more than 500mm irrigation applied per annum. Many vineyards in Australia, western USA, Israel and South Africa have been converted, during the past two decades, from systems where the whole surface is irrigated (flood, furrow, high output sprinklers) to micro-irrigation systems (eg. drip), where only a relatively small area of soil is wet, to derive the benefit of improved irrigation efficiency, particularly when combined with soil water monitoring devices such as neutron probe, tensiometer, gypsum block. The most recent innovation, ie. sub-surface drip irrigation, should lead to further improvements in efficiency because water losses due to evaporation from the wetted soil surface are eliminated.

Half-drying reduced total plant transpiration of woody fruit-producing species in a pot experiment (Poni *et al.* 1992); it is possible that partial drying of field vines could also increase water-use efficiency. Irrigation efficiency (either as 'tonnes fruit per ML irrigation applied' or as 'irrigation applied/irrigation requirement') is low in many vineyards in Australia and elsewhere, eg. vineyards in California may be receiving up to three times more water than necessary (Williams and Matthews 1990). Furthermore, over-irrigation not only wastes water but also causes environmental problems: in some horticultural areas of the Murray-Darling basin of Australia, up to 50%, or about 450 mm per annum, of applied irrigation water is lost below the root-zone (McCarthy 1997); this drainage water not only results in flow of saline water into river system but also contributes to a rising water table. Water is a scarce and valuable resource and supplies of water for irrigation are restricted in some regions in Australia, eg. the Barossa region of South Australia has an annual irrigation allocation of 100mm for vineyards but the actual requirement in some seasons will be 300mm or more (A. Thomson, PISA, South Australia, pers. comm.).

Chapter Three - General materials and methods

This chapter describes the materials and methods that were common to most of the experiments conducted as part of this thesis. Materials and methods that were specific to a particular experiment will be described in the relevant chapters. All experiments are tabulated in Appendix I.

3.1 Production of split-root grapevines

Split-root vines from single cuttings: Thick cuttings 40-50 cm long were selected in winter and the base of the cutting sawn for 15-20cm towards the tip with a bandsaw and callused in a heat-bed (25°C) inside a cool-room (2°C). Callused cuttings were planted such that each half of the cutting base was in a different plastic pot (Figure 3.1). The plants were then grown in a temperature-controlled greenhouse for several months, then transferred to a shade-house (Australia) or in the open (Germany). In the winter prior to experimental use, the plants were cut back to one- or two-node spurs.

Split-root vines by approach grafting: Vines were propagated from dormant cuttings in winter and subsequently grown in plastic bags in a greenhouse. In late spring, required combinations were positioned such that the single green shoots from each plant could be approach-grafted (Hartmann and Kester 1983) 20 to 25cm above the soil surface. The graft was wrapped in clear plastic tape and the plants grown in a shade-house for the remainder of the season. In the following winter, the original shoots were cut back to approximately 15cm above the graft union and a two-node spur retained on each side, both above and below the graft union, ie four, two-node spurs per plant (Figure 3.2). At the same time, the plants were transferred to two, seven litre pots and grown in the shade-house until required. The following combinations were used with *Vitis vinifera* cv. Shiraz and *Vitis champini* cv. Ramsey: Shiraz/Shiraz; Ramsey/Ramsey; Shiraz/Ramsey. The development of an effective graft union was tested on several plants of each combination; one of the root systems was removed by cutting one trunk below the graft union and subsequently monitoring the



Figure 3.1 A split-root grapevine produced from a single cutting



Figure 3.2 Close-up of the graft of an approached-grafted split-root grapevine (Shiraz/Shiraz): the two upper shoots are shown but the lower shoots are not visible.

growth of both shoots above the graft union. The fact that every one of the test plants (which were then discarded) had a successful graft union was used as an indication of the state of the remaining plants used in later experimentation.

In some of the experiments which used these plants, shoots were allowed to develop both above and below the graft union: these were designated as 'upper' and 'lower' shoots respectively. Furthermore, on half-dried plants, shoots on the trunk connected to the watered pot were designated as 'wet' and those on the trunk on the non-irrigated side as 'dry'. As a result, such half-dried plants had four different shoot types: lower/wet, upper/wet, upper/dry and lower/dry (Figure 3.3).

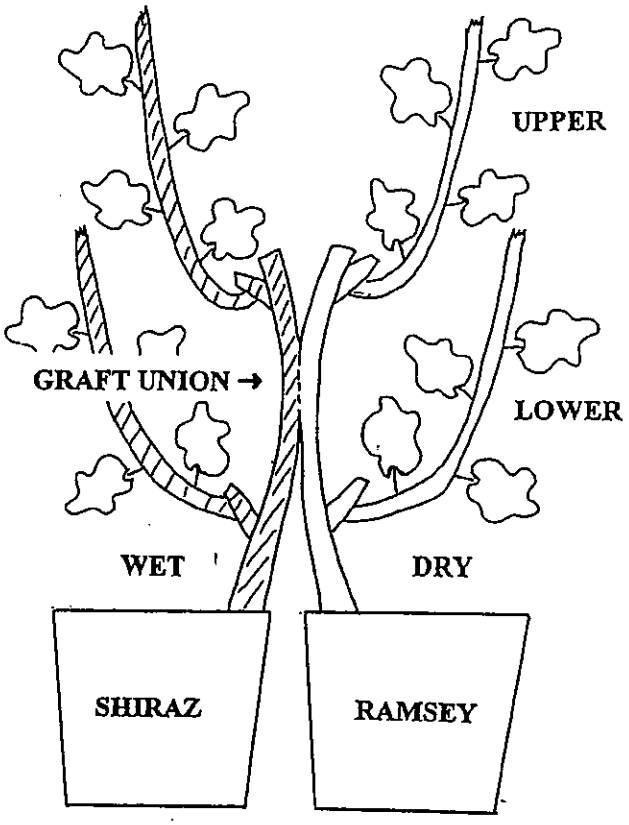


Figure 3.3 Diagrammatic representation of a half-dried, approached-grafted split-root grapevine (Shiraz/Ramsey combination) showing the different shoot types ie. 'lower/wet', 'upper/wet', 'upper/dry', 'lower/dry'. In this example, the pot containing Shiraz roots is irrigated ('wet'), the other pot is not irrigated ('dry').

Split-root vines from grafted rootlings: Bench-grafted vines (cv. Cabernet Sauvignon [*Vitis vinifera*] clone LC14 grafted to cv. Ramsey [*Vitis champini*]) were obtained from a commercial supplier: these plants had been grown for one season in an outside nursery after bench-grafting and were one year-old when converted into split-root plants. In winter, when the plants were dormant, the rootstock was sawn, with a bandsaw, from the base of the rootling to a point approximately one-third of the distance between the base and the union between the scion and the rootstock. The rootling was planted such that each half was in a different plastic pot and subsequently grown in a temperature-controlled greenhouse for several months, before planting in the field in late spring (Figure 8.1).

3.2 Potting media

Experiments 1-4, 8-12: Four parts coarse pine bark, two parts sharp white sand and 1 part coarse yellow river sand plus 1.5 g/l $FeSO_4$, 2.0 g/l Osmocote Long Life ®, 2.0 g/l pH amendment (= two parts dolomite, one part gypsum, one part agricultural lime); steam sterilised. Topsoluble Plant Food ® (21:5:18 N,P,K plus trace elements) was applied weekly during the growing season at the rate of 2.5 g/plant/week. The relationship between soil matric potential and volumetric water content was determined for this soil mix by the filter paper method of Greacen *et al.* (1989) [B. R. Loveys, unpublished data].

Experiments 5-7: 'Einheitserde' (standard soil mixture with a high organic matter content) with the addition of Basacote 6M ® (BASF, Germany) to continuously provide a source of mineral nutrition (14% N, 10% P_2O_5, 13% K_2O, 2% MgO and micro-elements.

3.3 Location of experiments

Experiments 1-4, 8-12: These were conducted on the Waite campus of The University of Adelaide using potted vines between January 1991 and March 1995; the plants in Exp. 1 were grown in a temperature-controlled greenhouse and the plants in the other experiments were grown in the open for the whole of the growing season. The soil surface in the pots

was not covered. The plants grown in the open were either placed on low benches (Exp. 2,3) or placed on 'Weedmat'® on the ground. The sides of the black plastic pots were covered with reflective insulation ('Sisalation'®) to reduce soil temperature. The polyethylene pipe used for irrigation was also insulated.

Experiments 5-7: These were conducted in a greenhouse at the Geilweilerhof Grapevine Breeding Research Institute of the Bundesanstalt für Pflazenzüchtung und Pflazenkultur, Siebeldingen, Germany between May and September, 1993. The temperature within the greenhouse was controlled by the use of automatic shutters which allowed the influx of external air.

Experiments 13, 14, 16: These were conducted in the Alverstoke vineyard on the Waite campus of The University of Adelaide, South Australia (34°58' S, 138°38'E; elevation 121m above sea level) using field vines between January 1993 and June 1996. The site was on a gentle slope with NW aspect and was relatively sheltered. Meteorological data was recorded by an automatic weather station 500m W of the site (Appendix II). The soil is a 'hard red pedal duplex' (Dr 2.23; Northcote 1988) [Appendix III]. The mean January temperature is 22.7°C and mean annual rainfall is 627mm of which 37% falls during the period from September to February inclusively. The region is classified as 'hot, moderately maritime, arid, sunny and not humid' (Dry and Smart 1988). Weeds were controlled under-vine by use of a mixture of residual and knockdown herbicides sprayed onto the soil surface in late winter/early spring. The bare surface which resulted during the season was desirable to check the wetting pattern from the drippers; infiltration rate was satisfactory but not maximised by this treatment. Alleys were mown two or three times during spring and sprayed with knockdown herbicide in early summer to kill volunteer growth. In the first two seasons, NPK fertiliser was applied to the soil surface in spring but none was applied in the last two seasons because the appearance of the foliage indicated that nutrient supply was more than adequate and vines were vigorous.

Experiment 15: This was conducted using field vines planted on the property owned by Dr J. Possingham at Blewitt Springs, South Australia. This site was 24km SSW of the Waite

campus, 180m above sea level, on an slope with SW aspect and was relatively exposed. The soil type is a 'bleached sand' (Uc 2.21; Northcote 1988). Rainfall and evaporation (Class A Pan) data were recorded at 9am and 3pm at the Southcorp Wines 'Seaview' vineyard, 5km WSW and 30m lower than the experimental site. Although the macroclimate is similar to the Waite site, due to the higher elevation and greater exposure to cool southerly winds of the Blewitt Springs site, all phenological stages occurred later than the Waite site, eg. harvest was seven weeks later in 1994/95. Soil management and nutrition was similar to the Waite experiment.

3.4 Shoot and leaf measurements

Shoot growth rate (SGR): A reference node at five to seven nodes below the shoot tip was labelled and the distance from that node to the shoot tip was measured at intervals of one or more days. SGR (cm/day) was calculated as the average increase in shoot length since the previous measurement. As each shoot stopped growing, ie. for two consecutive measurements the increment was zero, that shoot was discarded from the pool and the remaining shoots used to calculate SGR. Therefore, SGR was only representative of growing shoots. The percentage of shoots which had ceased to grow was determined at each measurement in some experiments. In some cases, shoots were replaced after the shoot tip was damaged by wind, machinery etc.

Mean internode length: Mean internode length of the shoot length increment (grown since the start of measurement) or of shoots grown over the whole season was calculated by dividing shoot length by node number. This method of determination calculates average internode length over the whole shoot (or increment) and does not distinguish between internodes formed at different times of the season. For this reason, in Experiment 14, the actual internode length between all nodes along the shoot was measured at the end of the season, or in Experiment 1, elongation of a particular internode was measured over time.

Shoot diameter: This was measured at the flat (ventral) side, in the middle of the internode between nodes 1 and 2, on mature shoots in winter.

Pruning weight: This is defined as the mass of mature, one year-old shoots (= 'canes') removed from the vine at pruning time in winter. All shoots from a single vine were bundled together and weighed in the field with a spring balance (accuracy within 100g); pruning weight (PWT) was expressed as kg/vine. In the 1995/96 season, vines were summer-pruned by trimming the upward-growing shoots; the winter pruning weight (measured in winter 1996) was corrected by estimation of the pruning weight equivalent of the trimmings. The dry weight/fresh weight ratios of samples of green shoots in summer (= 0.21g/g) and mature shoots in winter (= 0.45g/g) were determined after oven-drying at 60°C for three days. Assuming that dry weight of mature shoot is equal to dry weight of green shoots:

PWT correction factor = 0.47 fresh weight of green shoot trimmings

Leaf area per shoot: Leaf area per (main) shoot was determined non-destructively as follows: a sample (usually 5 to 10) of shoots was selected at random on each test vine (see individual experiments for details). For each shoot, the number of mature (N_m) and immature (N_i) main leaves was recorded. Also, the length (cm) of the main lobe (from the petiolar point to tip of lobe) of the most distal mature leaf ('a'), and of the leaves 2 ('b'), 4 ('c') and 6 ('d') nodes proximal to that leaf, was measured. On the same main shoots, the number of lateral shoots of each lateral leaf number class was recorded:

Mean mature leaf length (L_x) = (a + 2b+ 2c + 2d)/7

The relationship between mature leaf length (L, cm) and area per mature leaf (A, cm^2) was determined with a sample of 50 mature Cabernet Sauvignon leaves from the experiment in November 1993 using a Li-Cor leaf area meter (Model LI-3000):

$A = 0.04628L^{1.7239}$ ($r^2 = 0.78$, $p < 0.001$)

Mean mature leaf area (A_m) = $0.04628L_x^{1.7239}$

Mature leaf area per shoot (cm^2) = $N_m . A_m$

Immature leaf area at each sampling date was determined by collecting a sample of shoot tips from buffer vines (usually 5 each from 'control' and 'treated') and measuring total area

of immature leaves with a leaf area meter: this was used as a constant value for all shoots and was always less than 5% of total leaf area per shoot. Determination of immature leaf area was only necessary at sampling dates prior to veraison: after that time, there were no or few immature leaves:

Total main leaf area per shoot (MLA_S, cm^2) = mature leaf area per shoot (cm^2) + immature leaf area per shoot (cm^2)

Three lateral shoots from each leaf number per lateral class were collected from buffer vines and the leaf area of each shoot was measured with a leaf area meter: the relationship between mean leaf area per lateral shoot (y) and leaf number per lateral shoot (x) was determined at each sampling date; for example, in March 1995, $y = 64.7x - 81.7$ ($r^2 = 0.96$, $p < 0.001$).

Lateral leaf area per main shoot (LLA_S) = [number of lateral shoots with one leaf . mean leaf area (cm^2) of 1-leaf laterals] + [number of lateral shoots with two leaves . mean leaf area (cm^2) of 2-leaf laterals] + ... [number of lateral shoots with n leaves . mean leaf area (cm^2) of n-leaf laterals]

Total leaf area per main shoot (TLA_S, cm^2) = $MLA_S + LLA_S$

This method of TLA estimation was verified by a comparison with actual TLA (area of all main and lateral leaves measured with a leaf area meter) using 10 random shoots from buffer vines in November 1993: estimated TLA = 0.982 actual TLA ($r = 0.99$).

Leaf area per vine: Main shoot number per vine was determined in winter at the time of pruning:

Main leaf area per vine (MLA, m^2) = shoot number per vine .MLA_S (cm^2).10^{-4}

Lateral leaf area per vine (LLA, m^2) = shoot number per vine.LLA_S (cm^2).10^{-4}

3.5 Fruit sampling and fruit composition

Berry sampling: This was used exclusively for sampling fruit for determination of composition in Experiments 14 and 15. Generally berries were sampled for several weeks prior to harvest and at harvest: at each sampling date, a specified number of berries was randomly chosen from the test vines, selected from as many bunches as possible. The sample was immediately weighed on an electronic balance to determine mean berry weight and half the sample (chosen at random) was stored at -20°C for later analysis of berry colour and glycosyl-glucose (G-G); the other half was used to measure juice total soluble solids (TSS, °Brix), titratable acidity (TA) and pH. The harvest sample was either collected directly from the vine as above or selected at random from harvested bunches (see individual experiments for details).

Determination of TSS, TA and pH of juice: Berries were crushed in a citrus fruit press, the juice transferred to centrifuge tubes and centrifuged at 1500*g* for about five minutes. The supernatant was transferred to another container for measurement of TSS and pH. TSS was measured with a hand-held, temperature-compensated, digital refractometer while pH was measured with a pH meter as described by Iland *et al.* (1993). A five mL aliquot of the supernatant was diluted to 25 mL (1:5 dilution) with distilled water and stored at -20°C: this was later thawed and used to measure TA (g/L as tartaric acid) using an Erison-Compact titrator (Crison, version 3).

Determination of total anthocyanins, total phenolics and glycosyl-glucose of berry homogenate: A weighed sample (usually 50 berries), which had been stored in a 125 mL plastic container at -20°C, was partially thawed and processed cold (<10°C) according to the method developed by P. Iland (pers. comm). The sample was homogenised in the storage container at 24000 rpm with an Ultra-Turrax XT25 homogeniser for 30 seconds. After scraping any homogenate from the shaft to the vessel, the sample was homogenised for another 15 seconds. The homogenate was thoroughtly mixed and a scoop of approximately 1 g was transferred to a pre-tared centrifuge tube. The mass of the portion of homogenate taken was recorded. Aqueous ethanol (10 mL, 50% v/v) was added, the

tube capped and the contents mixed by inversion every 10 min during 1 h. After this period, the tube was centrifuged at 1500*g* for about 7 min. The volume of the supernatant extracted was measured. To measure anthocyanins and phenolics, an aliquot of the extract was acidified to c. pH 1 with 1M HCl and the absorbance of each determined spectrophotometrically at 520 and 280 nm respectively (Somers and Evans 1977).

To measure glycosyl-glucose (G-G), a further aliquot of the extract (usually 4 mL) was diluted with distilled water to c. 40 mL and adjusted to pH 2 with one drop of 5M HCl. The determination of 'total G-G' was carried out on the diluted extract according to the method described by Iland *et al.* (1996): glycosides are extracted from grape tissue and free glucose is removed; glycosides are then hydrolysed to release glucose and glucose concentration is determined by spectrophotometric enzymatic analysis using a hexokinase/glucose-6-phosphate (HK/G-6-PDH) Boehringer Mannheim enzyme assay kit. 'Red-free G-G' (μmol/berry and μmol/g berry mass) is an estimate of the concentration of glycosides other than anthocyanin glycoside and was obtained by subtraction of the concentration of 'anthocyanin-glucose' from the concentration of 'total G-G'.

The formulae used for calculation of anthocyanins (mg/berry and mg/g berry mass), total phenolics (absorbance units [OD^{280}]/berry and absorbance units [OD^{280}]/g berry mass), total G-G per berry (μmol/berry and μmol/g berry mass) and anthocyanin-glucose (μmol/berry and μmol/g berry mass) are presented in Appendix IV.

3.6 Yield components

Fruit weight (kg/vine) was measured at harvest; the weight of all samples taken previously was added to harvest weight to obtain the final fruit weight. Bunch number was counted as fruit was harvested (bunch number per vine) and mean bunch weight (g) calculated (= final fruit weight/bunch number). The berry samples used for determination of fruit composition were used to derive mean berry weight (g) unless otherwise specified and berry number per bunch calculated (= mean bunch weight/mean berry weight) by ignoring the weight of the rachis.

Fruit weight/pruning weight FW/PW = fruit weight (kg/vine)/pruning weight (kg/vine)

Fruit weight/volume irrigation applied (g/L) = fruit weight (kg/vine) / volume of irrigation water applied from budburst to harvest (L/vine)

3.7 Gas exchange measurements

Experiment 1: Rates of gas exchange of leaves were determined using a portable gas exchange system (LCA-3, Analytical Development Co., Hoddesdon, UK). The terminal part of the main lobe was inserted into the cuvette which was positioned normal to the sun; air temperature during measurement ranged from 25 to 33°C and maximum photon flux density Q (μmol/m^2/s) ranged from 690 to 1300.

Experiments 2,3,13,14: Rates of gas exchange of leaves were determined using a portable photosynthesis measuring system (Li-6200, Li-Cor, Lincoln, Nebraska, USA). The terminal part of the main lobe was inserted into a 1.0L chamber which was positioned normal to the sun. Measurements were conducted during cloudless periods on exposed leaves with a flow rate of 500 to 600μmol/s.

Experiments 4,10,12,14,15: Stomatal conductance of leaves was determined using a portable porometer (Delta-T AP4, Delta-T Devices, Cambridge, UK) according to the manufacturer's recommendations. The terminal part of the main lobe was placed into the cup on the head unit which was positioned normal to the sun. Measurements were conducted during cloudless periods on exposed leaves. The device was calibrated before use on every occasion using the supplied calibration plate; if the measurement period was longer than 1.5 hours, the device was re-calibrated.

Experiments 5-7: Rates of gas exchange of leaves were determined using a mini-cuvette system (H Walz, Effeltrich, Germany; Düring 1993). The distal part of the leaf blade was inserted into a cuvette chamber. Measurements were carried out at constant ambient conditions (light saturation at 850 μmol quanta/m^2/s; 350 ppm CO_2; leaf temperature 21°C

(Expts. 5,6), 25°C (Expt. 7); constant leaf to air water vapour pressure of the air entering the cuvette (dew point 13°C to 17°C depending on the experiment). Measurements of g_S in these experiments as mmol $CO_2/m^2/s$ were converted to mmol $H_2O/m^2/s$ by multipying the former by 1.56 (Düring 1987)

Correlation of stomatal conductance with SGR: SGR on a particular day was always calculated by averaging the increase in shoot length over the number of days since the previous measurement (this varied from the previous day up to seven days before). Therefore, in order to correlate SGR and g_S on a particular day , it was necessary, in some cases, to estimate SGR of that particular day by interpolation, especially where there were long intervals between measurements. Where SGR was measured daily or every second day, g_S was paired with the SGR calculated on that day or the following day: in the former case, because shoot length was normally measured in the morning, SGR on a particular day was representative of the previous morning to that morning. In other cases, g_S was paired with the SGR within 24 h.

3.8 Soil water measurement

Pot experiments: Gravimetric soil water content was determined by extracting two 1.5cm diameter cores from the middle of each pot: after oven-drying at 80°C for 48 hours, soil water content was calculated (g water/g soil). Volumetric soil water content was measured by time domain reflectometry [TDR] (Trase, Soilmoisture Equipment Corp., Santa Barbara, CA, USA) using 15cm wave guides inserted vertically from the soil surface.

Field experiments: Volumetric soil water content (%) was measured by using TDR (Exp. 13; Exp. 14, 1993/94 season only; Exp. 15) or capacitance sensors (Exp. 14, 1994/54 and 1995/96). TDR wave guides (50cm long) were permanently installed into undisturbed soil in early spring of 1992, 55cm from the vine line and 20cm from the edge of the original trench. A set of capacitance soil water sensors (EnviroSCAN soil water continuous monitoring system, Sentek Environmental Solutions, Adelaide, South Australia) was installed in a single plot of each treatment of Exp. 14 in winter 1994. PVC access tubes

were installed according to the manufacturer's recommendations in undisturbed soil, 25 to 30cm from the edge of the original trench and 60 to 65cm from the vine line, one on either side of each vine. Sensors were placed at 10, 20, 30, 40, 50, 60, 70 and 100cm depths in each access tube (two sets of sensors per vine). Readings were logged at 15 minute intervals. Summation of data from 20, 30, 40 and 50cm sensors was subsequently used for irrigation scheduling because most roots of nearby mature vines were found to be at a depth of 10 to 50cm. The data for Figures 9.2 and 9.3 were derived by interpolation (at 0000 and 1200h each day) from plots of original data over time produced by use of manufacturer's software; this was done so that data from different probes (summed over several depth classes) could be plotted on the same axes (this is not possible with the current version of manufacturer's software). Soil water retention curves were derived for soil at the site of the Waite experiment (Appendix V).

3.9 Leaf water potential (Ψ_L)

Ψ_L was measured with a pressure chamber (Scholander *et al.* 1965). Mature leaves (from the canopy exterior in the case of field vines) were detached from the shoot by cutting through the base of the petiole, placed into a plastic bag and measured within one minute. Where xylem sap was collected at the same time for later ABA analysis, large leaves in particular were selected.

3.10 Canopy measures

Bunch exposure index was determined by measurement of PAR in the bunch zone with a ceptometer (model SF-80, Decagon Devices, Cambridge, UK) using the method described by Botting *et al.* (1996). 'Leaf area/surface area ratio' and 'leaf layer number' (in the bunch zone by point quadrat technique) were measured using the methods described in Smart and Robinson (1991).

Leaf area/surface area ratio (LA/SA): In Experiment 14 in 1995/96, total surface area was calculated as 5.5 m^2 for all vines using the method described in Smart and Robinson (1991):

this included both 'up' and 'down' canopies but did not include the portion of the 'down' canopy which grew horizontally along the ground. As a result, in order to calculate the LA/SA of the vertical part of the canopy only, the leaf area per vine was corrected to exclude the LA of the part of the 'down shoots growing along the ground: this was done by determining the relationship between LA and PWT for the 'down' shoots ($LA = 4.8PWT$; $r^2 = 0.70$, $p<0.05$) and measuring the PWT of the horizontal portion of the 'down' shoots (28% and 24% of total 'down' PWT for 'control' and 'treated' respectively).

3.11 Statistical analysis

All statistical analyses were conducted using PRISM™ Version 2 (GraphPad Software Inc., San Diego, CA, USA). A paired or unpaired 't' test was used for comparison of two treatments, and a one-way analysis of variance for three or more treatments with Tukey's post-test for comparing pairs of treatments.

Chapter Four - Grapevine shoot growth is reduced when half of the root system is dried in a controlled environment

4.1 Introduction

Split-root plants have been used to study the effects of half-drying on shoot development of woody plant species. For example, Tan and Buttery (1982) found that stem and leaf dry weight of half-dried peach seedlings was reduced by 11 to 12% relative to control plants with both pots watered; similarly, leaf area of passionfruit was reduced by 15% (Turner *et al.* 1996). As for shoot growth, drying half of the root system reduced stomatal conductance of woody species (Tan and Buttery 1982; Poni *et al.* 1992; Khalil and Grace 1993) and the magnitude of the response was found to be strongly correlated with the amount of roots in the drying soil (Tan and Buttery 1982). Poni *et al.* (1992) found that total plant transpiration was decreased in response to half-drying and this was associated with changes in stomatal conductance. On the other hand, Turner *et al.* (1996) found that half-drying had no effect on stomatal conductance and suggested that the reduction in plant water-use was a consequence of reduced leaf area.

The first references to the use of split-root plants for the study of grapevine water relations appears to have been those of Düring (1990,1992) who used plants with roots divided between two pots grown in a controlled environment. He reported a relative reduction of net photosynthesis (Pn) and stomatal conductance (g_S) in response to drying of one half of the root system, without any associated change in shoot water status: four days after the start of drying, Pn and g_S had decreased to 70 and 50% respectively of the control with both pots watered. Using potted vines (Trebbiano/Teleki 8B) grown outside, Poni *et al.* (1992) maintained one half of the root system without irrigation for two months: 'treated' plants received the same amount of water per plant as 'control' plants with both pots watered. There was no effect on shoot growth rate (SGR). In this experiment, apple, peach and pear were used in addition to the grapevine and the results for all four species were pooled for some parameters: g_S, Pn and transpiration rate (E) of 'treated' plants

decreased to ca. 80% of 'control' after 9 days of soil drying without any change in leaf water potential Ψ_L. Plants were harvested at the end of the experiment and the fresh weight of leaves and stems of 'treated' plants (all species averaged) was reduced by 17% relative to the 'controls'.

In an attempt to reduce some of the variability introduced in field experiments, the following experiments were conducted to test the hypothesis *that, under controlled conditions, grapevine shoot growth is reduced when half of the root system is dried.*

4.2 Materials and methods

a. Experiment 1

One-year-old *Vitis vinifera* cv. Chardonnay (clone I10V1) split-root vines (from single cuttings) were grown in two, seven L pots in a temperature-controlled glasshouse for five months (mean maximum and minimum daily temperatures were 27 and 17°C respectively). Four weeks prior to the start of the experiment, each was cut back to a single lateral shoot which was trained vertically. Vines were blocked (four replicates) according to shoot height and stomatal conductance and treatments allocated at random. Treatments were: a) both pots irrigated twice daily (WW); b) one pot not irrigated from 0900h on day 2 (D2) until 1500h on D11 (WD); c) both pots not irrigated from D2 until D6 when one pot was irrigated (DD); from D11, all pots of all treatments were irrigated. One pot of the DD plants was watered on D6 because the leaves had started to wilt. On D1 (February 16) shoots averaged 120cm. A reference node (designated node '-6') was labelled seven nodes below the shoot tip and shoot length increase was measured daily or every second day; shoot growth rate (SGR) was calculated as average increase per day since the previous measurement. The internode length between nodes 0 and +1 was measured daily from D3 and the increase since the previous day calculated (when measurement of this internode started, it was the most distal visible internode). The rate of leaf elongation was determined by measuring the length of the main vein of leaves at nodes -2 and 0 from D2 to D11. Gas exchange was measured with a portable gas exchange system at one to three day intervals

in sunny conditions between 1100 and 1200h on the same four leaves per shoot. Leaf water potential was measured on D3, D6, D7 and D14 (average of two leaves per plant; leaves sampled were proximal to those used for gas exchange). Soil water content was measured by time domain reflectometry at 1430h daily or every second day. Maximum and minimum air temperatures during the experimental period were 19 to 28°C and 12 to 22°C respectively and RH ranged between 30 and 55%.

b. Experiment 2

Two-year-old *Vitis vinifera* cv. Shiraz (clone 12) split-root vines (approach grafts) were grown in two, seven L pots per plant in a shadehouse during spring and early summer with four shoots per plant (two shoots on each side, one above ['upper'] and one below ['lower'] the graft union). For two months prior to the start of the experiment on February 26 (D1), plants were transferred to the open. On January 18, the two upper shoots were pruned back to one strong lateral shoot at the base of the main shoot which was subsequently trained vertically upwards by attaching to a string (all lateral shoots were removed on this shoot as they appeared). The two lower shoots were cut back to six nodes on February 24 by removing the distal portion of the shoot and all laterals removed from the remaining nodes. Plants were blocked on the basis of stomatal conductance and treatments allocated at random (three replicates per treatment). Treatments were: a) both pots irrigated daily (C); b) one pot not irrigated from D1 until D23, the other pot irrigated daily (T). From D1 to D13 inclusively, there were two, 15 minute irrigations per day; from D14 to D22 the frequency was increased to four, 15 minute irrigations per day because soil water measurements indicated excessive soil drying between irrigations.

The increase in the length of the two upper shoots (reference node = six nodes below the shoot tip) was measured daily and the SGR calculated as cm/d since the previous measurement. The increase in node number per shoot was determined at intervals from four to six days. For the T plants, the shoots on the side with the irrigated pot were designated as 'wet' and those on the side of the non-irrigated pot as 'dry'. Soil water content was measured every three days on average by time domain reflectometry. Gas

exchange measurements were conducted every 3.5 days on average between 1300 and 1600h using a LiCor photosynthesis system on the same four leaves per shoot (the lower shoots were only measured on D18). Leaf water potential was measured between 1300 and 1530h on D18 and D22 on one leaf per shoot.

c. Experiment 3

As for Experiment 2: two-year-old *Vitis champini* cv. Ramsey split-root vines (approach grafts). Gas exchange measurements of leaves of lower shoots were only conducted on D21.

4.3 Results

a. Experiment 1

The reduction in g_S, Pn and SGR (Figure 4.1) for DD plants coincided with the decrease in soil water content from D1 to D6 (Figure 4.2). From D6 to D11, when DD plants were converted to WD, all three parameters partially recovered to 60 to 70% of the control (WW) level, with full recovery taking place after both pots had been irrigated on D11. For WD, the reduction in g_S, Pn and SGR relative to WW plants also coincided with the decrease in water content of the dried pot but the rate at which the shoot parameters decreased was slower than for DD. The maximal reduction of SGR (74% of WW) occurred on D5 at about the time that the soil water content reached its lowest level (8%); similarly, the maximal reduction of g_S and Pn (ca. 66% of WW) occurred two days later. Recovery of all three parameters appeared to start prior to rewatering of the 'dry' pot on D11, followed by complete recovery by D14. Ψ_L was significantly lower on DD plants than either WW and WD by D3 (Table 4.1). After one pot was rewatered on D6, Ψ_L had recovered to the WD level by D7, but was still significantly lower than WW. By D14, Ψ_L of DD was still lower than WW, but the difference was not significant. By comparison, Ψ_L of WD was not significantly lower than WW at any time except on the afternoon of D7.

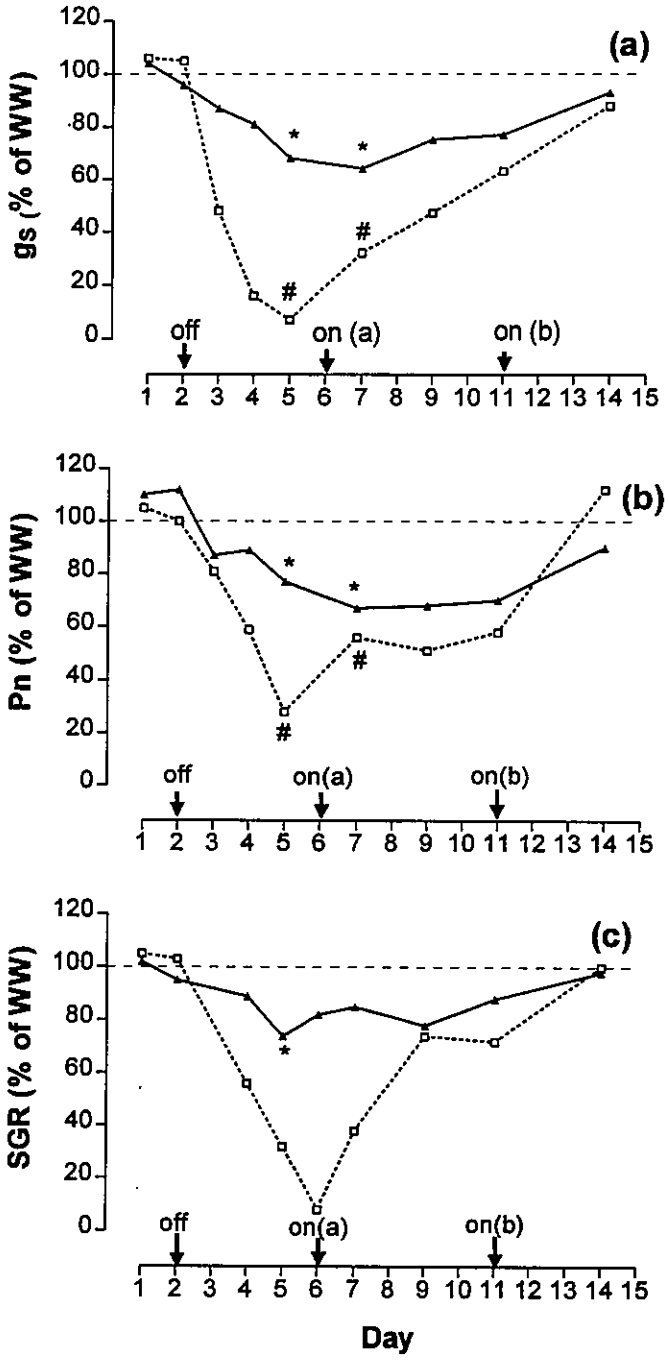


Figure 4.1 Effect of wet/dry combinations on gas exchange and shoot growth, Chardonnay, Exp. 1. WD (▲) and DD (□) treatments expressed as % of WW; on(a): DD changed to WD; on(b): all pots irrigated. * indicates those days when WD significantly different ($p<0.05$) from WW and # those days when DD significantly different ($p<0.05$) from WD.
a). stomatal conductance (g_s; mmol/m^2/s): DD significantly different ($p<0.05$) to WW on D3 to 11 inclusively.
b). net photosynthesis (Pn; µmol /m^2/s): DD significantly different ($p<0.05$) to WW on D3 to 11 inclusively
c). shoot growth rate (SGR; cm/day): DD significantly different ($p<0.05$) to WD and WW on D4 to 7 inclusively

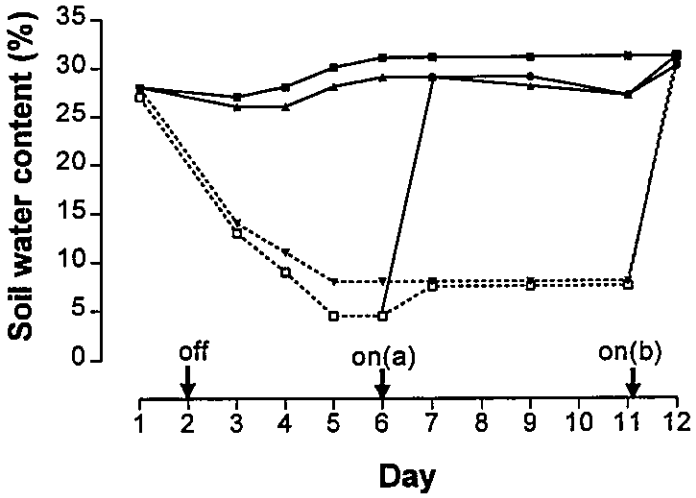


Figure 4.2 Volumetric soil water content (%) measured by TDR: average of both pots WW (■); irrigated pot of WD (▲); non-irrigated pot of WD (▼);average of both pots DD to day 6 and non-irrigated pot only thereafter (□); irrigated pot of DD from D7 (●); Chardonnay, Exp. 1. On(a): DD changed to WD; on(b): all pots irrigated.

The rate of internode elongation of DD shoots was lowest up to D6, but not after rewatering of one pot of DD plants on D6. Similarly, the only differences in the rate of leaf elongation between DD and the other treatments occurred prior to, but not after, D6 (Table 4.2). There was no difference between WW and WD plants for the rates of internode or leaf elongation. From D1 to D14, SGR and g_S (both expressed as % of WW) were positively correlated: r = 0.71* and 0.96*** for WD and DD respectively .

Table 4.1 Effect of wet/dry combinations on leaf water potential (MPa; mean ± se); Chardonnay, Exp. 1 (see text for explanation of treatments).

Day	Time (h)	WW	WD	DD	Signif. diff ($p<0.05$)	Signif. diff. ($p<0.01$)
3	1500	- 0.56 ± 0.03	- 0.72 ± 0.05	- 1.09 ± 0.06	WD and DD	WW and DD
6[a]	0530	- 0.42 ± 0.04	- 0.43 ± 0.00	- 0.68 ± 0.05		WW and DD; WD and DD
7	1440	- 0.49 ± 0.04	- 0.63 ± 0.06	- 0.68 ± 0.03	WW and DD; WW and WD	
14	1130	- 0.39 ± 0.03	- 0.36 ± 0.02	- 0.45 ± 0.06		

[a] DD to WD on day 6 (pm); all pots watered from day 11

b. Experiment 2

Shoot growth rate (SGR) increased for both control (C) and treated (T) plants from the start of measurement to reach a maximum on D25 (Figure 4.3). The SGR of T plants was

Table 4.2 Effect of wet/dry combinations on leaf length[a] (cm) at nodes '0' and '-2'(mean ± se); Chardonnay, Exp. 1.

	WW	WD	DD	Signif. diff. (p<0.05)	Signif. diff. (p<0.01)
Node '0'					
Length on day 2	2.5 ± 0.4	2.2 ± 0.1	2.2 ± 0.1		
Increase from day 2 to 6	4.3 ± 0.5	3.4 ± 0.5	1.3 ± 0.2	WW & DD	WW & DD
Increase from day 7 to 11[b]	2.4 ± 0.3	2.1 ± 0.7	2.5 ± 0.3		
Node '-2'					
Length on day 2	4.8 ± 0.5	4.6 ± 0.3	4.3 ± 0.1		
Increase from day 2 to 6	4.3 ± 0.6	4.8 ± 0.3	2.5 ± 0.4	WD & DD	
Increase from day 7 to 11[b]	2.1 ± 0.4	2.4 ± 0.3	2.3 ± 0.2		

[a] Length of main vein; [b] DD changed to WD from day 6

significantly lower from D17 to D25. The relative reduction in SGR of T plants occurred during the period when soil water content of the 'dry' pot reached the minimum value and SGR stayed at that level until rewatering on D23 (Figure 4.4). The initial reduction of both SGR and g_s of T plants relative to C (Figure 4.5a) coincided with the decrease in soil water content of the 'dry' pot from D2 (Figure 4.4); SGR decreased to a minimum of 52% of the C plants on D17 and g_s decreased to a minimum of 72% of C on D12 (Figure 4.5a). Recovery of SGR appeared to start after D19, g_s after D18, and both recovered before the 'dry' pot was rewatered on D23; this coincided with the period when the 'dry'

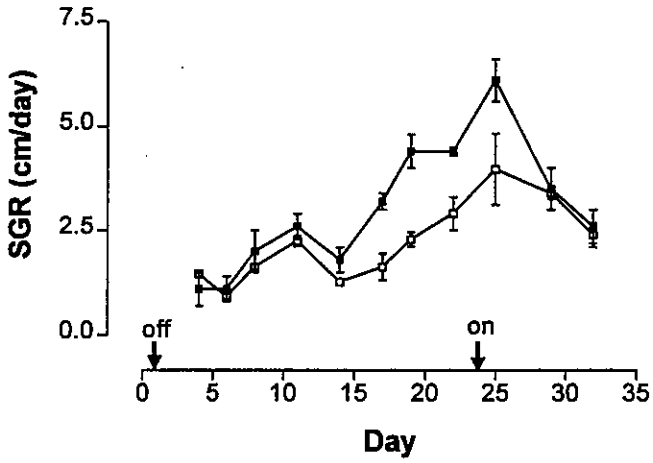


Figure 4.3 Effect of partial drying on shoot growth (SGR, mean ± se, cm/day) of Shiraz, Exp. 2: 'control' vines (C, ■) and 'treated' vines (T, mean of 'wet' and 'dry' shoots, □). T significantly different (p<0.05) to C from D17 to D25. One pot of T not irrigated from D2 to D23.

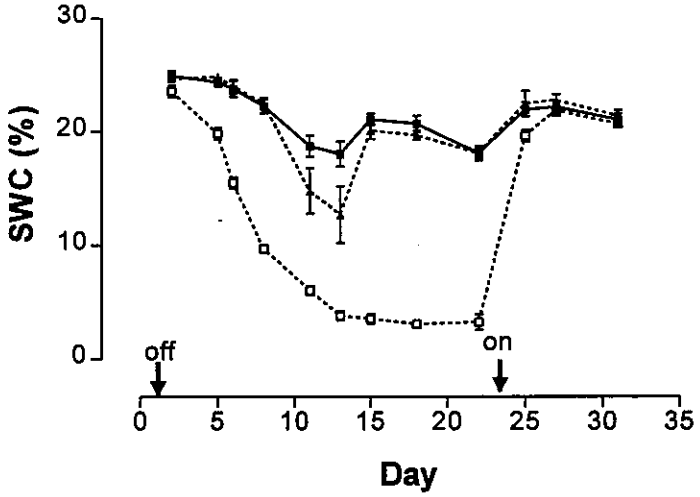


Figure 4.4 Volumetric soil water content (SCW, %) measured by TDR: average of both pots of 'control' (C, ■); irrigated pot of 'treated' (T, ▲); non-irrigated pot of T (□); Shiraz, Exp. 2 .One pot of T not irrigated from D2 to D23.

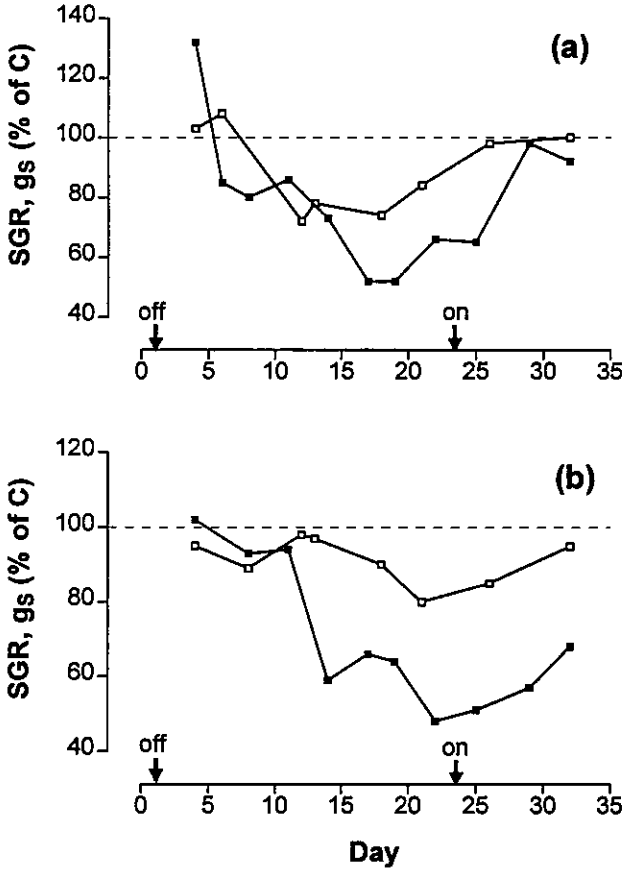


Figure 4.5 Effect of partial drying on shoot growth rate (SGR, ■) and stomatal conductance (g_s, □): 'treated' (T) as % of 'control' (C). One pot of T not irrigated from D2 to D23.

a). Shiraz, Exp. 2: SGR and g_s of T significantly different ($p<0.05$) to C for D17 to D25 and D12 to 18 respectively.

b). Ramsey, Exp. 3. SGR and g_s of T significantly different ($p<0.05$) to C for D14 to D32 and D21 to D26 respectively.

pot reached its minimum water content (8%). SGR and g_S completely recovered by D29 (Figure 4.5a). There was no difference between 'wet' and 'dry' shoots of T plants for either SGR or g_S (except on D18 when 'dry' shoots had significantly lower g_S).

Table 4.3 Effect of half-drying on shoot growth components (increment from D1 to D25) of Shiraz (Exp. 2) and Ramsey (Exp. 3). Both pots irrigated (C); one pot not irrigated from D1 to D23 (T).

	C	T	T as % of C	Signif.
Shiraz				
Length (cm)	78 ± 7	56 ± 8	-28	<0.05
Node number	9.3 ± 0.6	7.5 ± 0.5	-19	<0.05
Mean internode length[a] (cm)	8.4 ± 0.4	7.5 ± 0.6	-11	ns
Ramsey				
Length (cm)	47 ± 6	33 ± 1	-30	<0.05
Node number	7.8 ± 0.6	6.2 ± 0.4	-20	<0.05
Mean internode length[a] (cm)	6.0 ± 0.4	5.3 ± 0.2	-12	ns

[a] Mean internode length = length/node number

The shoot length increment to D25 was reduced by 28% in response to treatment, in association with a reduction in number of new nodes and mean internode length (Table 4.3). For Ψ_L, there was no significant difference between shoots of C and T plants; similarly there was no significant difference between 'wet' (W) and 'dry' (D) shoots of T plants (Table 4.4). A comparison of upper (U) and lower (L) shoots of both treatments on D18 showed that g_S of UD shoots was significantly lower than that of LW, and g_S of LD shoots was lower than that of C shoots (significant only for L shoots; Table 4.5). There was no significant difference between any shoots for Ψ_L on the same day or three days later (Table 4.4). Stomatal conductance and SGR were found to be linearly related when mean values of upper shoots were calculated for the period D11 to D22, and C and T values were plotted on the same axes (Figure 4.6a).

Table 4.4 Effect of half-drying on leaf water potential [a] (MPa; mean ± se); Shiraz, Exp. 2 (see text for details of treatments); no horizontal comparisons are significantly different.

Day	C (upper shoots)	T (upper shoots)		T (lower shoots)	
		Wet	Dry	Wet	Dry
18	-0.89 ± 0.05	-0.94 ± 0.00	-1.01 ± 0.10		
22	-0.97 ± 0.03	-1.02 ± 0.03	-1.01 ± 0.03	-1.00 ± 0.2	-0.95 ± 0.1

[a] Measured between 1300 and 1530 h

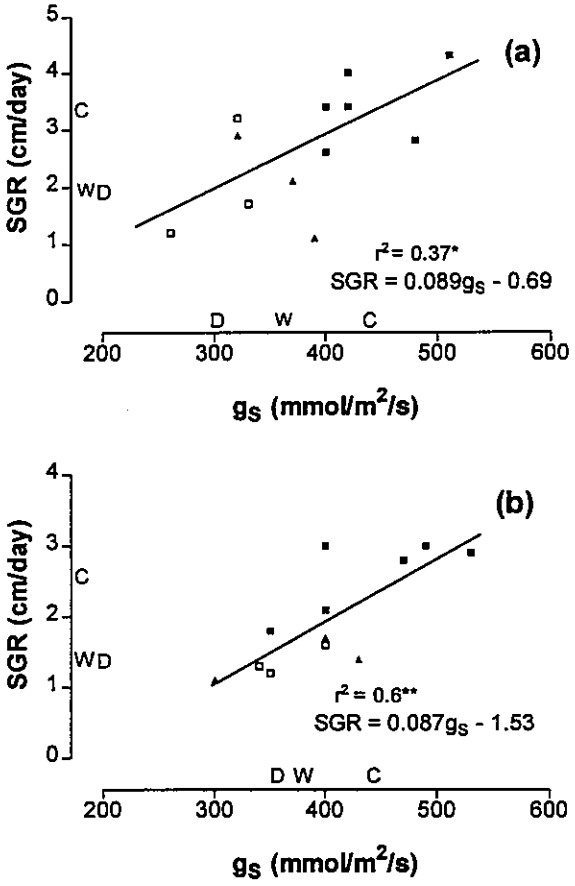


Figure 4.6 Relationship between shoot growth rate (SGR, mean per shoot, D11-D22, cm/d) and stomatal conductance (g_S; mean per shoot, D12-D21, mmol/m^2/s) for six control shoots(C, ■), three 'wet' (W, ▲) and three 'dry' (D, □) shoots of half-dried plants. Letters on axes represent mean values of all shoots of each type.
a). Shiraz, Exp. 2
b). Ramsey, Exp. 3

c. Experiment 3

SGR showed a similar pattern to Figure 4.3 and increased for both C and T plants from the start of measurement to reach a maximum on D25; SGR of T plants was significantly lower from D14 to D32 (data not shown). The relative reduction in SGR of T plants occurred during the period when soil water content of the 'dry' pot reached its minimum and SGR stayed at that level until rewatering on D23 (Figure 4.4[1]). The initial decrease of SGR and g_S of T plants relative to C (Figure 4.5b) coincided with the decrease in soil

[1] Because the soil water content of Ramsey pots in Expt 3 was identical to that of Shiraz in Expt 2, Figure 2 is also used in this instance

water content of the 'dry' pot from D2 (Figure 4.4). SGR decreased to 59% of the C level by D14 whereas g_S decreased to a minimum of 80% of the C level on D21 (Figure 4.5b). Recovery of both SGR and g_S coincided with the rewatering of the 'dry' pot on D23 and was not complete for either by D32 when measurements were terminated (Figure 4.5b).'Wet' and 'dry' shoots of T plants were not significantly different for either SGR (data not shown) or g_S (Table 4.5), but the 'dry' shoots tended to have the lowest g_S. The shoot length increment to D25 was reduced by 30% in response to treatment, in association with a reduction in number of new nodes and mean internode length (Table 4.3). There was a strong linear relationship between g_S and SGR when mean values of upper shoots were calculated for the period D17 to D26, and C and T values were plotted on the same axes (Figure 4.6b).

Table 4.5 Effect of half-drying on stomatal conductance (mean ± se; mmol/m²/s) of upper and lower shoots of C and T ('wet' and 'dry') of Shiraz, Exp. 2 (D18) and Ramsey, Exp. 3 (D21). Means followed by same symbol are significantly different ($p<0.05$).

	C	T	
Shiraz		**Wet**	**Dry**
Upper	430 ± 20	360 ± 30	280 ± 20 #
Lower	470 ± 60 *#	410 ± 50	270 ± 40 *
Ramsey			
Upper	540 ± 60	470 ± 70	410 ± 0
Lower	610 ± 50	580 ± 30	430 ± 20

4.4 Discussion

The DD plants behaved as expected: the reduction in g_S, Pn and SGR by 70% or more relative to WW was very similar to the results of Tan and Buttery (1982), Düring (1992) and Turner *et al.* (1996). They observed a similar response within four to seven days, coincidental with a decrease in soil water content and associated with a significantly lower Ψ_L. Similarly, recovery of both gas exchange and SGR was delayed by several days after rewatering whereas Ψ_L recovered almost immediately. SGR partially recovered to the WD level after rewatering one pot but this was not the case for either g_S or Pn, which remained lower than the WD level until both pots were watered on D11. This suggests that SGR is more responsive to soil drying than g_S and also it recovers more rapidly.

SGR decreased in response to drying of half the root system with both cultivars of *Vitis vinifera* and with Ramsey (*Vitis champini*), indicating that the response is not restricted by genotype. SGR of half-dried plants decreased by 30 to 50% relative to the control after five to 22 days of drying (Figures 4.1c, 4.5). This was a similar response to Gowing *et al.* (1990), but the magnitude of the response was greater than that reported by Tan and Buttery (1982) and Turner *et al.* (1996) for the shoot growth components of other species. By comparison, Poni *et al.* (1992) were not able to detect any effect on shoot length after several weeks of half-drying with *Vitis vinifera* cv. Trebbiano, but it is unlikely that the different response was the result of a difference in cultivars. In this study, SGR decreased in response to half-drying with all cultivars tested and the lowest rate of shoot growth relative to the control occurred coincidentally with the minimum value of soil water content in the 'dry' pot.

As for SGR, both g_S and Pn of half-dried plants decreased coincidentally with the reduction in soil water content of the non-irrigated pot for the *Vitis vinifera* cultivars but the change in gas exchange was relatively delayed for Ramsey. In the latter case, the lowest value of g_S relative to the control was not reached until one week after the soil water content of the 'dry' pot had stopped decreasing, suggesting that shoot growth may be more responsive to half-drying than gas exchange with this species; Gowing *et al.* (1990) had a similar result with half-drying of apple. The magnitude of the response of g_S and Pn to drying of half the root system in my experiments, ie. 20 to 35% decrease, is similar to that reported by Poni *et al.* (1992) and Düring (1992) for *Vitis vinifera*, greater than that for passionfruit where g_S was not affected at all (Turner *et al.* 1996) or peach (Tan and Buttery 1982) but less than that reported for sycamore (Khalil and Grace 1993). Therefore, unlike the fully-dried treatment, half-drying of the root system only results in partial stomatal closure. This may be beneficial because plant water-use efficiency is increased with partial stomatal closure (Düring 1992); this aspect is discussed in more detail in Chapter 5.

Recovery of both gas exchange and SGR of half-dried plants relative to controls appeared to start before the rewatering of the 'dry' pot in the case of *Vitis vinifera* cultivars but it is not possible to make the same conclusion with any degree of certainty in the case of

Ramsey. SGR of fully-dried Chardonnay increased from 8% to 72% of the control value between D6 and D11 while one pot remained dry (Figure 4.1c). For Shiraz, the evidence was less convincing: SGR increased from 52% to 66% of the control value between D17-19 and D23 (Figure 4.5a). Furthermore, this relative recovery of the shoot function of half-dried plants appeared to coincide with no further decrease in soil water content of the 'dry' pot. For example, in Exp. 1, SGR and g_S recovered from D5 to D7 and minimum SWC was reached on D6 (Figures 4.1, 4.2); in Exp. 3, SGR and g_S recovered from D18-19 and minimum SWC was reached on ca. D18 (Figures 4.4, 4.5a). Both SGR and g_S recovered completely within a few days of both pots being watered and one could speculate that this may have taken place even if the 'dry' pot had not been rewatered. Khalil and Grace (1993) observed a partial recovery of g_S during the day prior to rewatering of the 'dry' pot in their half-drying experiment; however, in their case, stomates were almost fully closed prior to the partial recovery. This appears to be the only report of this phenomenon. Additional experiments will need to be conducted to determine if complete recovery of SGR of half-dried grapevines can occur while half of the root system remains in 'dry' soil: this is the subject of Chapter 5.

The reduction in SGR and g_S of half-dried grapevines in these experiments was not associated with any significant change in shoot water relations, except for D7 in Exp. 1. This one instance may have been a consequence of excessive drying of the 'wet' pot. The irrigation frequency was increased from D14 in Exps. 2 and 3 when it became obvious that the water requirements of the half-dried plants were not being met by the schedule in place to D14 (Figure 4.4). Düring (1992) decreased the leaf area of WD plants relative to WW in order to reduce transpiration but this was not done in my experiments. The lack of any effect on Ψ_L confirms the observations of Gowing *et al.* (1990) and others that changes in stomatal conductance are more closely linked to changes in soil water status than to the leaf water status.

The rates of leaf and internode elongation were only affected on DD plants prior to D6 in Exp. 1, a typical response to water stress (Williams and Matthews 1990). After D6, when one pot was watered, and Ψ_L had recovered, there was no longer any significant effect on

expansive growth. Similarly, the decrease in shoot length in response to half-drying in Exps. 2 and 3 appeared to be more of an effect on the rate of initiation of nodes than on internode elongation. However, it is difficult to draw any firm conclusions because average internode length is calculated over the whole shoot length increment and thus it is not possible to differentiate between internodes which have elongated at different stages of shoot development. Gowing *et al.* (1990) found that the rate of leaf initiation, ie. the rate of node production, was more sensitive to half-drying than the rate of leaf elongation.

If the 'wet' (W) and 'dry' (D) shoots of half-dried plants are compared, there was no difference in SGR of upper (U) shoots for either cultivar [lower (L) shoots not measured]. However, g_S of D shoots tended to be lower than that of W shoots for both cultivars at the time when mean g_S of treated plants was at its lowest level relative to controls (Table 4.5). One possible explanation is that the graft union of these approach-grafted Shiraz and Ramsey vines was not 'perfect' and the signal was not fully translocated from the D trunk to the W shoots. On the other hand, there was no significant difference in Ψ_L between W and D shoots of half-dried Shiraz: this may indicate that the graft union did not interfere with the movement of water from the W trunk to the D shoots (which presumably was occurring by D18 because the SWC of the 'dry' pot had reached its minimum level by that time; Figure 4.4).

Stomatal conductance of D shoots of half-dried plants tended to be lower than that of control shoots and there was no difference between the upper and lower 'dry' shoots of T plants. However, although g_S values of LW shoots of half-dried plants tended to be similar to L shoots of controls, those of UW shoots tended to be less than either LW of half-dried or U shoots of controls (Table 4.5). This may indicate that the passage of any signal from the 'dry' trunk across the graft union to the 'wet' trunk is incomplete; also, the strength of the signal received by the UW shoot may be greater than that received by the LW shoot. Further work will need to be carried out to confirm this hypothesis (see Chapter 10).

The observations from these experiments suggest that grapevine species may respond differently to half-drying of the root system, eg. the decrease in g_S in response to half-

drying was relatively delayed for Ramsey (*Vitis champini*) in comparison with the *Vitis vinifera* cultivars. This may have important implications for commercial viticulture because *Vitis vinifera* scions are grafted to rootstocks of non-*Vitis vinifera* parentage in most vineyards throughout the world and it is known that many rootstocks are more vigorous and more drought tolerant than *Vitis vinifera* (May 1994). This will be investigated in more detail in Chapter 6. There was a positive linear relationship between gas exchange and shoot growth rate for half-dried plants, either when expressed as a percentage of control plants over time (Exp. 1) or when actual values per shoot were used (Exps. 2,3; Figure 4.6). This suggests that the two physiological processes may be influenced by the same root signal, as proposed by Gowing *et al.* (1990). On the other hand, SGR may simply respond to changes in assimilate supply. These possibilities will be further discussed in Chapters 10 and 11.

The water requirements of half-dried plants grown in containers appear to be supplied by half the root system so long as the 'wet' half is irrigated frequently; this confirms the observations of others (Turner *et al.* 1996). The experience from Exps. 2 and 3 indicates that, if water is not applied frequently, the 'wet' pot will dry excessively and there is the risk of an hydraulic effect on shoot growth and gas exchange. The large decrease in SGR for treated Ramsey relative to the control between D11 and D14 may be partly ascribed to that effect although the fact that there was no significant change in g_s during that time tends to counter this idea. It is possible that even the control pots were not being irrigated frequently enough up to D14 because there was an actual decrease in SWC (Figure 4.4) and SGR of control Shiraz (Figure 4.3) from D11 to D14.

Changes in Pn in response to half-drying in Exp. 1 were strongly correlated with changes in g_s; this is similar to the results of Tan and Buttery (1982), Düring (1992), Poni *et al.* (1992) with other woody plant species, and suggests that stomatal conductance is the dominant influence on changes in Pn. This appears to be first report of a significant decrease in shoot growth in response to partial drying of the root system of grapevines.

4.5 Conclusions

a. *Half-drying of the root system significantly reduced shoot growth of grapevines growing under controlled conditions.*

b. *Changes in both shoot growth and stomatal conductance in response to half-drying took place in the absence of any change in shoot water status suggesting the involvement of a non-hydraulic signal in mediating this response.*

c. *Recovery of both shoot growth rate and stomatal conductance appeared to start before rewatering of the dried half of the root system and at the time when the minimum soil water content of the dried half was reached.*

d. *Shoot growth and stomatal conductance appear to be influenced by the same non-hydraulic signal but shoot growth appears to be more sensitive; on the other hand, shoot growth may be responding to changes in assimilation rate.*

e. *There appeared to be differences between grapevine genotypes in response to half-drying: Vitis vinifera may respond more rapidly than Vitis champini.*

Chapter Five - Shoot growth and stomatal conductance of half-dried grapevines can recover without rewatering of the dried half of the root system.

5.1 Introduction

An important observation from the experiments described in Chapter 4 was that recovery of both shoot growth and stomatal conductance appeared to start before rewatering of the dried half of the root system. It was hypothesized that this may have taken place even if the 'dry' pot had not been rewatered. The only previous reference to this phenomenon appears to be that of Khalil and Grace (1993) who observed a partial recovery of g_S during the day prior to rewatering of the 'dry' pot; however, in their experiment, stomata were almost fully closed prior to the partial recovery, unlike the experiments described in Chapter 4 where stomatal closure, prior to recovery, was only partial, ie. there was no more than 35% reduction in g_S relative to the control. It is interesting that gas exchange of half-dried plants in the experiments of Poni *et al.* (1992) appeared to recover relative to the controls in the absence of rewatering of the dry half of the root system but the authors made no reference to this. They may have overlooked this phenomenon because the actual values of g_S and Pn for control and half-dried treatments (means of four species: apple, pear, peach and grape) were plotted over time. Using their data, derived by interpolating from their graphs, g_S of the half-dried treatment as % of the control was plotted over time (Figure 5.1).

It is obvious that recovery, after partial stomatal closure, commenced 10 days after the onset of half-drying and was completed ca. 18 days later. No soil water content data were provided by Poni *et al.* (1992) so it is not possible to conclude if recovery coincided with no further decrease in soil water content of the dried container.

If the shoot function of partially dried plants is affected by a positive signal produced by roots in contact with drying soil, and if recovery of shoot function coincides with no further decrease in water content of the soil surrounding those roots, then it follows that recovery

may take place because there are no more roots being dried and thus no further production of the signal. The first part of this hypothesis, i.e that recovery occurs because there are no

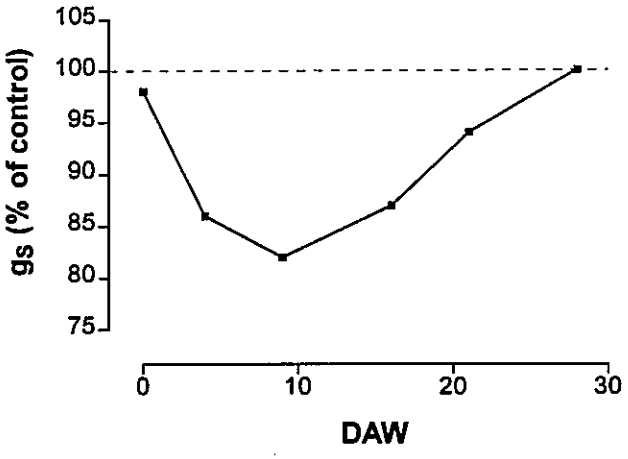


Figure 5.1 Effect of drying half the root system of split-root plants on stomatal conductance (g) (as % of control with the whole root system watered); mean of apple, peach, pear and grape. Time scale = days after water 'off' (DAW). Data reworked from Poni *et al.* (1992).

more roots being dried, will be tested in an experiment described in this chapter. The question of signal production is addressed in Chapter 10.

The total growth of roots has been measured in split-root experiments but there do not appear to have been any temporal studies on either root growth or the proportion of the root system that is dried in the non-irrigated container. Results from several split-root experiments indicate that the magnitude of the response to soil drying is directly proportional to the number and/or length of roots dried (Tan and Buttery 1982; Ebel *et al.* 1994) and the proportion of root tips dried may be particularly important (Jensen *et al.* 1989).

It is possible that the 'wet' roots supply water to the 'dry' roots. As a result, roots maintained in dry soil preserve their water-absorbing capacity until rewatered, allowing the plant to resume root growth under favourable soil conditions. On the other hand, the dried roots may die and the plant may shed those roots as part of its strategy in reducing carbon expenditure under stress conditions. However, in perennial plants, such strategies are more common for species which are endemic to habitats where prolonged drought is common

(Kosola and Eisenstatt 1994). Saab and Sharp (1989), Poni *et al.* (1992) and others have suggested that the plant tends to equilibrate with the wettest part of the root system and that roots in drying soil are rehydrated during the night. Furthermore, transfer of water not only keeps roots alive but also allows them to grow in dry soil (Glenn and Welker 1993). If roots remain alive and continue to grow in the 'dry' container of half-dried plants after the soil water potential has decreased to a level at which root growth is inhibited (should the whole root system be exposed to such conditions), then this is circumstantial evidence of water movement from 'wet' to 'dry' roots. Increased root growth in the 'dry' pots relative to 'wet' pots of split-root plants has been observed with some species (Tan and Buttery 1982; Poni *et al.* 1992) and the relative root growth of 'dry' and 'wet containers was measured in Experiment 6 described in this chapter. More compelling evidence of water movement from 'wet' to 'dry' roots could be provided by actual measurement of flow and this is the subject of the experiment with tritiated water described in this chapter to test the third hypothesis.

Turner *et al.* (1996) found that water uptake by the 'wet' roots of half-dried plants increased relative to those of the 'control' plants. It is likely that the water-absorbing role of the 'wet' roots gradually increases as the soil water content in the 'dry' container decreases and the roots therein consequently reduce uptake as there is a decrease in the water which is readily extractable by the roots. Eventually, a point may be reached at which there is no further water uptake from the soil by the 'dry' roots, but this may occur before the water in the soil of the 'dry' container is completely depleted (Figure 4.2).

The 'new' root system (i.e excluding the original root ball) of grapevines growing in pots, where the roots are able to explore new soil, will initially be made up of fine (< 2mm diameter), white roots. These roots may change to a brown colour relatively quickly: this is the result of the oxidation of phenols released from vacuoles of dead or collapsed epidermal cells and it appears to be a transitional stage in the 'suberisation' of fine roots (the hypodermis develops suberin lamellae inside its cell walls). The factors that regulate suberisation are not well understood but it is known that it occurs in response to dry soil conditions in the field; some roots will become suberised and brown to their tips. When

soil conditions become favourable, the suberised roots may resume extension growth from the original root apical meristem or they may produce new lateral roots. It is probable that suberisation enables the primary roots to survive during periods of water stress or other unfavourable conditions (Mullins *et al.* 1992; Mohr 1996). Roots which undergo secondary thickening have an exterior periderm, are generally thicker than 2mm, and are most likely to survive long-term unfavourable conditions to provide the structural part of the root system. Although most of the absorption of water and nutrients is done by the fine roots (and particularly by the unsuberised white roots), the role of the thicker roots in absorption is not clear (Mohr 1996)

The experiments described in this chapter were conducted to test the following hypotheses:

a. That recovery of shoot growth and stomatal conductance coincides with no further decrease in soil water content of the dried half of the root system;

b. That recovery occurs because there are no more roots being dried; and

c. That the roots in dried soil can survive because water moves from 'wet' roots to 'dry' roots.

5.2 Materials and methods

The following experiments were conducted to test the first hypothesis:

a. Experiment 5

Three, two-year-old Kober 5BB (*Vitis berlandieri* x *Vitis riparia*) split-root vines from single cuttings were grown in a glasshouse for several weeks prior to the start of the experiment. All were trained to a single vertical shoot which was reduced from 14 to 5 mature leaves on D0 (May 3[1]). On D3, one of the two, three L pots per vine was irrigated for the last time; the other three L pot was irrigated twice per day (0730, 1200h) for the next 14 days. Gas exchange measurements were conducted twice per day between 0900 and 1200h on the same two leaves per plant: the data in Table 5.1 are the means of four measurements. At the time of gas exchange measurement, air temperature and relative

[1] Northern Hemisphere

humidity in the glasshouse ranged from 19 to 27°C and 52 to 60% respectively. Soil water content in the top 10cm was determined gravimetrically on D7.

b. Experiment 7

Two-year-old Kober 5BB (*Vitis berlandieri* x *Vitis riparia*) split-root vines from single cuttings were grown in two, 5L pots outside for one month and moved into the glasshouse on June 22. They were trained to a single vertical shoot (all laterals removed) and had 13 ± 1 leaves at the start of the experiment on July 5 (D0). Plants were blocked on the basis of preliminary stomatal conductance measurements and treatments allocated at random (three replicates per treatment). The treatments were: a) both pots irrigated (WW); b) one pot irrigated, the other not irrigated from day 1 (WD); c) both pots not irrigated from day 1 (DD). From D8, one of the pots of the DD treatment was irrigated. Irrigation was normally done once per day. The increase in shoot length (reference node = six nodes below shoot tip) was usually measured every day (0800 h). Gas exchange measurements were conducted on the same leaf per plant (leaves changed on D7) in the morning between 1000 and 1200h and again in the afternoon between 1300 and 1500h (two to three times per period); on D4, measurements were carried out nine times between 0830 and 1630h. From D7 (pm) to D9 (am), there were no gas exchange measurements due to equipment failure. Soil water content was determined gravimetrically in the top 10cm every two to five days. Leaf water potential was measured on D2, D4, D7, and D18 at approximately 1300h on one leaf per plant

c. Experiment 8

Three-year-old *Vitis vinifera* cv. Shiraz (clone 12) split-root vines (approach grafts) were grown in two, seven L pots per plant and grown in the open. One week prior to the start of the experiment on January 16, all vines were thinned back to four shoots per plant (two 'upper' and two 'lower'; see Exp. 2); those shoots were topped, reduced to 10-12 mature leaves per shoot (at the time, there were ca. 20 leaves per shoot) and all lateral shoots removed except for one terminal lateral, usually at the most distal node. The main shoot

and the terminal lateral were trained vertically upwards. Each pot was irrigated with two, 2 L/h drippers. Treatments were: a) both pots irrigated four times daily ('control'); b) one pot not irrigated from January 18 (D3) until D18, the other pot irrigated four times daily ('treated'). Treatments were chosen at random with five 'treated' and two 'control' vines. For the 'treated' plants, the shoots on the side with the irrigated pot were designated as 'wet' and those on the side of the non-irrigated pot as 'dry'. All pots were irrigated from D18. Soil water content was measured every second day on average by time domain reflectometry. The increase in the length of the terminal laterals of the two upper shoots per plant (reference node = most basal node) was measured every two days on average and the SGR calculated as cm/day since the previous measurement. Stomatal conductance was measured on the same four leaves per shoot every second day on average between 1030 and 1230h with a Delta T porometer; for the control vines, there was no significant difference between upper and lower shoots measured on D1 so thereafter only upper shoots were measured and the average per plant calculated. Leaf water potential was measured on one leaf per shoot on D10 between 1400 and 1500h (all shoots on 'treated', upper only on 'control'); the ambient temperature at the time was 38-40°C.

d. Experiment 10

Three-year-old *Vitis vinifera* cv. Shiraz (clone 12)/*Vitis champini* cv. Ramsey approach graft combinations (each plant had both Shiraz and Ramsey roots and shoots; see Chapter 3.1 for further explanation). Each pot was irrigated with two, 2 Lph drippers. Treatments were: a) both pots irrigated three times daily ('S+R+'); b) the pot with the Ramsey roots was not irrigated from May 8 (D2), the other pot containing Shiraz roots was irrigated daily ('S+R-'); c) the pot with the Shiraz roots was not irrigated from May 8 (D2), the other pot containing Ramsey roots was irrigated daily ('S-R+'). There were four replicates per treatment in a completely randomised design. Only the Shiraz shoots were measured. Plants were grown in the open for the whole season before use. On March 4, all vines were thinned to four shoots per plant (two 'upper' and two 'lower'; see Exp. 2); those shoots were topped, reduced to eight mature leaves per shoot and all lateral shoots removed except for one terminal lateral, usually at the most distal node. The main shoot and the

terminal lateral were trained vertically upwards. Soil water content was measured every two to three days on average by time domain reflectometry; due to similar results, the data from 'wet' pots of S+R- and S-R+ were pooled with that of S+R+, and similarly for data from 'dry' pots of S+R- and S-R+. Stomatal conductance was measured on the same four leaves per shoot on D1,8,15 and 21 between 1030 and 1200h with a Delta T porometer. Leaf water potential was measured on two leaves per shoot between 1300 and 1430h on D15 (ambient temperature was 30°C).

The following experiment was conducted to test the second hypothesis namely that recovery occurs because there are no more roots being dried.

e. Experiment 6

Four, two-year old 110 Richter (*Vitis berlandieri* x *Vitis rupestris*) split-root vines were moved to a glasshouse on May 4 and transplanted to glass-sided containers (20 x 20cm section, 47 cm high with the single glass side at an angle of 20° such that roots growing vertically downwards intercept the glass wall; Figure 5.12. The glass sides were covered to exclude light and covers were only removed for up to 15 minutes per day for measurements. The root ball for each half of the root system was reduced to approximately 12 x 12 x 10cm and planted next to the glass wall such that the base of the root ball was, on average, 10cm below the soil surface. This resulted in at least 34cm of new soil between the base of the root ball and the base of the container. All plants were trained to a single shoot (all laterals removed) with 12 leaves per shoot at the start of the experiment. From May 22 (D6) until June 5 (D20), one container of each plant was not irrigated ('dry'); the other was irrigated twice daily ('wet'). The container with the most root development at the start of the experiment was chosen as the 'dry' treatment. 'New' roots were defined as those which had grown from the original root ball since re-potting.

Gas exchange measurements were conducted twice each day between 0900 and 1200h using a Walz infrared gas analyser on the same two leaves per plant from D1 (one leaf per plant was changed each time on D8 and D15). At the time of gas exchange measurement, air temperature and relative humidity in the glasshouse ranged from 20 to 27°C and 52 to

60% respectively. An index of the rate of soil drying was determined by daily measurement of the average depth (relative to the soil surface) of the margin between wet and dry soil in each container on the glass wall (Figure 5.12). The growth rate of individual roots in each container was measured daily from D8 to D19: three to four fine, white roots (<2mm diameter) were selected (from among those which had grown downwards for at least the two days prior to the start of measurement) and the mean increase in length per day for each container was calculated. The increase in the area of new roots which had grown in contact with the glass wall (mainly below the base of the root ball) was determined every second day on average: a transparent plastic sheet was placed on the glass wall and the roots were drawn on the plastic with a marking pen. The position of the wet/dry margin was also marked. The area of the roots on the plastic sheet was measured with a continuous belt planimeter (Delta-T Devices Ltd., Cambridge, UK) and the average increase (cm^2/day) of all roots and percentage of 'new' root area dried was calculated.

On D21, roots from both wet and dry containers of one representative plant were harvested: the root ball was carefully (and easily) removed and the soil at different depth classes (0 to 12, 12 to 18, 18 to 26 and >26 cm) excavated from the container with a hand trowel. Roots were extracted by hand, weighed immediately ('fresh weight') and dried for five days at 50°C, then reweighed ('dry weight'). The correlation between root dry weight and root area on the glass (measured on D18) was calculated. The root areas for the different depth classes (0 to 5, 5 to 10, 10 to 15, 15 to 20, > 20 cm) were measured on the remaining three plants on D2: only the apparently living roots were included, while the relatively few dead and decaying roots were excluded. Leaf water potential was measured on D12 at 0730h on two leaves per plant. Photographs of the root growth on the glass walls were taken on D12 and D19 (the containers were tilted such that the glass walls were vertical).

The following experiment was conducted to test the third hypothesis namely: that the roots in dried soil can survive because water moves from 'wet' roots to 'dry' roots.

f. Experiment 11

In late January, tritiated water (^3H_2O) was applied to roots in the 'wet' pot of *Vitis vinifera* cv. Ramsey split-root (approach grafted) plants at a time when the volumetric water content of the soil in the 'dry' pot had decreased to 7% as a result of half-drying. Several white roots in the 'wet' pot were carefully teased out at 1000h, placed in a vial containing 0.37MBq ^3H_2O (total volume 10mL) and left for 10 minutes. Leaf disks (11mm diameter) were sampled at 1100h and at hourly intervals thereafter until 1700h, and again at 0800h and 1400h the following day. The plants used were the same as for Exp. 9 (Chapter 10): four shoots per plant (two shoots on each side ['wet' and 'dry' sides], one above ['upper'] and one below ['lower'] the graft union). Leaf disks were sampled from all shoots, ie. lower/wet LW, upper/wet UW, upper/dry UD, lower/dry LD, frozen and thawed several times before counting in a Beckman scintillation counter after addition of five mL scintillation cocktail.

5.3 Results

a. Experiment 5

Average soil water content of 'wet' and 'dry' pots was 0.46 and 0.24 g/g respectively on D7. A significant reduction in net photosynthesis (Pn) and stomatal conductance (g_s) occurred five days after irrigation of one of the pots was stopped (5DAW) and recovery without rewatering was almost immediate (Figure 5.2). Relative to the average of the previous seven days, g_s and Pn on D8 (5DAW) were reduced by 33 and 32% respectively (Figure 5.2). Changes in Pn were strongly correlated with changes in g_s and Pn/g_s ratio (Figure 5.2).

b. Experiment 7

Stomatal conductance (Figure 5.3) and SGR of DD plants decreased relative to WW in response to the decrease in soil water content of the dried containers (Figure 5.3). By the afternoon of D5, g_S had decreased by 73% relative to the control, and by D7, SGR had decreased by 45%. Over the same time period, soil water content decreased from ca. 0.55 g/g to a minimum of ca. 0.25 g/g on D7. After rewatering of one of the containers on D8 (while the other remained dry), there was a partial and rapid recovery within 3 days for both g_S (am and pm) and SGR. During the next 10 days, with one container still dry, g_S and SGR recovered to the level of the WW plants. For half-dried WD plants, both SGR and g_S

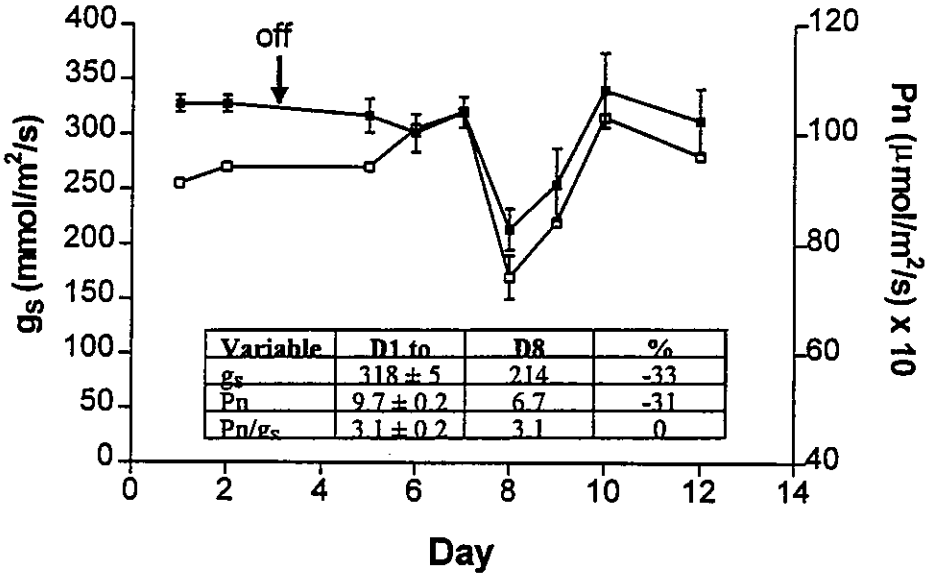


Figure 5.2 Effect of half-drying 5BB split-root vines from D3 on stomatal conductance (g, mean ± se, mmol/m^2/s, ■) and assimilation rate (Pn, mean, µmol/m^2/s, □); Exp.5. Insert: mean values (± se) of g_S, Pn and Pn/g_S (µmol/mmol x 10^2) for D1-D7 compared with values on D8.

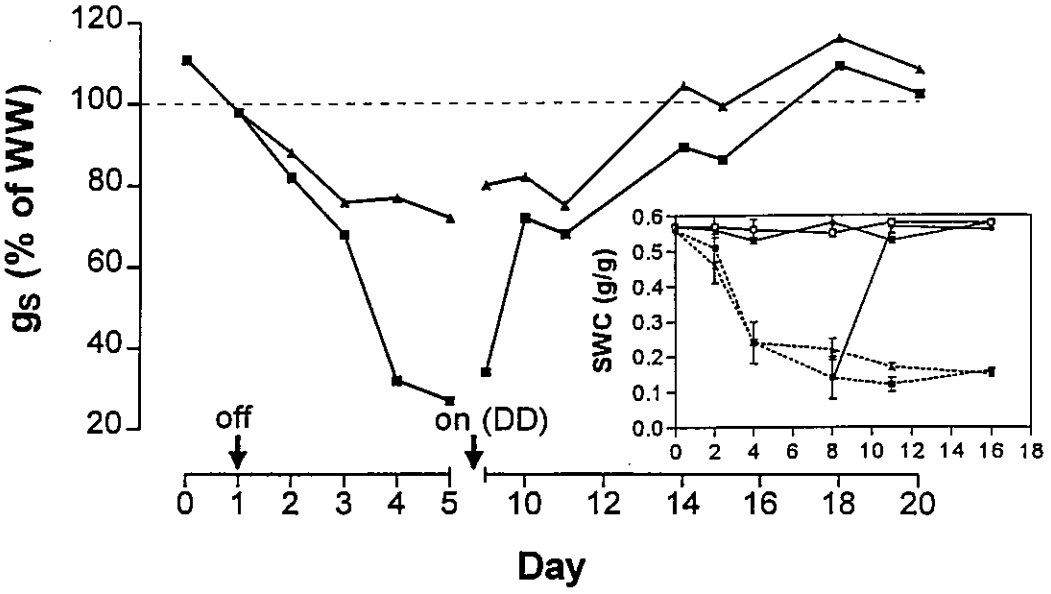


Figure 5.3 Effect of wet/dry combinations of 5BB split-root vines on stomatal conductance (g_S, mmol /m^2/s) of WD (▲) and DD (■) treatments expressed as % of WW; Exp. 7. Off: one pot of WD and both pots of DD not irrigated from D1; on: DD changed to WD. g_S measured in pm (mean of two to three measurements between 1300 and 1500h). DD and WD significantly different ($p<0.05$) to WW on D3 to D11 inclusively. Insert: Gravimetric soil water content (SWC, g/g):average of both pots WW (□); irrigated pot of WD (●);non-irrigated pot of WD (▲);average of both pots DD to D8 and non-irrigated pot only thereafter (■).

(measured in the morning; Figure 5.3) were not significantly different to the controls over the whole period of measurement. However, g_S measured in the afternoon decreased relative to WW, coincidentally with the decrease in soil water content of the 'dry' pot (Figure 5.3), and g_S was significantly lower on D3 by which time the soil water content was ca. 0.25 g/g (compared to ca. 0.55 g/g in the 'wet' pot). Recovery of g_S (pm) of WD plants relative to controls after D11 coincided with no further decrease in soil water content and was completed by D14, at which time the soil water content of the 'dry' and 'wet' pots was ca. 0.16 and 0.55 g/g respectively (Figure 5.3). Stomatal conductance of DD plants was significantly lower than WW from 1100 h on D4: the DD average (1200 to 1600h) was 64% lower than WW (Figure 5.4). By comparison, g_S of WD plants was only significantly less than WW at 1500h.

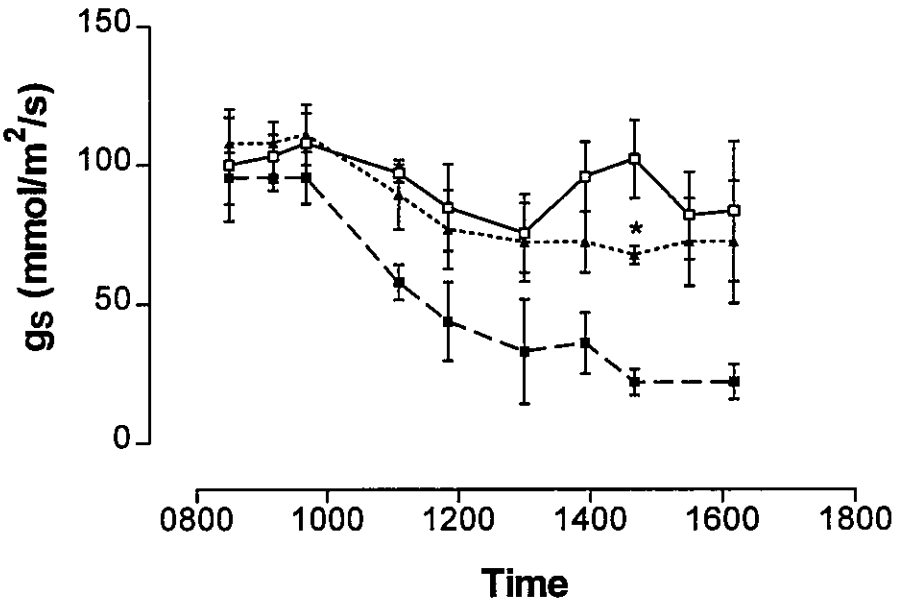


Figure 5.4 Effect of wet/dry combinations of 5BB split-root vines on diurnal change on D4 of stomatal conductance (g_S, mean ± se, mmol /m^2/s); WW (□), WD (▲) and DD (■), Exp. 7.
Off: one pot of WD and both pots of DD not irrigated from D1; on: DD changed to WD. * indicates WD significantly different ($p<0.05$) to WW; DD significantly different ($p<0.05$) to WW from 1100h.

Half-drying had no significant effect on Ψ_L relative to the control; on the other hand, Ψ_L of DD plants was significantly reduced relative to both WW and WD on D7 (just prior to rewatering of one container of DD plants on D8). By D18, when one container of both WD and DD plants was 'dry', there was no significant difference between any treatment combination (Table 5.1). There was no correlation between Ψ_L and g_S measured concurrently on the same shoot for any treatment (data not presented). Gas exchange parameters for WW and WD plants are presented in Table 5.2 for three of the days when the g_S (pm) of WD plants was significantly lower than that on WW: for WD, lower Pn was associated with lower g_S and higher Pn/g_S.

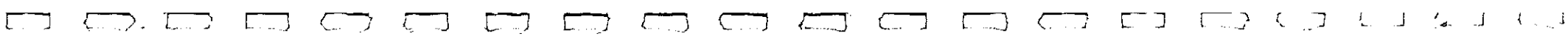
Table 5.1 Effect of wet/dry combinations on Ψ_L (mean ± se, MPa) at 1300h. Mean of three plants, one leaf per plant. One pot of WD and both pots of DD not irrigated from D1; DD changed to WD on D8; Exp.7. On D7, DD significantly different to WW ($p<0.01$) and WD ($p<0.05$).

Day	WW	WD	DD
2	- 0.81 ± 0.06	- 0.86 ± 0.04	- 0.78 ± 0.03
4	- 0.75 ± 0.03	- 0.73 ± 0.06	- 0.84 ± 0.06
7	- 0.78 ± 0.04	- 0.84 ± 0.06	- 1.04 ± 0.04
18	- 0.50 ± 0.02	- 0.56 ± 0.02	- 0.64 ± 0.03

Table 5.2 Effect of wet/dry combinations on net photosynthesis (Pn, mean ± se,μmol/m^2/s), stomatal conductance (g_s, mean ± se, mmol /m^2/s), and Pn/g_s ([μmol CO_2/mmol CO_2] x 10^2) on D3, D4 and D9; 5BB, Exp. 7. One pot of WD and both pots of DD not irrigated from D1; DD changed to WD on D8. Measured between 1300-1530h.

Day	Pn		g_s		Pn/g_s	
	WW	WD	WW	WD	WW	WD
3	11.3 ± 0.9	9.2 ± 0.8	222 ± 19	168 ± 18	8.0	8.5
4	7.7 ± 0.9	6.0 ± 0.6	94 ± 13	72 ± 8	12.8	13.0
9	11.6 ± 0.5	9.6 ± 0.8	150 ± 8	120 ± 9	12.1	12.5

The lower 6 to 8cm of 'dry' pots of WD plants was still moist with many white roots on D22. By comparison, there were fewer new, white roots at the bottom of the 'wet' pots of WD plants (Figure 5.5a). There were relatively few new roots in the lower 3 to 4cm of the pots of WW plants, and many fewer than the 'wet' pots of WD plants. For the DD plant, of which one pot had been rewatered 14 days previously, there was much new root growth in the 'wet' pot (Figure 5.5b).



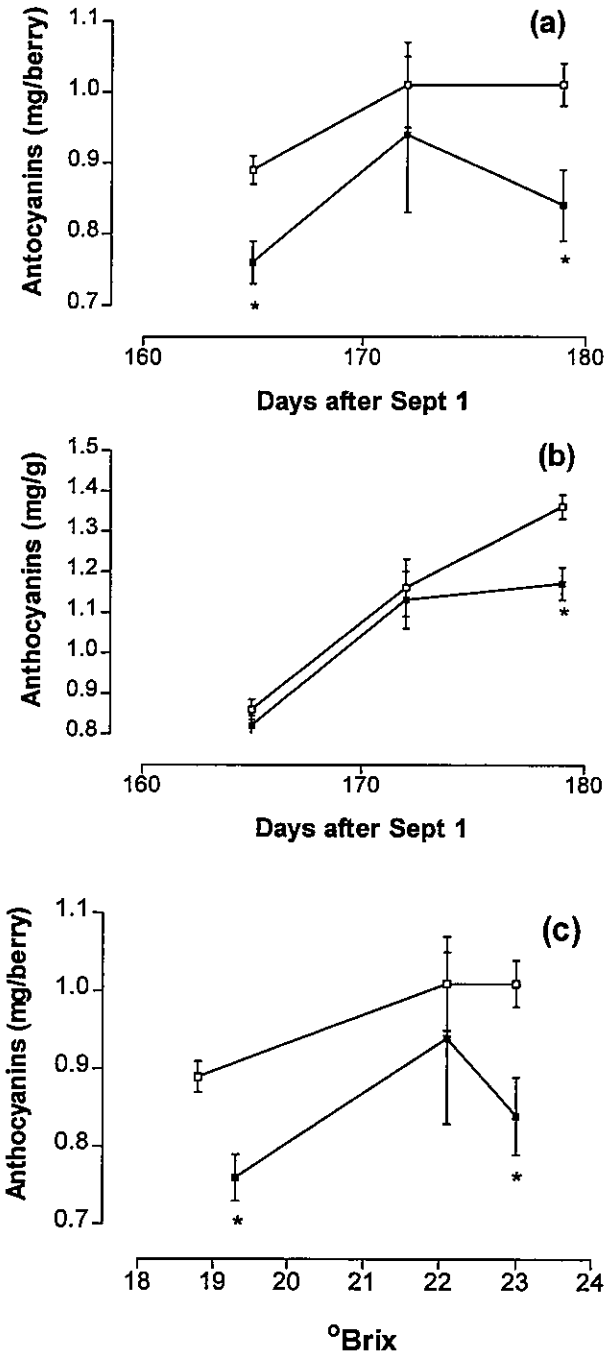


Figure 9.18 Anthocyanins (mean ± se) of Cabernet Sauvignon/Ramsey split-root vines, 1995/96, Exp. 14. Both sides of vine irrigated (C, ■); one side of vine not irrigated from D45 until D179 [= 27/2/96] (T, □). * indicates means are significantly different ($p<0.05$).

a) mg/berry vs time;
b) mg/g berry mass vs time;
c) mg/berry as a function of the change in TSS (°Brix) of homogenate.

Correlations: *Individual vines*: Values for T and C vines were pooled for selected parameters within individual seasons (Table 9.9) and over all seasons (Figure 9.19) to investigate the relationships between shoot function, canopy density and fruit composition. Averages from set to veraison were used for SGR and g_S. The indices of vigour (SGR, shoot weight and lateral number or lateral leaf area) were all strongly and positively inter-correlated within each season, and positively correlated with canopy density indices in the last two seasons.

Table 9.9 Correlation matrices (mean values per vine, 'treated' [T] and 'control' [C] data pooled): stomatal conductance (g_S, mean, set to veraison, mmol/m^2/s); shoot growth rate (SGR, set to veraison, cm/day); pruning weight (PRWT, kg); leaf layer number (LLN); lateral no/sht (LN); leaf area on lateral shoots, harvest (LLA, m^2 per vine); mean shoot weight, winter (SWT, g); bunch exposure index (BEI, harvest); leaf area/surface area, harvest (LASA); TSS (°Brix); pH (at same °Brix); total anthocyanins (COL, mg/g berry mass; at same °Brix); total glycosyl-glucose (G-G, µmol/g berry mass; at same °Brix). *, ** and *** indicate significance at $p<0.05$, 0.01 and 0.001 respectively.

a. 1993/94

	g_S	SGR	PRWT	LLN	LN	SWT	pH
SGR	0.59	-	-	-	-	-	-
PRWT	0.57	0.71 *	-	-	-	-	-
LLN	0.59	0.58	0.53	-	-	-	-
LN	0.66	0.80 *	0.79 *	0.53	-	-	-
SWT	0.59	0.69	0.96 ***	0.52	0.89 **	-	-
pH	0.71 *	0.81 *	0.92 **	0.71 *	0.87 **	0.94 ***	-
°Brix	0.03	-0.5	-0.02	-0.31	-0.05	0.06	-0.18

b. 1994/95

	g_S	SGR	LLA	PRWT	PAR	Brix	COL
SGR	0.94 ***	-	-	-	-	-	-
LLA	0.70*	0.69	-	-	-	-	-
PRWT	0.63	0.82 *	0.42	-	-	-	-
BEI	-0.74 *	-0.87 **	-0.60	-0.72 *	-	-	-
Brix	-0.44	-0.24	0.08	-0.08	0.07	-	-
COL	-0.90 **	-0.81 *	-0.71 *	-0.51	0.71 *	0.49	-
G-G	-0.82 *	-0.62	-0.50	-0.36	0.57	0.62	0.96 ***

c. 1995/96

	g_S	SGR	LLA	PRWT	LASA
SGR	0.85 **	-	-	-	-
LLA	0.67	0.86 **	-	-	-
PRWT	0.53	0.76 *	0.90 **	-	-
LASA	0.47	0.76 *	0.95 ***	-0.92 **	-
COL	-0.63	-0.84 **	-0.93 ***	-0.90 **	-0.89 **

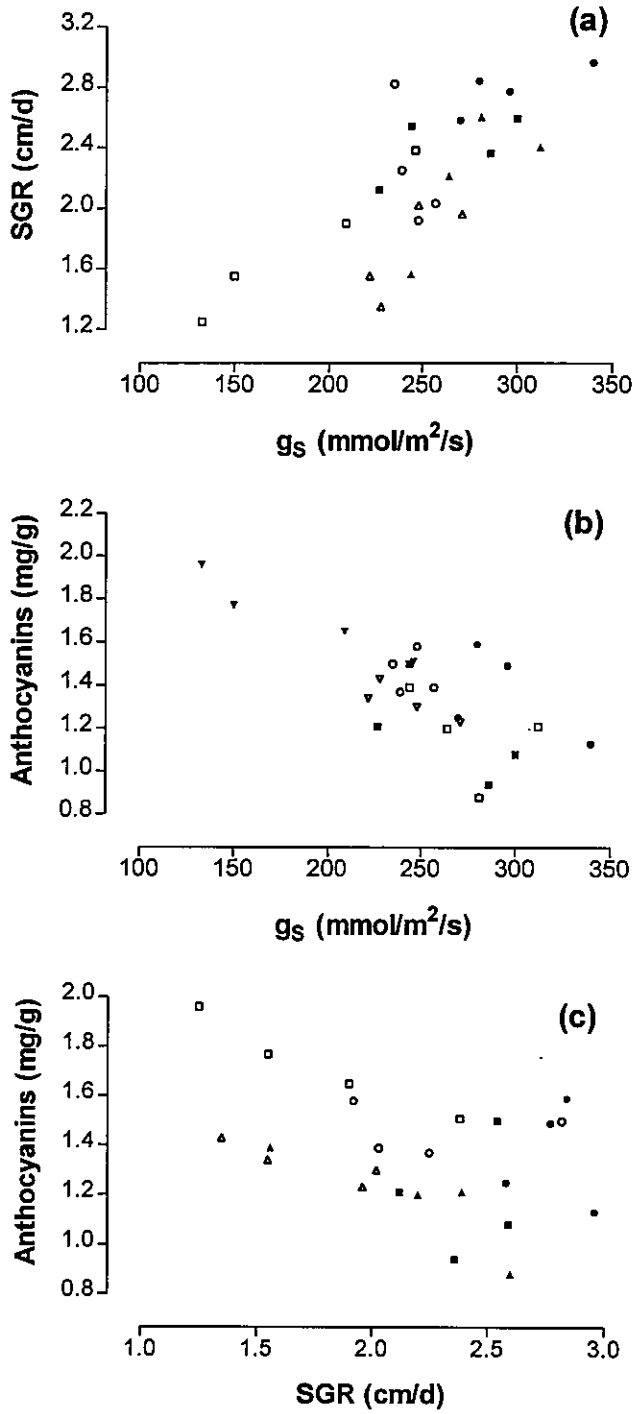


Figure 9.19 Relationships between stomatal conductance (g_S, mmol/m^2/s), shoot growth rate (SGR, cm/day) and anthocyanins (mg/g berry mass) of C (irrigated on both sides, closed symbols) and T (partial drying, open symbols) Cabernet Sauvignon (Exp.14) over three seasons: 1993/94 (circles), 1994/95 (squares), 1995/96 (triangles). Both SGR and g_S : mean from set to veraison.

a. SGR vs. g_S : r = 0.74***.
b. Anthocyanins vs. g_S: r = 0.73***.
c. Anthocyanins vs. SGR: r = 0.45*.

Stomatal conductance was strongly positively correlated with SGR in the last two seasons and negatively correlated with bunch exposure in 1994/95. Colour was strongly negatively correlated with vigour indices and canopy density in the last two seasons, and with g_S in 1994/95, but only weakly correlated with SGR and g_S in 1993/94. pH was strongly correlated with all parameters except °Brix in 1993/94. G-G was strongly correlated with colour (positive) and g_S (negative) in 1994/95. The relationships between SGR (as an index of vigour), g_S and colour (anthocyanins mg/g berry mass) were analysed by pooling individual vine data over three seasons (Figure 9.19). SGR and g_S were strongly correlated on this basis, as well as during individual seasons. Colour and g_S were also strongly correlated but the relationship between SGR and colour was not as strong. Colour (mg/g berry mass) was used for this analysis rather than colour (mg/berry) in order to remove any seasonal effect of berry size on the latter. However, in the last two seasons, berry weights were almost identical and so the relationships between colour (mg/berry) and both SGR and g_S were analysed by pooling the individual vine data from those two seasons only: colour (mg/berry) was negatively correlated with both SGR ($r = 0.6^*$) and g_S ($r = 0.61^*$).

Treatment means over time: The relationship between g_S and SGR was also analysed in a different manner in 1994/95. From D75 to D141 (set to veraison), daily values within three days of each other were paired and the linear regression lines calculated for T as % of C (Figure 9.20a) and for actual treatment means (C and T values analysed separately; Figure 9.20b). These analyses further confirmed the strong positive relationship between g_S and SGR. However, the relationship was stronger for T vines than C (Figure 9.20b).

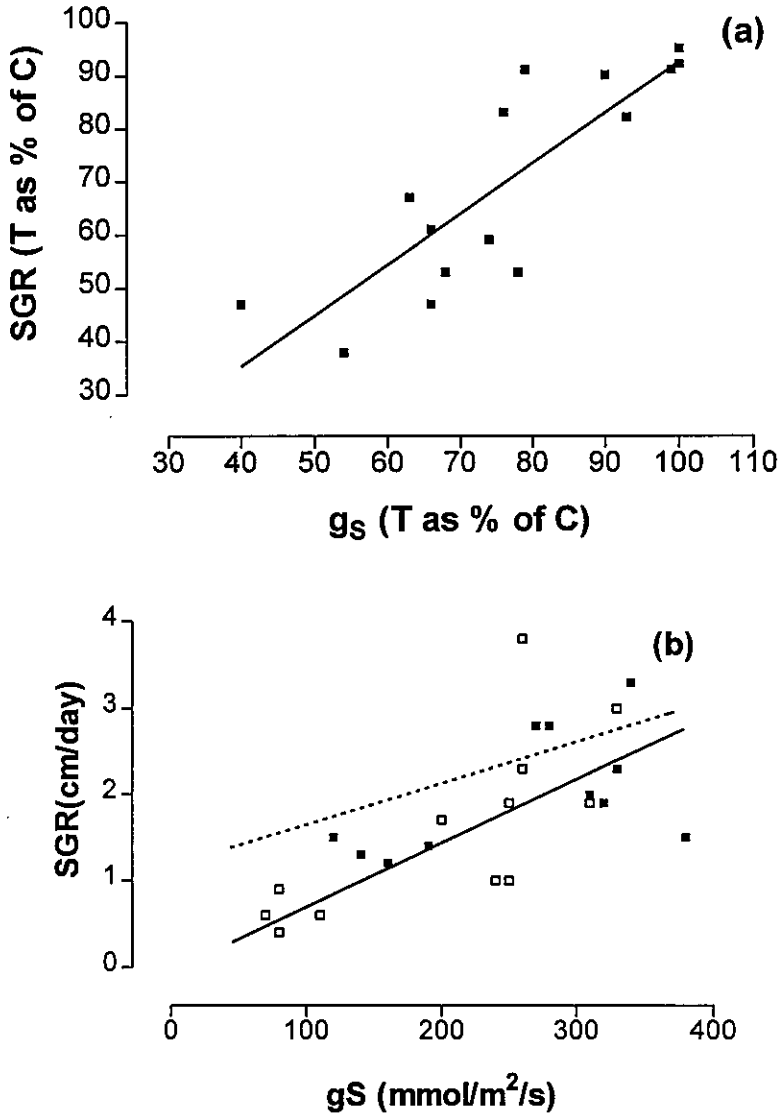


Figure 9.20 Relationship between stomatal conductance (g_s) and shoot growth rate (SGR) from D75 to D141; Cabernet Sauvignon/Ramsey split-root vines, 194/95, Expt 14. C: both sides of vine irrigated; T: one side of vine not irrigated during PRD periods; g_s and SGR values within three days of each other.

a. T as % of C. Equation of line: SGR = $0.95g_s$ - 2.64; $r^2 = 0.71$***.

b. Actual values of C (■) and T (□). Equation of line: SGR = 0.005 + 0.84 ($r^2 = 0.23$, ns) for C (dotted line; SGR = 0.008 - 0.09 ($r^2 = 0.55$*) for T (solid line).

9.3.2 Blewitt Springs

Soil water and irrigation: Soil water content (0 to 50cm) of the 'dry' side of T vines was lower than the 'wet' side on each occasion when measured until the end of January (Table 9.10); however, during February and March there appeared to be no difference between C vines or either side of T vines. Over the whole season from start of PRD to harvest, 98mm less irrigation was applied to T vines than C (Appendix VI). During the PRD periods, an average of 17.2 L/day was applied to each C vine (Table 9.1).

Table 9.10 Volumetric soil water content (SWC, mean ± se, %), 0 to 50cm depth, of C (both sides of vine irrigated, mean of both sides) and T (one side of vine not irrigated at any one time from D98 until D181) Cabernet Sauvignon/Ramsey split-root vines, Exp. 15. All measurements during middle of drying period except for D141(end of period) and D192 (both sides of 'treated' on). 'Wet' and 'dry' refer to irrigated and non-irrigated sides respectively of each T vine at any point in time.

Date	DAS	Side not irrigated	C	T	
				Wet	Dry
14/12/94	106	W	22.0 ± 0.6	21.0 ± 0.4	6.3 ± 0.2
28/12/94	120	E	10.4 ± 0.3	10.3 ± 0.3	5.9 ± 0.2
11/1/95	134	W	15.4 ± 0.7	15.1 ± 1.3	5.9 ± 0.3
18/1/95	141	W	17.8 ± 0.3	15.3 ± 1.5	5.2 ± 0.3
25/1/95	148	E	15.8 ± 0.9	16.6 ± 0.9	5.4 ± 0.3
8/2/95	162	W	7.3 ± 0.5	8.0 ± 0.4	7.3 ± 0.4
22/2/95	176	E	6.5 ± 0.2	7.0 ± 0.4	6.2 ± 0.3
10/3/95	192	-	6.0 ± 0.3	6.8 ± 0.4	6.5 ± 0.2

Shoot growth: SGR of C and T vines decreased after veraison. SGR of T vines was significantly less than that of C vines from D120, ie. 20 days after the start of PRD, by which time the SGR of T vines had decreased to 63% of C (Figure 9.21). By D170, SGR of T vines had decreased to ca. 30% of C; at that time, 15% and 50% of C and T shoots respectively had ceased to grow. Overall, from set to veraison, SGR of T vines averaged 30% less than C (Table 9.11) resulting in significantly shorter shoots at maturity. The overall decrease in vegetative growth in response to treatment was reflected by the significantly lower pruning weight (-23%) which was due entirely to a decrease in mean shoot weight (Table 9.11), which in turn would have been due to a reduction in lateral shoot development on T vines (observed visually, not measured).

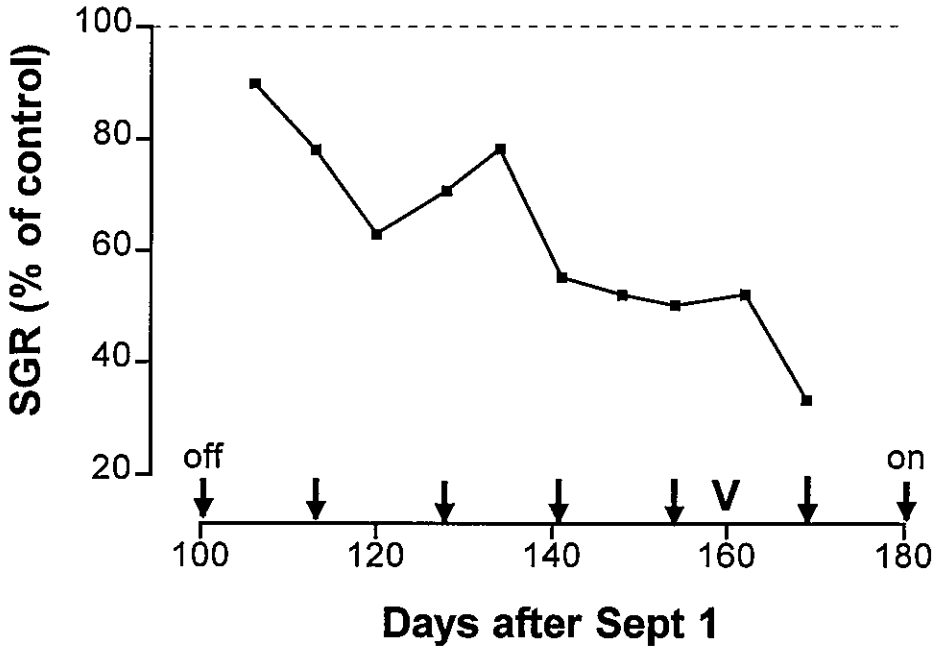


Figure 9.21 Effect of partial drying on shoot growth rate (SGR, cm/d) of 'treated' (T) as % of 'control' (C): Cabernet Sauvignon/Ramsey split-root vines, Exp.15. C: both sides of vine irrigated; T:one side of vine not irrigated from D98 ('off') until D181 ('on'). Arrows indicate days when irrigation of 'treated' switched from one side to the other. V indicates veraison date. T significantly different ($p<0.05$) to C from D128 to D169.

Table 9.11 Shoot growth and canopy measurements of C (both sides of vine irrigated) and T (one side of vine not irrigated during PRD periods) Cabernet Sauvignon/Ramsey split-root vines, Exp. 15.

Variable	C	T	% diff.	Signif
Pruning wt (kg/vine)	4.73 ± 0.46	3.62 ± 0.29	-23	<0.05
Shoot no /vine	44 ± 3	44 ± 3	0	ns
Shoot wt (g)	108 ± 16	82 ± 6	-24	ns
SGR (cm/day), set to veraison	1.74 ± 0.23	1.21 ± 0.22	-30	<0.05
Bunch exposure index, veraison +30d	151	195	+29	ns

Gas exchange: Stomatal conductance of C vines decreased from set until veraison, then decreased further after veraison; g_S of T vines followed a similar pattern but was significantly lower than C from set until veraison, averaging 18% less over the whole period (Table 9.12).

Table 9.12 Stomatal conductance (mean ± se, mmol/m^2/s) of C (both sides of vine irrigated) and T (one side of vine not irrigated during PRD periods) Cabernet Sauvignon/Ramsey split-root vines, Exp. 15.

Measurement period (DAS)	C	T	% diff.	Signif.
106 -128	414 ± 8	364 ± 10	-12	<0.01
134 -154	345 ± 28	248 ± 16	-28	<0.05
162 -182	192 ± 15	155 ± 13	-19	ns
Set to veraison	326 ± 7	266 ± 5	-18	<0.01

Canopy measures: As a result of decreased shoot growth (Table 9.11), the canopy of T vines was slightly more open than C, with a 29% increase in bunch exposure.

Yield components: There was no significant effect of treatment on any yield component (Table 9.13). Fruit weight/pruning weight ratio was 30% higher for T due to the reduction in pruning weight per vine and fruit weight/volume irrigation was increased by 53% because of the reduction in amount of irrigation to T vines.

Table 9.13 Yield components of C (both sides of vine irrigated) and T (one side of vine not irrigated during PRD periods) Cabernet Sauvignon/Ramsey split-root vines, Exp.15.

Variable	C	T	% diff.	Signif
Fruit wt (kg/vine)	9.69 ± 0.45	10.04 ± 0.81	+4	ns
Bunch no/vine	110 ± 4	114 ± 4	+3	ns
Bunch wt (g)	88 ± 5	89 ± 9	+1	ns
Berry no/bunch	80 ± 3	81 ± 8	+2	ns
Berry wt (g)	1.11 ± 0.04	1.09 ± 0.03	-2	ns
Fruit wt/pruning wt	2.10 ± 0.20	2.78 ± 0.24	+30	ns
Fruit wt/volume irrign [a] (g/L)	4.8	7.7	+60	-

[a] Irrigation over whole season estimated at 2002 and 1304 L/vine for 'control' and 'treated' respectively; see Appendix VI for details

Fruit composition: There was no effect of treatment on the timing of veraison nor any other phenological stage (Table 9.14). T vines ripened at the same rate as C vines. Compositional parameters were compared at the same °Brix at harvest: there were no significant differences for any parameter, although anthocyanins of T vines were slightly higher than C, both on a per-berry and per-berry mass basis (Table 9.14).

Table 9.14 Fruit composition at harvest (D237) of C (both sides of vine irrigated) and T (one side of vine not irrigated during PRD periods) Cabernet Sauvignon/Ramsey split-root vines, Exp. 15.

Variable	C	T	% diff.	Signif
TSS (°Brix)	23.6 ± 0.2	23.6 ± 0.4	0	ns
Titratable acidity (g/L)	8.0 ± 0.1	7.4 ± 0.3	-7	ns
pH	3.57 ± 0.03	3.59 ± 0.02	0	ns
Berry wt (g)	1.11 ± 0.04	1.09 ± 0.03	-2	ns
Total anthocyanins (mg /berry)	1.15 ± 0.14	1.26 ± 0.1	+10	ns
Total anthocyanins (mg /g berry mass)	1.04 ± 0.14	1.17 ± 0.11	+12	ns
Phenolics (ab. units /berry)	48 ± 4	51 ± 3	+6	ns
Phenolics (ab. units /g berry mass)	43 ± 4	47 ± 3	+9	ns

9.3.3 Comparison of Waite and Blewitt Springs experiments

Actual SGR of Blewitt Springs vines was lower than that of Waite vines in 1994/95, probably due to a combination of more shoots per vine and higher yield at Blewitt Springs (Table 9.2). However, this did not explain the lower SGR at Blewitt Springs relative to the 1995/96 season at Waite because shoot number and fruit weight per vine were similar. The relative effect of PRD treatment on SGR in the Blewitt Springs experiment was greater than for any season of the Waite experiment. By contrast, actual g_S of vines in the Blewitt Springs experiment was higher than for any season of the Waite experiment. This was probably due to the difference in climatic conditions between the two sites, ie. lower temperature and VPD at Blewitt Springs. The relative effect of PRD treatment on g_S in the Blewitt Springs experiment (-18%) was the same as the three-year average of the Waite experiment.

Average irrigation rate (L/vine/day) during the PRD period for the Blewitt Springs experiment was more than twice as much as for the last two seasons at the Waite site (Table 9.1). Fruit weight for Blewitt Springs vines was higher than for Waite in the same season, but similar to Waite in 1995/96 (Figure 9.16), and was correlated with shoot number per vine (Tables 9.6, 9.12). Fruit weight/pruning weight ratio of Blewitt Springs vines was similar to Waite in 1995/96, but berry weight was ca. 50% more for Blewitt Springs (Tables 9.6, 9.13).

9.4 Discussion

Soil water and irrigation scheduling: The irrigation strategy adopted for these experiments successfully maintained 'half' of the root system of T vines in a drying phase during the PRD periods. At the same time, the 'wet' side of T vines and both sides of C vines were maintained at a relatively higher water content. At the Waite site, the data suggest that the amount of water applied late in the season during each irrigation period, e.g after D120 and D140 in 1994/95 and 1995/96 respectively, was inadequate to refill the soil reservoir (20 to 50cm depth) to field capacity on the 'wet' side of the T vines. However, the amount was adequate for the C vines. Analysis of data recorded by individual sensors at different depths on T vines indicates that this apparent deficit over the whole 20 to 50cm was the result of a progressive drying of the soil at 50cm and deeper layers on the T vines during the course of the season. In turn, this was a consequence of insufficient length of irrigation periods later in the season which did not replenish these deeper layers to field capacity. This implies that the T vines were not only subjected to PRD treatment, they also had an inadvertent RDI treatment (Goodwin and Jerie 1992) in the latter part of the season because there was replacement of less than the full irrigation requirement on the 'wet' side during some of the PRD periods. However, in RDI experiments, pre-dawn Ψ_L of RDI-treated vines was significantly lower than that of well-watered control vines (Goodwin and Macrae 1990). This caused Goodwin and Jerie (1992) to define a 'mild stress' as "..a difference of less than 0.2 MPa in Ψ_L" relative to well-watered, non-stressed vines. Furthermore, in other irrigation experiments, eg. McCarthy and Staniford (1984), Matthews and Anderson (1988,1989), vines subjected to 'deficit' treatments typically have Ψ_L which are 0.3MPa or more lower at midday than fully-irrigated vines. On the other hand, the Ψ_L of PRD vines in this study was never less than that of the controls. In particular, there was not a statistically significant difference at those times during the 1994/95 and 1995/96 seasons when there may have been an apparent deficit in the soil on the 'wet' side. On those relatively few occasions when the actual mean Ψ_L of PRD vines was less than that of controls, the difference was never more than 0.1 MPa.

More detailed analysis of the capacitance probe data will provide information on the likely differential water use by roots at different soil depths; of particular interest is the rate of water use by roots on the 'wet' side of the vine as the SWC on the 'dry' side declines. However, because this was not one of the aims of this study, it has not been included. The monitoring of soil water status was principally carried out in the experiments with field vines: a) to ensure that drying of part of the root-zone was being successfully achieved through a combination of spatial separation of drippers, the use of the plastic membrane and the wide row x vine spacing; b) to schedule irrigation so as to maintain the soil of the root-zone of 'control' and the wet side of 'treated' vines within prescribed limits; and c) to determine the optimal time of alternation of the drying from one side of 'treated' vines to the other.

Sap flow: There was no attempt in this study to correlate the absolute values of sap flow rate with either soil water content or estimated whole-plant transpiration. Rather the relative flow rates of the two halves of the trunk, which were presumably indicative of the flow rates from the two halves of the root system, were used to show that: a) flow from the 'wet' roots was generally greater over a 24 hour period than flow from the 'dry' roots; b) the change in relative flow rate from one side of the vine to the other coincided with changes in irrigation from one side to the other; and c) the flow from 'dry' roots was sufficiently high during the day to allow a significant flux of a chemical signal such as ABA from drying roots. It is possible that roots in drying soil are rehydrated during the night by water supplied by 'wet' roots as proposed by Glenn and Welker (1993).

Vegetative growth: Vegetative growth of field-grown Cabernet Sauvignon was substantially reduced at both sites, and in all seasons, in response to PRD. Average shoot growth rate (set to veraison) of PRD vines was reduced by 18 to 30%, pruning weight by 19 to 29%, total leaf area per vine by 25% and total lateral shoot length/main shoot by 55 to 66%, relative to control vines. The magnitude of the response with fruit-bearing Cabernet Sauvignon grafted to Ramsey in the field was less than that achieved with de-fruited, self-rooted Shiraz in the pot experiment (Chapter 7). Apart from the obvious

differences of genotype and crop load, there is also the likelihood that a larger proportion of the root system was dried in the pot experiment; the strength of the signal may be related to the proportion of roots dried (Blum and Johnson 1993).

Mean reduction in SGR of field vines in response to PRD was between 18 and 30% relative to the control. This was greater than the g_S response, adding support to the hypothesis that vegetative growth is more sensitive to PRD than gas exchange. The decrease in components of vegetative growth in response to PRD was of a similar order to that recorded in irrigation deficit experiments where the amount of water applied to deficit treatments has been substantially less than the well-watered controls (McCarthy and Staniford 1984; Hepner *et al.* 1985; Matthews and Anderson 1988,1989; Goodwin and Jerie 1992; Naor *et al.* 1993; McCarthy 1997). However, in these cases, there was also a significant reduction in yield (12 to 33% less than control), with the response depending on the timing and duration of the deficit. The main yield component affected in these other experiments was berry weight and it is notable that this yield component was not significantly affected in response to PRD in any season and at either site.

In all seasons during the first period of soil drying, the relative decrease in shoot growth rate in response to PRD coincided with the decrease in soil water content on the 'dry' side, with some recovery in SGR at the end of the first period. Although there were several 'minor' recoveries subsequently during the first two seasons, there were no obvious recoveries towards the end of PRD periods in the last season. This suggests that the timing of the alternation of drying improved as the seasons progressed.

The average rate of shoot growth over the season decreased over the three seasons at the Waite site. This may have been a consequence of improved use of the PRD technique and earlier start of PRD (in the last season). It may also have been a consequence of an increase in shoot number and crop load per vine: an increase in both components is known to decrease shoot vigour (Tassie and Freeman 1992). This may also explain the relatively high shoot growth rate of vines of both treatments in the first season between flowering

and veraison. In subsequent seasons, actual SGR reached a peak soon after flowering and declined thereafter, as is typical for grapevines in this environment (by veraison + two weeks in the last two seasons, SGR of both treatments had reduced to less than one cm/day). The actual rates of shoot growth were similar to those measured in other field experiments with mature vines: for example, Matthews *et al.* (1987) reported a maximum SGR of 4.4 cm/day just after flowering for cv. Cabernet Franc. In the first two seasons, SGR responded to partial drying within three to four weeks, but not until six weeks after the start of PRD in the last season. This difference can possibly be explained in terms of soil water status: in 95/96, SWC in the root-zone on the 'dry' side of T vines remained relatively high during the first two PRD periods and did not drop below the refill point set for the irrigation scheduling of the 'wet' side (Figure 9.3a). As a consequence, SGR of T vines did not significantly decrease below that of C vines until early in the third PRD period. By comparison, in the previous season, the SGR of different treatments became significantly different for the first time during the second PRD period, coincidentally with substantial decrease in SWC on the 'dry' side (Figure 9.2a). In the last season, PRD started ca. 40 days earlier than the previous two seasons but the inhibition of SGR in response to PRD was earlier by only 10 days or so. Therefore, it may be difficult to sufficiently dry the soil prior to flowering at the Waite site in order to induce a shoot growth response to PRD. On the other hand, if a soil has a relatively low water-holding capacity, this may be more readily achieved; for example, at Blewitt Springs, SGR responded to PRD in the first period, coincidentally with a substantial decrease in SWC on the 'dry' side of the vine. The implications of relatively high SWC in spring for the practical implementation of PRD will be discussed in Chapter 11.

Pruning weight is a useful index of overall vegetative growth of a grapevine. The significant decrease in pruning weight in response to PRD was almost entirely due to an effect on mean shoot weight, rather than shoot number per vine. The actual shoot weight values indicate that the vines of both treatments were still excessively vigorous by the 1995/96 season, in spite of the lighter pruning and larger crop load: more than 60g per shoot is indicative of high vigour according to Smart and Robinson (1991). Lower shoot

weight in response to PRD was not only due to shorter shoots but also lower shoot weight per unit length, which in turn was not due to an effect of PRD on shoot thickness but rather reduced lateral growth. Decreased shoot thickness can occur in response to water stress of grapevines but it is less sensitive than axial growth (Williams and Matthews 1990).

The reduction in shoot length in response to PRD of field vines was not a consequence of any effect on internode elongation; this was also observed in pot experiments. The rate of elongation of specific internodes was not examined in the field experiments. However, measurement of the length of individual internodes on mature shoots suggests that this was not affected by PRD during the periods of maximal inhibition of shoot growth rate. The decrease in SGR in response to PRD of both field and potted vines was the result of a decrease in the rate of node initiation and earlier termination of shoot growth. This response does not appear to have been recorded with other studies with half-dried, split-root plants, although Turner *et al.* (1996) did observe that leaf expansion was terminated earlier on half-dried passionfruit. Furthermore, whereas duration of shoot growth and production of nodes are known to be sensitive to water stress(Williams and Grimes 1987), internode elongation is the major component of shoot growth that is affected by water stress (Smart and Coombe 1983; Williams and Matthews 1990). Therefore, the response of shoot growth to PRD is different to that commonly observed in deficit irrigation experiments. The actual mean internode length per shoot in 1995/96 was within the range considered by Smart and Robinson (1991) to be desirable, ie. six to eight cm. On the other hand, the node number and total shoot length of untrimmed shoots of both treatments were indicative of high shoot vigour. This is a paradox because a high vigour shoot should have both more nodes per shoot and longer internodes. Smart *et al.* (1990) suggested that mean mature shoot (cane) weight and mean weight per unit of cane length were better indicators of shoot vigour than mean internode length: the decrease of mean cane weight in response to PRD ranged from 22 to 35% in the Waite experiment.

The reduction in growth of lateral shoots, in response to partial drying, was the most substantial of any component of vegetative growth: both lateral number and mean lateral

length were affected with decreases of up to 66% relative to the control. As expected, the trimming of 'up' shoots in 1995/96 stimulated lateral shoot development on those shoots (particularly number per main shoot). However, the associated stimulation of lateral development on the untrimmed, 'down' shoots was not anticipated; and neither was the relatively greater degree of inhibition of mean lateral shoot length on those shoots than the 'up' shoots in response to PRD (i.e 41 and 29% decrease relative to control respectively).

The degree of lateral shoot development is an important indicator of vine balance (Smart and Robinson 1991) and excessively vigorous vines are characterised by many and long lateral shoots. Lateral growth may continue after the growth of main (primary) shoots has terminated and late-season improvement in water status can induce growth of lateral shoots (Smart and Coombe 1983; Reynolds and Naylor 1996). The degree of within-canopy shading is strongly correlated with the degree of lateral shoot development and the proportion of lateral leaf area to main leaf area (Smart 1992; P. R. Dry, unpublished data). Lateral number per shoot is positively correlated with incidence of primary bud necrosis in seeded grapevine cultivars (Dry and Coombe 1994). Late-season development of lateral shoots is said to be undesirable because it competes with fruit ripening (Kasimatis 1987; Reynolds and Naylor 1996). According to Koblet (1977), lateral shoots are net importers of photosynthate, unless leaves are fully expanded. Lateral shoot development, as a component of vegetative growth, has been infrequently measured in water deficit studies of grapevines in the field: Williams and Matthews (1990) stated that leaf area on lateral shoots was affected more by water deficit than main leaf area, and Goodwin and Jerie (1992) reported that RDI reduced lateral shoot development (but did not provide any data). Therefore, the detailed measurements of lateral shoot development and the observed response of this component to partial drying in this study are a useful addition to the body of knowledge which indicates an association between lateral shoot development and fruit composition.

If the 'down' shoots had also been trimmed, it is possible that there may have been even more lateral development on the 'up' shoots because they are inherently more vigorous

than the 'down'. Therefore, the deliberate non-trimming of the shoots of the lower curtain of vertically-divided training systems where the shoots of the lower curtain are positioned downwards, e.g 'Scott Henry' (Smart and Robinson 1991) and 'Smart-Dyson' (Smart 1994), may be a useful strategy in order to limit lateral development of the shoots of the upper curtain and is worthy of further investigation.

As a result of the decrease in lateral shoot development in response to PRD, the difference in total leaf area (TLA) between treatments was mainly due to a difference in lateral leaf area (LLA) rather than main leaf area (MLA). This is not surprising because a large proportion of the main leaf area had been determined before the PRD treatment started to exert its influence on vegetative growth, i.e prior to fruit set. On the other hand, most of the lateral leaf area developed after set, when the response to PRD was strongest. For example, in 1995/96, between D110 and harvest, PRD had no significant effect on the increase in MLA (ca. $5m^2$ for both treatments) but the effect on the increase in LLA over that time was very significant, i.e an increase of $5.1m^2$ for control compared to an increase of $2.0m^2$ for PRD-treated (Figure 9.11b). A greater effect on lateral leaf area than main leaf area has also been reported from irrigation deficit experiments: for example, 50% decrease in amount of water applied over the whole season resulted in 16 and 40% reductions in MLA and LLA respectively with Thompson Seedless (unpublished data of Williams and Grimes, cited in Williams and Matthews 1990). The values of LLA as a percentage of TLA (ca. 38% and 28% for C and T vines respectively) indicate that the vines of both treatments were excessively vigorous.

The response of leaf number (and thus the rate of leaf initiation) for both main and lateral leaves was greater than the response of leaf size (and thus the rate of leaf expansion). This is consistent with the prior observation that partial drying of grapevines appears to have more effect on the rate of node initiation than expansive growth (e.g internode elongation).

The actual values of leaf area per vine in the Waite experiment were relatively high and this suggests that the method used may have over-estimated the actual leaf area. For

example, intensively-irrigated, cane-pruned Cabernet Sauvignon on Ramsey had a maximum leaf area of ca. $14m^2$ (Sommer and Clingeleffer 1996) but these vines were planted at a lower density than the Waite experiment. On the other hand, the TLA values for the Waite control vines were similar to those reported for minimally-pruned Cabernet Sauvignon on Ramsey (Sommer and Clingeleffer 1996) and to well-irrigated, self-rooted Thompson Seedless (unpublished data of Williams and Grimes, cited in Williams and Matthews 1990). The magnitude of the decrease in TLA in response to PRD of field vines was similar to or slightly greater than that reported from experiments on deficit irrigation (Williams and Matthews 1990).

The fact that there was no difference in trunk diameter after three seasons of PRD at the Waite site may indicate that there was no detrimental effect on carbohydrate partitioning to the permanent woody parts of the vine. If the mean diameter of the upper parts of the root system below the split, but above the soil surface, is representative of the size of the root system, then the fact that this was not affected by treatment may indicate that PRD decreases the shoot/root ratio of field vines as well as potted vines (Chapter 7). However, this can only be confirmed by actual excavation and measurement of the root system. Differences in diameter between the west and east sides of the upper parts of the root system were measured on some vines, but there was no effect of treatment or direction: in the most extreme case, which happened to be a C vine, the east side was 40% less than the west side. Nevertheless, this may indicate that, for some vines, there may have been a difference in the size of root systems on each side of the plastic membrane, and this may explain why the response to drying was greater on one side of the vine than the other in some cases.

The effect of PRD on the frequency distribution of shoot length and on lateral shoot development was similar to the effect achieved with a combination of lighter pruning and 'Ruakura Twin Two Tier' trellis system (canopy division into four curtains) on vigorous Cabernet Franc vines (Smart *et al.* 1990). This result indicates PRD has the potential to devigorate shoots more economically than with the use of elaborate trellis systems.

Gas exchange: The gas exchange response of fruit-bearing field vines to PRD was similar in many respects to that of non-fruiting vines in pots and in the field. For example, stomatal closure was only partial, the decrease in g_S during the first PRD period coincided with decrease in soil water content on the 'dry' side, and the response of g_S was relatively less than the response of shoot growth rate.

The actual average g_S values of both control and treated vines from set to veraison did not differ greatly between seasons or sites, with the possible exception of treated vines in 1994/95 at the Waite site, and were comparable with those reported for *Vitis vinifera* field vines in semi-arid climates (eg. Winkel and Rambal 1993; Correia *et al.* 1995). The relatively low average g_S, and large % decrease (relative to control), of treated vines in 1994/95 at the Waite site can be explained in part by the low values of g_S during the fourth PRD period: although g_S of both treatments decreased substantially (Figure 9.13), the relative decrease was greater for T vines than C vines (Figure 9.5b). If the values recorded during the fourth period were not included in the calculation of mean seasonal g_S, then the 1994/95 mean would be more comparable with the means of the other seasons. Therefore, it is reasonable to conclude that the mean reduction in g_S of field vines in response to PRD is likely to be between 12 and 20% relative to the control, or 40 to 60 mmol/m^2/s less than the control in absolute terms. This is similar to the mean difference (ca. flowering to harvest) reported by Winkel and Rambal (1993) for Carignan (*Vitis vinifera*) growing in 'wet' and 'dry' sites: the latter site was lower by 37 and 76 mmol/m^2/s for daily maximum and midday values of g_S respectively. These differences, where stomatal closure was only partial, are less than those reported from situations where well-watered vines have been compared with moderately to severely stressed vines: for example, differences in excess of 250 mmol/m^2/s at midday (Correia *et al.* 1995). In situations of severe water stress, stomata may be closed for much of the day, often in association with perturbed shoot water relations (Smart and Coombe 1983). The advantages of partial stomatal closure for improved water-use efficiency have been previously discussed (Chapter 5).

There is a high degree of variability associated with the measurement of stomatal conductance on leaf areas of less than one cm^2 due to the phenomenon of non-uniform stomatal behaviour ('patchiness') under stress conditions. As a result, Düring and Loveys (1996) recommended that five to ten readings at different locations on the same leaf should be used with field vines. Despite the likely occurrence of patchiness, and the use of a porometer with a cup of less than one cm^2, single measurements of leaves has produced remarkably consistent results with respect to the g_S response to PRD. One can only assume that the use of a relatively large number of leaves per plant, careful leaf selection and repetitive measures over space and time minimised this potential source of variability.

As for the other experiments described in this thesis, there was a strong correlation between g_S and shoot growth rate, suggesting that the two physiological processes are influenced by the same root signal in response to partial drying. Stomatal conductance was also strongly correlated with concentration of secondary metabolites in fruit. However, since the relationship is negative, it would not be appropriate to propose an explanation that invokes increased assimilation rate and thus an increase in substrate for secondary metabolite synthesis.

Yield components: There was no significant effect of treatment on any yield component at either site or in any season. This result was unexpected because some decrease in yield per vine was anticipated from the experience of deficit irrigation experiments in a similar environment with cultivars of *Vitis vinifera* where there had been a 50% reduction in the amount of water applied, eg. a decrease of the order of 20 to 30% has been frequently reported by McCarthy and Staniford (1984), Grimes and Williams (1990) and others. Equally unexpected was the complete absence of any effect of PRD treatment on berry weight: of all the yield components of the grapevine, this is the component which is most sensitive to water stress (Smart and Coombe 1983; Williams and Matthews 1990). Frequently, the decreased yield measured in deficit irrigation experiments can be largely attributed to an effect on berry weight, eg. McCarthy and Staniford (1984); Matthews and Anderson (1988,1989); McCarthy (1997). The lack of any berry weight response in this

study to such a substantial reduction in the amount of water applied is perhaps without precedent in viticultural research, and particularly where a deficit has been imposed between flowering and veraison because this is the period when berry size is most sensitive to stress (Williams and Matthews 1990; McCarthy (1997). Furthermore, because there was no effect of treatment on any other yield component, there was not the complicating factor of yield component compensation. For example, a change in bunch number per vine will usually result in an effect on berry weight and/or berry number per bunch.

The fact that bunch number per shoot was not affected by treatment is surprising in view of the substantial reduction in both shoot vigour and canopy density. One can only assume that the improved radiation microclimate of the renewal zone in response to treatment was not sufficient to affect long-term bud fruitfulness. It is also surprising in view of the dependence of bud fruitfulness on water status (Williams and Matthews 1990): a 50% reduction in irrigation (similar to Waite in 1995/96) significantly decreased fruitfulness of Cabernet Franc (Matthews and Anderson (1989). It is possible that any increase in fruitfulness in this experiment, as a result of decreased canopy density, was counteracted by a decrease in cytokinin production in response to soil drying (Mullins *et al.* 1992).

The actual yield per vine in the Waite experiment increased over the three years of cropping in response to lighter pruning and increased shoot and bunch number per vine for both treatments. The fruit yield of ca. 14 t/ha, for both treatments for the second and third crop in the Blewitt Springs and Waite experiments respectively, is within the appropriate range for commercial vineyards in the same environment with the same amount of irrigation as the control vines. However, the yield of the PRD-treated vines was greater than would normally be achieved with the same amount of water applied with conventional irrigation. The actual amount of irrigation water applied to control vines in the Waite experiment, ie. 1.34 ML/ha in 1995/96, was less than that applied to commercial vineyards in a nearby region with similar growing season rainfall and the same yield per hectare. For example, 2.6 ± 0.45 ML/ha was the average amount of irrigation water applied to nine vineyards surveyed in the Southern Vales region of South Australia with an average yield

of 14 ± 1.5 t/ha (A. Thomson, PISA, South Australia, pers. comm.). The ratio of fruit weight to volume of irrigation water applied (FW/VI) may be used as an index of irrigation efficiency: the FW/VI calculated for the surveyed Southern Vales vineyards is 5.4, compared to 10.1 for the Waite control vines in 1995/96. This suggests that the control vines in the Waite experiment were irrigated more efficiently than those in commercial vineyards, probably through the use of the soil water monitoring equipment. Furthermore, because the amount of irrigation water applied to PRD-treated vines was substantially reduced relative to the control vines, the irrigation efficiency of PRD vines was even higher, i.e 20.2 in 1995/96. The increase in the value of the FW/VI index over the three years of the Waite experiment was not the result of a reduction in the amount of water applied (in fact the opposite is the case) but rather the result of a real increase in fruit weight per vine over that time (Figure 9.14). The control vines in the Blewitt Springs experiment were irrigated to the same degree as comparable vineyards in the same (Southern Vales) region, and as a result, the FW/VI value was also similar; however, at 7.7, the FW/VI value of PRD-treated vines was higher than the district average for vines with the same yield. If the irrigation of the vines in the Blewitt Springs experiment could have been managed with the same degree of precision as in the Waite experiment, it is likely that the FW/VI values would have been higher. Nevertheless, the success of PRD at Blewitt Springs, with far less control than at Waite, is indicative of the potential of this technique on a commercial scale. The difference in berry weight between the 1995/96 Waite experiment and the 1994/95 Blewitt Springs experiment, with similar yield per vine in each case, may be partly due to the greater amount of irrigation applied at the Blewitt Springs site; it may also have been a consequence of poorer set, resulting in fewer berries per bunch.

The ratio of fruit weight to pruning weight (FW/PW) has been used as an index of vine balance (Smart 1992). It equates inversely and approximately with leaf area/fruit weight ratio. FW/PW has been termed 'crop load' by some authors (reviewed by Jackson and Lombard 1993) but this term will not be used in this discussion. FW/PW increased over time in the Waite experiment as the vines became more balanced with age. By the 1995/96

season, although the PRD-treated vines were relatively more balanced, both treatments were still unbalanced and excessively vigorous according to the standards of Smart and Robinson (1991). The percentage increase in FW/PW ratio in response to PRD-treatment in each season was similar to that reported by Hepner *et al.* (1985) from a drip irrigation experiment with Cabernet Sauvignon where irrigation amount over the whole season was decreased by reducing replacement of pan evaporation from 0.45 to 0.27. These authors also found that vegetative growth was affected more than fruit growth by irrigation 'cut-back'. However, a significant increase in FW/PW ratio in response to a water deficit is not common to all experiments because the decrease in pruning weight may be associated with a yield decrease of similar magnitude: for example, halving of amount of irrigation applied had no significant effect on FW/PW (McCarthy and Staniford 1984).

Canopy measures: Several different indices were used to quantify the degree of within-canopy shading and the extent of bunch exposure. There is no single index which can adequately characterise the whole canopy. Nevertheless, the bunch exposure index (by ceptometer) has been shown to be a useful index in other studies (Botting *et al.* 1996). In all seasons, as a result of decreased vegetative growth, the canopy of PRD-treated vines was more open, with better bunch exposure, than control vines. The effect of PRD on bunch exposure was relatively greater in the second than the third season, probably in response to the change from a single canopy to a divided canopy; this had the result of decreasing canopy density and increasing bunch exposure of both treatments, but relatively more for the control than PRD-treated vines.

Fruit composition: There was no significant effect of treatment on time of flowering or veraison. The time of ripening (as indicated by the rate of sugar accumulation) was advanced in the first two seasons in the Waite experiment but not in the last, nor in the Blewitt Springs experiment. The only other reference to an effect of partial drying on phenology is that of Turner *et al.* (1996) who reported earlier flowering of passionfruit in response to half-drying of potted plants. Differences in fruit composition reported from deficit irrigation studies have been attributed to an effect of time of maturity (via an effect

of crop load) [Smart and Coombe 1983]; however, the differences in fruit composition (particularly secondary metabolites) between treatments in this study are not likely to be attributable to a difference of just one week in maturity in two out of three seasons. It is notable that moderate levels of water stress prior to ripening may have no effect on the rate of ripening (Matthews and Anderson 1988) because the accumulation of sugar is said to be much less sensitive to water stress than berry growth (Williams and Matthews 1990).

The fruit was harvested at a sugar concentration that is appropriate for red wine production, i.e 22 to 24 °Brix, and the levels of both pH and titratable acidity for both treatments indicate that the fruit would have been most suitable for this purpose. It is very important that treatments are compared at the same °Brix because of the strong correlation between sugar concentration and secondary metabolite concentration in other studies (McCarthy *et al.* 1996; Botting *et al.* 1996). In this study, all comparisons between treatments were made with samples for which the treatment means were within 0.1°Brix.

At the same °Brix, pH was significantly lower in fruit from PRD-treated vines in the first two seasons in the Waite experiment. This may have been a response to the differences in canopy density in those two seasons: PRD reduced canopy density and it is a common observation that both juice and wine from vines with shaded canopies tend to be relatively high in pH (Smart 1992). There was a strong correlation between pH and canopy density (as indicated by leaf layer number) and factors which tend to affect canopy density such as SGR and lateral shoot number. In the last season, pH of T vines was lower than C but not statistically significant; the relative lack of response in that season may be a function of the decreased difference between treatments with respect to canopy density in that season, arising from the change to a divided canopy for both treatments. Similarly, the lack of a treatment effect on pH in the Blewitt Springs experiment may be due to the relatively minor effect of treatment on canopy density in that case. The pH response in this study is similar to that reported from some deficit irrigation experiments but, in general, the response of pH to water stress is variable (Williams and Matthews 1990).

Grape quality for winemaking cannot be adequately defined by means of °Brix, pH or titratable acidity, because these measures give little indication of the potential quality of the wine. Colour and phenolic content are widely recognised as important quality components of cultivars used for red wine and have been use to test the effects of irrigation and canopy management on fruit composition of such cultivars (Williams and Matthews 1990; Jackson and Lombard 1993). Abbott *et al.* (1993) proposed that a measure of the total concentration of glycosylated flavour compounds of juice may indicate the potential wine quality of grapes, and this lead to the development of the glycosyl-glucose (G-G) assay as a measure of total glycosides (Williams *et al.* 1995). Subsequently, the 'red-free' assay for pigmented grape berries was developed by Iland *et al.* (1996) in which the contribution of anthocyanins was subtracted from total G-G.

PRD significantly increased the concentration of secondary metabolites (anthocyanins, phenolics, G-G) in two out of three seasons in the Waite experiment when fruit was compared at the same stage of ripeness (relative to °Brix); although anthocyanin concentration was increased in response to PRD in the first season at Waite, and in the Blewitt Springs experiment, the difference was not statistically significant. Anthocyanin concentration, per g berry mass, of grape cultivars used for production of red wine is strongly correlated with wine colour parameters (intensity and hue) and wine quality. The non-pigmented phenolic compounds contribute to the astringency and stability of wine and there is increasing evidence of a correlation between G-G and wine quality (D.G. Botting, University of Adelaide, pers. comm.). Therefore, it is likely that the increase in secondary metabolite concentration of the fruit observed in this study in response to PRD, particularly of the order of magnitude measured in 1994/95, would have resulted in significant differences in wine quality had the fruit been made into wine.

The relative increase in anthocyanin concentration (per g berry mass), measured in response to PRD in the last two seasons, was similar to, or greater than, that recorded in deficit irrigation experiments with Cabernet Sauvignon (Hepner *et al.* 1985) or with other cultivars of *Vitis vinifera* (McCarthy and Staniford 1984; Matthews and Anderson

1988,1989; McCarthy *et al.*1996; McCarthy 1997). In those other studies, the size of the irrigation deficit was comparable to the difference in irrigation volume between treatments in this study. Furthermore, the PRD-induced increase per g berry weight was achieved without the 'advantage' conferred by berry weight loss in those other studies. Measurement of the amount of anthocyanin per berry over time during the ripening period has provided evidence that there was a significant difference between treatments at least two weeks before harvest which was independent of the rate of sugar accumulation.

It is difficult to compare the absolute values of anthocyanin and phenolic concentration recorded in this study with those of others with the same cultivar because different methods of extraction and measurement have been used in each case. However, it is possible to compare the values in this thesis with those derived from studies with different cultivars but with the same analytical procedure (described in Iland *et al.* 1996) at the same stage of sugar ripeness. For example, when compared at the same °Brix, the actual values of anthocyanin concentration per g berry mass over all seasons and sites in my experiments with Cabernet Sauvignon were 5 to 72% higher than for Shiraz (*Vitis vinifera*) grown in the Riverland region of South Australia (Botting *et al.* 1996); taking climatic and cultivar differences into consideration, the results are similar. Also, values of G-G (per berry mass) for Cabernet Sauvignon were comparable with those recorded for Shiraz in the Riverland where values ranged from ca. 1.5 to 3.6 μmol/g at a similar °Brix value (Botting *et al.* 1996; McCarthy 1997).

Total G-G concentration (both per berry and per g berry mass) increased in response to PRD in the only season in which this parameter was measured. In a deficit irrigation experiment with Shiraz, McCarthy (1997) found that G-G concentration of fruit from the non-irrigated treatment was significantly higher than the fully-irrigated treatment; however, there was no significant difference in 'red-free' G-G. Similarly, although 'red-free' G-G concentration increased in response to PRD, the difference was not statistically significant. This is the first report of measurement and detection of G-G in fruit or wine of Cabernet Sauvignon. The G-G assay is an estimate of the concentration of all glycosylated

secondary metabolites in the fruit and it does not quantify the individual secondary metabolites which may differ in their contribution to the flavour of wine (Williams *et al.* 1995). The importance and validity of 'red-free' G-G remains to be clarified. Nevertheless, these assays may prove to be a valuable tool for prediction of potential wine quality: recent work with Shiraz has shown a strong positive correlation between G-G concentration of berry homogenate and both wine aroma and flavour (D.G.Botting, pers. comm.).

Berry size may be important in determining the degree of extraction (dilution) of skin cell contents, which are the primary source of secondary metabolites in Cabernet Sauvignon and similar *Vitis vinifera* cultivars used for red wine production. Large berries tend to have a relatively greater solvent (pulp cell sap) to solute (skin cell sap) ratio than small berries, as a result of lower surface to volume ratio. An improvement in anthocyanin concentration (on a per berry mass basis) in response to water stress has frequently been reported from deficit irrigation studies (Smart and Coombe 1983; Williams and Matthews 1990). This response, which has been attributed to a direct effect of water status on fruit composition, is, in many cases, simply the result of an inhibition of berry expansion in response to water stress, ie. a decrease in solvent volume. It is not the result of an increase in secondary metabolite concentration on a per berry basis. However, this is not the case for all deficit irrigation studies: for example, Matthews and Anderson (1988) were able to show an increase in anthocyanin concentration per berry by expressing anthocyanin concentration on a berry surface area basis. The absence of any effect of PRD on berry size made this approach unnecessary and, therefore, the interpretation of data on secondary metabolite concentration per g berry mass is this study is not confounded by differences in berry weight, as is often the case in other experiments, eg. McCarthy (1997).

It is possible to conclude that the increase in secondary metabolite concentration per g berry mass in response to PRD was almost entirely due to an increase in concentration per berry. This distinction is important because it indicates a direct effect of PRD treatment on secondary metabolite accumulation and/or synthesis in the berry. Furthermore, once a high

concentration of secondary metabolites per berry has been achieved, by whatever means, manipulation of berry size by pruning level can be used to further increase concentration per g berry mass.

What is the explanation for the increase in concentration of secondary metabolites on a per berry basis in response to PRD? There are several possibilities: for example, it may be a response to the decrease in canopy density induced by PRD. The influence of the canopy microclimate on fruit composition has been well documented (Smart *et al.* 1990). Fruit from shaded canopies has relatively higher K concentration and pH, but reduced concentration of anthocyanins and phenolics (both per berry and per berry weight) [Smart 1992; Botting *et al.* 1996]. For Cabernet Sauvignon in particular, levels of anthocyanins and phenolics were correlated with the degree of bunch exposure in the field (Carbonneau 1985; Crippen and Morrison 1986; Morrison and Noble 1990) and experiments in controlled environments have shown that exposure of bunches to visible radiation enhances both sugar accumulation and colour development (Dokoozlian and Kliewer 1995a,b). Leaf removal in the bunch zone (Smith *et al.* 1988) or partial defoliation of the whole canopy (Hunter *et al.* 1991) between fruit-set and veraison was effective in increasing skin anthocyanin concentration of Cabernet Sauvignon. Canopy shading has been linked with undesirable 'vegetative' characters in Cabernet Sauvignon wines (Allen *et al.* 1996) and overall wine quality is lower from shaded canopies (Smart *et al.* 1990). It is difficult to separate the possible effects of leaf shading from those of bunch shading in field situations (Jackson and Lombard 1993). Anthocyanins per g berry mass were strongly correlated with canopy indices (bunch exposure index and LASA); Botting *et al.* (1996) found the same with Shiraz.

Another possibility is that the effect of PRD on fruit composition is via an effect on shoot vigour. There are very few reports indicating a direct link between shoot vigour and fruit composition, and those reports are limited to effects on TSS, TA and pH. Working with vines in a controlled environment, Jackson (1986) found a direct correlation between shoot vigour and fruit composition (acid and pH) which was independent of shading. Total K

uptake by actively growing vines is closely related to shoot vigour (Freeman and Kliewer 1983): much of this K will end up in the fruit (resulting in high must pH and reduced wine quality), particularly if there is a high proportion of shaded leaves within the canopy (Smart 1992). Indices of shoot vigour were strongly correlated with pH (Table 9.9a). Reynolds and Naylor (1994) suggested that the increase in sugar concentration in fruit of several *Vitis vinifera* cultivars grown in containers may be due to decreased lateral shoot growth with concomitant re-allocation of carbohydrate to the fruit.

There does not appear to have been any reports showing a direct link between shoot vigour and concentration of secondary metabolites in fruit. There was a strong correlation between anthocyanin concentration per g berry mass (Table 9.9b,c; Figure 9.19) and various indices of shoot vigour but this is not evidence of a causal relationship. In the past, the association between excess vigour and fruit composition has focussed on the indirect effects via the canopy microclimate; in field experiments, it is difficult to separate possible direct from indirect effects. Price *et al.* (1996) found a higher concentration of flavonol compounds in fruit of low vigour than high vigour Pinot Noir *(Vitis vinifera)* but also pointed out that low vigour vines usually have a higher proportion of well-exposed bunches.

The greatest response to PRD with respect to secondary metabolite concentration occurred in 1994/95, rather than in the following season. This may be circumstantial evidence in support of an indirect effect of PRD via an effect on canopy density because the difference between treatments with respect to canopy density (as indicated by the bunch exposure index) was relatively greater in 1994/95 than in 1995/96.

Any differences in fruit composition between treatments cannot be attributed to lower-than-optimal values of leaf area to fruit weight (LA/FW) because actual values of LA/FW for both treatments in all seasons, and at both sites, were higher than the recommended range for Cabernet Sauvignon, ie. 6 to 15 g/cm^2 (Smart and Robinson 1991; Poni *et al.* 1993).

9.5 Conclusions

a. *Partial drying of the root-zone of field vines, with alternation of the drying from one half of the root system to the other, substantially reduced vegetative growth without any decrease in fruit weight. The timing of the alternation was successful in limiting the recovery of shoot growth.*

b. *The irrigation strategy adopted in each season and at both sites resulted in a substantial reduction (up to 50%) in the amount of irrigation applied, without any detrimental effect on fruit yield.*

c. *Fruit quality was significantly improved by PRD, with concentration of secondary metabolites (anthocyanins, phenolics, glycosyl-glucose) increased by up to 45%: this was not associated with any change in berry weight.*

d. *The component of vegetative growth most responsive to PRD was lateral shoot development; other components such as main shoot length, were also affected but to a lesser extent.*

e. *The reduction in vegetative growth in response to PRD significantly reduced canopy density and increased bunch exposure.*

f. *Some of the responses of fruiting vines to PRD were typical of vines subjected to water stress, eg. reduced shoot growth rate, but many of the typical symptoms were absent, eg. there was no effect on internode elongation, berry size or* Ψ_L.

g. *Both shoot growth and stomatal conductance were reduced in response to PRD without any significant effect on shoot water status (as indicated by Ψ_L), suggesting that a non-hydraulic signal is produced by roots in drying soil which affects shoot function.*

Chapter Ten - The concentration of ABA in xylem sap increases in response to partial drying of the root system of grapevines.

10.1 Introduction

There is strong evidence from the experiments described in preceding chapters that changes in shoot growth rate and stomatal conductance of grapevines in response to partial drying of the root system are mediated by a non-hydraulic signal produced by the roots in drying soil. Furthermore, it is likely that the signal is 'positive' in nature (Davies and Zhang 1991) as opposed to 'negative', because a negative signal could not account for the observed changes in stomatal conductance and SGR.

In the last 15 years or so, a large body of evidence has accumulated to link both stomatal function and growth processes with ABA. This evidence (reviewed by Gowing *et al.* 1993; Davies *et al.* 1994; Dodd *et al.* 1996) suggests that ABA, produced by dehydrating cells, can move to the guard cells of leaves to restrict water loss and slow further dehydration; at the same time, ABA limits leaf area development by inhibiting production of new leaves and leaf expansion and sustains root growth. Originally it was thought that ABA was synthesized in the leaf mesophyll and moved to the guard cells and to growing points to exert an effect. However, recent results have provided evidence that increased xylem ABA concentration [X-ABA] was root-sourced and quantitatively sufficient to account for the physiological changes (reduction of stomatal conductance and leaf growth rate) observed in the shoots of a wide range of species droughted under controlled conditions (Loveys and Düring 1984; Zhang *et al.*1987; Zhang and Davies 1989a, 1990a,b; Tardieu *et al.* 1992a; Jackson *et al.* 1995; Liang *et al.* 1996). Similar evidence has come from field experiments with almond (Wartinger *et al.* 1990), maize (Tardieu *et al.* 1992b), *Ceanothus* (Tenhunen *et al.* 1994) and grapevine (Loveys 1984a; Loveys and Düring 1984; Loveys 1991; Correia *et al.* 1995) showing an apparent effect of xylem ABA on leaf conductance. Many reports have shown that there is a good relationship between [X-ABA] and soil water status (Zhang and Davies 1990b; Khalil and Grace 1993).

ABA can be synthesized in roots in many species (Davies and Zhang 1991). In split-root experiments, the half of the root system in drying soil contained significantly increased concentrations of ABA compared to the other (watered) half of the root system (Zhang *et al.* 1987; Khalil and Grace 1993) and concentration of ABA in roots increased with decreasing soil water content. Since these increases in root ABA took place without any change in leaf water status, it was unlikely that the roots were the recipients of ABA transported from water-stressed leaves (Jackson 1993).

Both bulk leaf ABA concentration and xylem sap ABA concentration [X-ABA] have been measured in experiments, but it is the latter which has been most strongly correlated with shoot function (Loveys 1984a; Tardieu *et al.* 1991, 1992b; Liang *et al.* 1996). Dodd *et al.* (1996) have suggested that bulk leaf ABA concentration may be a consequence, and not a cause, of chemical signalling. Sensitivity of stomata to [X-ABA] may depend on Ψ_L (Tardieu *et al.* 1993): stomatal sensitivity to the ABA signal may increase as Ψ_L decreases (Tardieu and Davies 1992; Trejo and Davies 1994). This suggests that high conductance of droughted plants early in the day can be explained by low stomatal sensitivity to a significant ABA signal; as Ψ_L decreases to its minimum value at midday (which may be no lower than that of well-watered plants), stomata are sensitised to the ABA signal (Dodd *et al.* 1996).

These results seem to confirm that ABA satisfies the criteria for a chemical root signal, ie. root-sourced, able to move from the root to the shoot and able to affect shoot function in the field.

The hypothesis to be tested in the experiments described in this chapter is *that the reduction in stomatal conductance in response to partial drying of the root system of grapevines is associated with an increase in the concentration of ABA in xylem sap.*

10.2 Materials and methods

a. Details of experiments

Experiment 8: Three-year-old *Vitis vinifera* cv. Shiraz split-root vines (approach grafts) were also used to determine the relationship between g_S and xylem sap ABA concentration [X-ABA]. All vines had four shoots per plant (lower/wet LW; upper/wet UW; upper/dry UD; lower/dry LD; see Chapter 5 for explanation of shoot nomenclature and other experimental details). Treatments were: a) both pots irrigated daily ('control'; two replicates); b) one pot not irrigated from D3 until D18, the other pot irrigated daily ('treated'; four replicates). Stomatal conductance was measured on the same four leaves per shoot every second day on average between 1030 and 1230h. On D10, because large differences in g_S between shoot types were measured in the morning, the decision was made to collect xylem sap from leaves between 1400 and 1500h, even though the air temperature was high, eg. between 38 and 40°C at the time of sampling. Leaf water potential was measured on two leaves per shoot (all shoots on 'treated', upper shoots only on 'control') and xylem sap was collected at the same time.

Experiment 9: The same plants were used as for Experiment 3 (*Vitis champini* cv. Ramsey split-root approach grafts) except they were one year older; the experimental procedure was the same as for Experiment 8. One pot of the six 'treated plants' was not watered from D1 until D25 while the other pot was watered four times per day, as were both pots of two 'control' plants. At the start of the experiment, plants had 16 mature leaves plus one lateral shoot on each of four shoots per plant. Soil water content was measured by TDR. Stomatal conductance was measured with a Delta T porometer on leaves of the upper shoots only of 'control' and 'treated' plants between 1000 and 1100h, up to and including the morning of D23; the 'control' plants were only used to provide a reference value of g_S. In the afternoon of D23, between 1300 and 1420h (air temperature 25 to 26°C), stomatal conductance was also measured on three leaves per shoot on all shoot types (as for Exp. 8)

on one selected 'treated' plant in conjunction with sampling of xylem sap for ABA analysis. Immediately after g_S was measured on each leaf, the leaf was removed, Ψ_L determined and xylem sap was sampled.

Experiment 16: Cabernet Sauvignon/Ramsey vines in the field from Exp. 14 in the 1995/96 season were used (see Chapter 9 for further details). Xylem sap samples were collected from leaves during two separate PRD periods (nos. 8 and 10; refer to Figure 9.3): these periods were chosen because mean SGR of T vines had reached its lowest level relative to C (Figure 9.7c). Also, these particular periods were chosen because the east side of the vine was the 'wet' side during these periods; prior experience had shown that maintenance of soil water content above the designated refill point was achieved more readily with the east side than with the west side (see Chapter 9 for further discussion). For xylem sap sampling, the day before the start of each PRD period was designated as Day 1, i.e January 9 and January 28 for periods 8 and 10 respectively: during period 8, xylem sap was collected on Days 2, 4, 7 and 8; during period 10, on Days 2,4 and 6 (for technical reasons, no sap collection or g_S measurement was possible after Day 6 in period 10). During period 8, xylem sap was collected both in the morning (between 0830 and 0945h) and in the afternoon (between 1345 and 1500h), but only morning collection took place during period 10. Three to four leaves per plant were used for each sample so as to obtain a total of 40 to 50 μL per sample: large, well-exposed leaves, of similar maturity to those used for gas exchange measurement, from both 'up' and 'down' shoots on the same side of the vine on each occasion, ie. east in the morning, west in the afternoon. An attempt was made to measure stomatal conductance on the same days as xylem sap collection; however, this was not always possible due to the weather conditions at the time. As a result, stomatal conductance was only measured during sunny conditions between 1030 and 1130h on Days 2,5,7 and 9 (period 8) and Days 2 and 6 (period 10); also, Day 6 in period 8 was not used for either stomatal conductance measurement or sampling of xylem sap due to the very high temperature (maximum = 43°C; Figure 10.2) on that day. Ψ_L was measured concurrently with xylem sap collection.

b. Collection of xylem sap sample

ABA concentration was measured in xylem sap rather than in leaf tissue for the reasons outlined in 10.1. A Scholander pressure chamber was used to express xylem sap from leaves through the cut end of the petiole. Leaves were placed into the chamber within 30 seconds of collection. After determination of the balancing pressure, one sample was collected from each leaf by applying an over-pressure of 0.3 MPa or less. Sap was collected with a micro-pipette and placed into pre-weighed, one mL Eppendorf vials. The vials were kept in ice before and after sap collection and light was excluded (except for a brief period during weighing). Vials were weighed and subsequently stored in a -50°C freezer for three weeks before ABA analysis.

c. ABA analysis

Samples of expressed sap were assayed for ABA without any prior purification using stable isotope dilution analyis (Loveys and van Dijk 1988). Twenty ng of [$^2H_3$]ABA standard (200μL of 0.1μg/mL) was mixed thoroughly with the thawed xylem sap sample in the Eppendorf vial used for storage and the mixture was dried in a Speedivac vacuum centrifuge for ca. 30 minutes. The methylating agent ethereal diazomethane (200μL) was added and the tubes were covered with foil for 20 minutes or so until the yellow colour had disappeared; the foil was then removed and the samples were dried in a fume-hood. Methanol (100μL) was added, the mixture transferred to a new Eppendorf tube and centrifuged. The supernatant was transferred to gas chromatography (GC) vial glass inserts and dried in a Speedivac. Methanol (20μL) was added and the sample was analysed by gas chromatography/mass spectrometry using selected ion monitoring. Ions monitored were m/z 190 (base peak), 193, 162, 165. Quantification was by interpolation from a previously constructed calibration curve relating peak area ratio (m/z 190/193) to mass ratio (ABA/[$^2H_3$]ABA).

10.3 Results

Experiment 8: Stomatal conductance of LD and UD ('dry') shoots was significantly less than that of control and LW, but not UW shoots. Also, g_S of UW shoots was significantly less than LW (Table 10.1). Ψ_L of LD shoots was significantly higher than LW. [X-ABA] was higher for 'dry' shoots but only statistically significant for the difference between LD and the 'wet' shoots; there was no difference between LW and UW, nor between 'dry' shoots and control.

Table 10.1 Effect of half-drying on stomatal conductance (g_S, mean ± se, mmol/m^2/s), leaf water potential (Ψ_L, MPa) and xylem sap ABA concentration ([X-ABA], mean ± se, nmol/mL) of different shoot types on a single split-root ('treated') plant on D10 ('dry' pot not watered from D3); Shiraz, Exp.8. Refer to text for explanation of shoot types.

Shoot type	g_S	Ψ_L	[X-ABA]
Treated			
LD	349 ± 57	-1.02	0.75 ± 0.18
UD	349 ± 48	-1.12	0.41 ± 0.12
UW	485 ± 38	-1.15	0.23 ± 0.03
LW	753 ± 53	-1.23	0.25 ± 0.13
Control[a]	626 ± 49	-1.17	0.34 ± 0.10
Significant diff.	LD, UD & LW***; LD, UD & Con**; UW & LW*	LD and LW*	LD and UW*; LD and LW*

[a] Mean of upper shoots only

There was a strong negative curvilinear relationship between g_S and [X-ABA] when values for individual leaves on all shoot types of 'treated' plants were pooled: $g_S = 5118.4[\text{X-ABA}]^{-0.539}$; $r^2 = 0.61$*** (Figure 10.1).

Experiment 9: Soil water content of the 'dry' pot of 'treated' plants decreased to 6.6% by D9 and remained at that level for the remainder of the experiment; SWC of the wet pots of both 'treated' and 'control' plants was ca. 22% over the same period. By D18, mean g_S of

'treated' shoots was 17% less than 'control' ('up' shoots only for both); five days later, 'treated' plants had partially recovered relative to the 'control' (11% lower g_S for

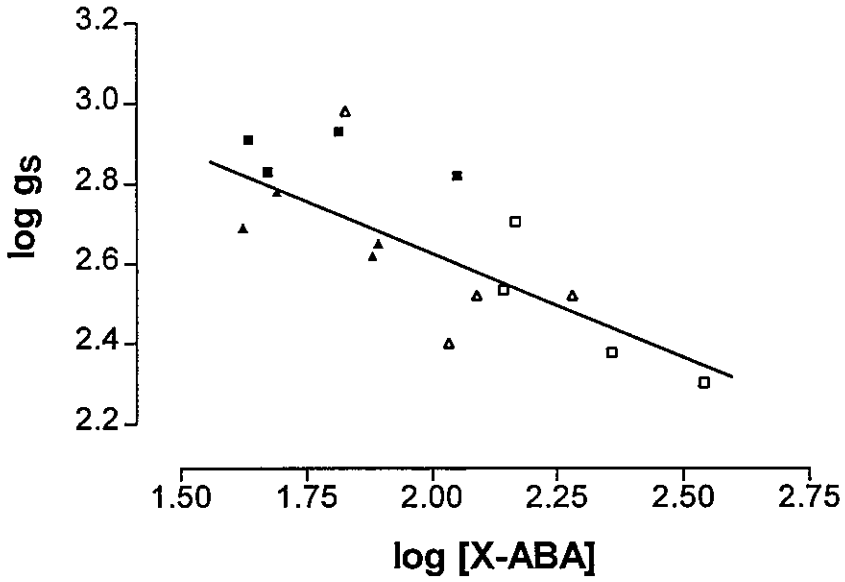


Figure 10.1 Relationship between stomatal conductance (g_S, mmol/m^2/s) and xylem sap ABA concentration ([X-ABA], nmol/mL) of four shoot types of half-dried Shiraz on D10 (code for symbols: upper shoots = triangles, lower = squares; wet shoots = solid, dry = open); Exp. 8. One pot not irrigated from D3 to D18. Regression line calculated for log/log plot of pooled values of all shoot types.

'treated'). On D23, the 'treated' plant with the lowest mean g_S relative to the 'control' (-29%) was selected for measurement of [X-ABA]. Stomatal conductance and [X-ABA] of the 'dry' shoots was lower and higher respectively than 'wet' shoots but only statistically significant for the comparison between LD and LW shoots (Table 10.2). There was a negative correlation ($r = -0.6$, $p<0.05$) between [X-ABA] and g_S when leaves from all shoot types were pooled. There was no difference between shoot types for Ψ_L.

Table 10.2 Effect of half-drying on stomatal conductance (g_S, shoot mean, mmol/m^2/s), leaf water potential (Ψ_L, shoot mean, MPa) and xylem sap ABA concentration ([X-ABA], shoot mean ± se, nmol/mL) of different shoot types on a single split-root ('treated') plant on D23 ('dry' pot not watered from D1); Ramsey, Exp. 9.

Shoot type	g_S	Ψ_L	[X-ABA]
LD	283*	-0.84	0.50* ± 0.12
UD	293	-0.87	0.45 ± 0.14
UW	370	-0.85	0.35 ± 0.10
LW	503*	-1.02	0.20* ± 0.01

* Significantly different ($p<0.05$)

Experiment 16: Maximum air temperature ranged from 23 to 43°C during period 8 (Figure 10.2) and from 19 to 34°C during period 10; however, the temperature was lower during measurements of stomatal conductance in period 8, ie. 21 to 30 °C. Photon flux density was sufficiently high to permit full stomatal opening on every occasion when g_S was measured.

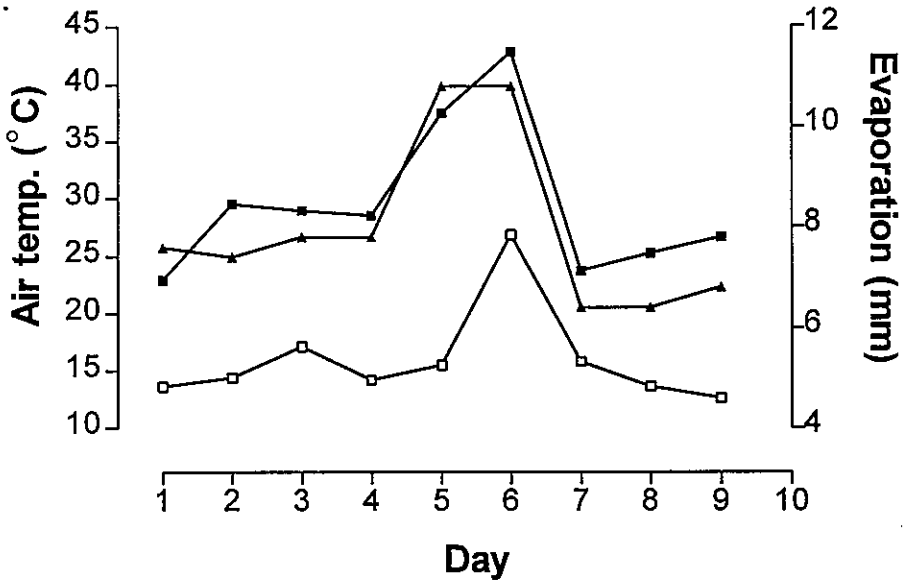


Figure 10.2 Maximum (■) and minimum (□) air temperature (°C) and class A pan evaporation (mm/d, ▲) during PRD period 8; Exp.16, Cabernet Sauvignon/Ramsey, Waite, 1995/96.

'Control' g_S averaged 235 ± 17 mmol/m^2/s from Day 2 to 9 inclusively in PRD period 8. [X-ABA] of 'control' vines remained relatively constant during PRD period 8 whereas [X-ABA] of 'treated vines increased from Day 2 to Day 4 and declined thereafter (Figure 10.3). Although the concentration was higher in the afternoon than the morning on each occasion, the changes over time in response to treatment were similar and, as a consequence, mean [X-ABA] per day was used in subsequent analyses.

Soil water content (15-55cm depth summed, maximum value per day) on the non-irrigated ('dry') side of the treated vines decreased from ca. 120mm on Day 1 to level off at ca. 85mm on Days 5 to 6 during both PRD periods (Figure 10.4a,b). For sensors at 20cm and

40cm depth, SWC levelled off at 22mm (=22% v/v) and 19mm (=19% v/v) respectively. At the same time, SWC (15-55cm depth summed, maximum value per day) on the 'wet'

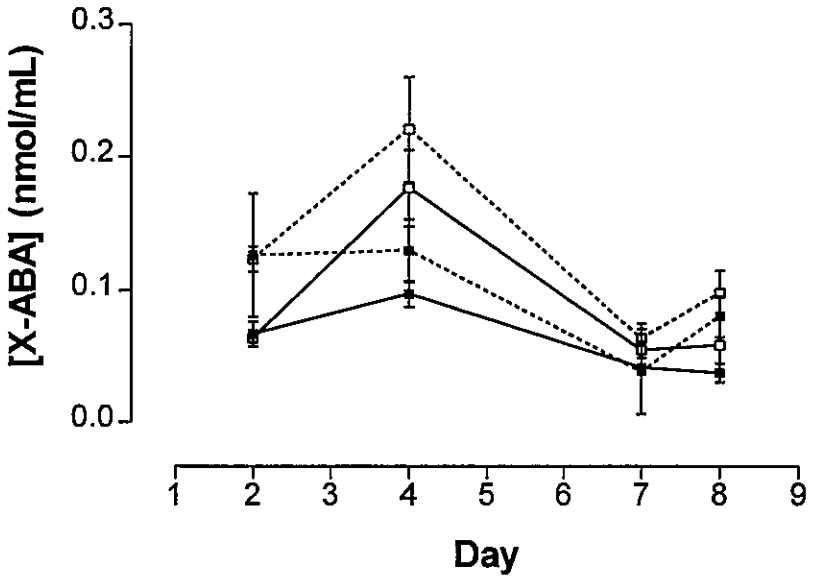


Figure 10.3 Effect of partial drying on xylem sap ABA concentration ([X-ABA], mean ± se, nmol/mL) during PRD period 8; Exp.16, Cabernet Sauvignon/Ramsey, Waite, 1995/96. PRD period started on Day 2. Both sides of vine irrigated (■); partial drying (□). Sap collected in morning (solid line) and afternoon (dotted line).

side was maintained between 120 and 140mm in period 8, and between 107 and 137mm in period 10. The relative increase of 'treated' [X-ABA] during the initial part of the PRD periods coincided with actual decrease in soil water content on the 'dry' side of the vine and relative decrease of stomatal conductance (Figure 10.4a,b). In period 8 (Figure 10.4a), the decrease of 'treated' [X-ABA] relative to the 'control' from Day 4 coincided with the relative recovery of g_S after Day 5 and slowing of the rate of soil drying (minimum level of SWC in the root-zone on the 'dry' side was reached ca. Day 5).

There was a strong negative linear relationship between g_S and [X-ABA] (mean per day) of individual vines on Days 4 to 5 in period 8, at the time when mean g_S and [X-ABA] of 'treated' vines (relative to 'control') reached their lowest and highest levels respectively (Figure 10.5). Similarly, there was a significant negative correlation ($r = -0.40$; $p<0.05$) between g_S and [X-ABA] (am) from Day 2 to Day 9 inclusively in period 8 (individual

vine values of both treatments pooled; Day 4 [X-ABA] paired with Day 5 g_S, Day 8 [X-ABA] paired with Day 9 g_S).

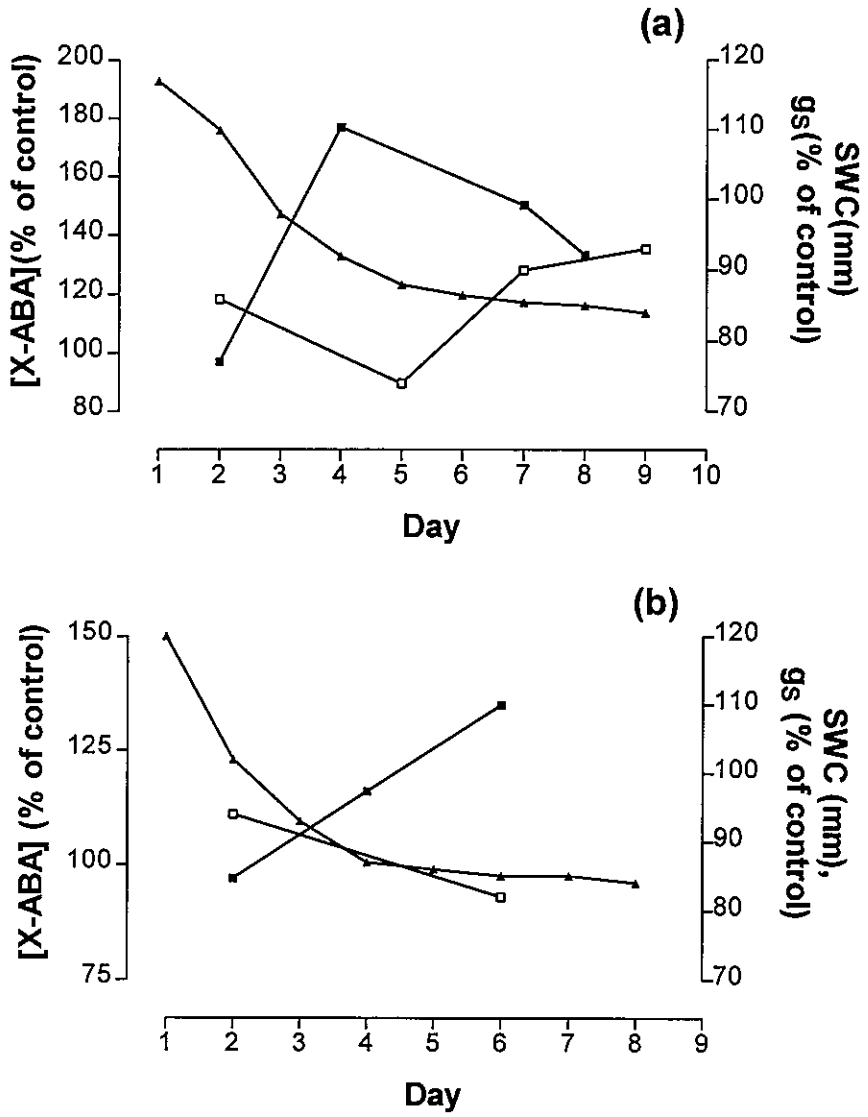


Figure 10.4 Effect of partial drying on leaf xylem sap concentration ([X-ABA], mean of am and pm samples, ■) and stomatal conductance (g_S, □): 'treated' (partial drying) as % of 'control' (both sides of vine irrigated); Exp.16, Cabernet Sauvignon/Ramsey, Waite, 1995/96. Volumetric soil water content (SWC, maximum value per day, mm, ▲) of 'dry' side of vine, 15-55cm depth summed (see Figure 9.3 for complete data set). PRD periods started on Day 2.

a. PRD period 8
b. PRD period 10

There was no statistically significant difference between treatments for Ψ_L on any occasion; however, mean Ψ_L of 'treated' vines measured in the morning over both periods averaged 0.06 MPa less than 'control' (with no difference in the afternoon in period 8).

There was no correlation between g_S and Ψ_L (both measured in the morning) during either period.

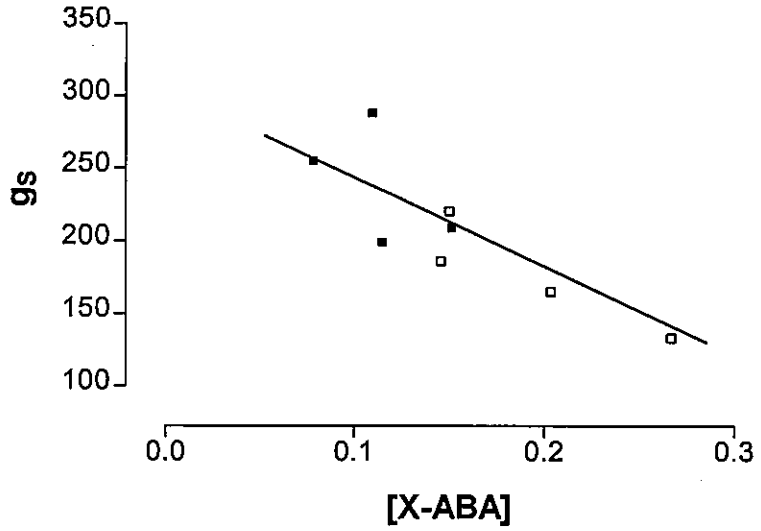


Figure 10.5 Relationship between stomatal conductance (g_S, mean per vine, am, Day 5, mmol/m^2/s) and leaf xylem sap concentration ([X-ABA], mean per vine of am and pm on Day 4, nmol/mL) of 'control' (■) and 'treated' (partial drying, □) during PRD period 8; Exp.16, Cabernet Sauvignon/Ramsey, Waite, 1995/96. PRD period started on Day 2. Regression line calculated for both treatments pooled: g_S = -702[X-ABA] + 314; r^2 = 0.72***.

10.4 Discussion

Pot experiments: The differences in stomatal conductance between shoot types in Exps. 8 and 9 cannot be explained in terms of shoot water status (see Chapter 5.4 for further discussion). The correlation between g_S and [X-ABA] provides circumstantial evidence in support of the hypothesis that ABA is produced by drying roots and is transported to the shoots where it exerts an effect on shoot function. This is further supported by the observation that g_S and [X-ABA] of 'dry' shoots of approach-grafted, split-root plants tended to be lower and higher respectively than 'wet' shoots in Exps. 3 (only g_S measured; see Chapter 4), 8 and 9. This difference between 'wet' and 'dry' shoots may be explained in terms of an incomplete passage of the signal from the 'dry' trunk to the 'wet' trunk across the graft union, perhaps due to some characteristic of the approach-graft union; however, in the absence of information on the anatomy of approach-graft unions

(Hartmann and Kester 1983), it is difficult to support this explanation. Also, the fact that the movement of water from the 'wet' trunk to the 'dry' trunk across the graft union does not appear to be impaired (Exp. 11, Chapter 5) casts further doubt on this proposition. An alternative explanation may be that the passage of ABA across the graft union is unimpaired, but is subsequently diluted by the relatively strong transpiration stream in the 'wet' trunk during the day; thus [X-ABA] is lower in the 'wet' trunk (and shoots) than in the 'dry'. [X-ABA] may vary not only in response to changes in the amount of ABA entering the transpiration stream but also in response to changes in sap flow rate through dilution (Jackson 1993).

There were no significant differences between upper and lower 'dry' shoots with respect to either g_S or [X-ABA]. However, there were differences between upper and lower 'wet' shoots for both variables, but these were not consistent in all respects. For example, in Exp. 8, g_S of the LW shoot (of the half-dried treatment) was higher than that of the UW shoot over the whole course of the experiment, and relative to the control (watered on both sides), the LW shoot did not appear to respond at all to half-drying. By comparison, the UW shoot responded in concert with the 'dry' shoots, but to a lesser degree (Figure 5.7). However, at the time of the greatest difference in g_S between UW and LW shoots, there was no difference in [X-ABA] (Table 10.1). On the other hand, in Exp. 9, lower g_S of the UW shoot was associated with a higher [X-ABA] (Table 10.2). It is likely that the differences between the conditions under which these experiments were conducted may account for the apparent differences in [X-ABA];. For example, it is possible that the less-than-ideal atmospheric conditions under which both g_S was measured and the xylem sap collected in Exp. 8 may have resulted in the large degree of variation between plants for the same shoot type. In the case of Exp. 9, individual plant variation was avoided by sampling only from one plant; however, there was still a relatively large degree of variation between leaves on the same shoot. Also, g_S was only measured in the morning in Exp. 8 and it is possible that the difference between UW and LW shoots at that time was not indicative of the situation in the afternoon when xylem sap was sampled, particularly under the atmospheric conditions prevailing at the time; by comparison, in Exp. 9, g_S was

measured concurrently with collection of xylem sap. These results are indicative of the difficulties associated with the explanation of long-term physiological responses on the basis of instantaneous measurements of plant hormones, even if such measurements are conducted at what appear to be strategic times, and thus emphasise the importance of temporal studies (as in Exp. 16).

Field experiment: [X-ABA] was higher in the afternoon than the morning in Exp.16. This was a similar result to Loveys (1984a) and Correia *et al.* (1995) for well-watered and droughted grapevines respectively in the field, but differs from field studies with other species which have tended to show a lack of diurnal variation in [X-ABA] (Wartinger *et al.* 1990; Tardieu *et al.* 1992a). There have been relatively few reports of actual values of [X-ABA] for grapevines: for field-grown vines (*Vitis vinifera*), Loveys (1984a) reported values ranging from 0.15 to 0.50 nmol/mL while Correia *et al.* (1995) reported values of 0.57 to 0.91nmol/mL in the morning, and 0.57 to 1.79 nmol/mL in the afternoon [these are values before correction for symplast contamination: corrected values are generally 30 to 40% lower]. The values of both potted and field vines in this study are lower than those of Correia *et al.* (1995), but very similar to those reported by Loveys (1984a) for grapevines and by Khalil and Grace (1993) for half-dried sycamore grown in a controlled environment, ie. ca. 0.2 to 0.4 nmol/mL (sap collected at 'midday'). However, it may not be valid to compare actual values of [X-ABA] between different experiments because shoot function may respond to the flux of ABA rather than [X-ABA], and flux is dependent on both [X-ABA] and flow rate of the transpiration stream.

The fact that actual [X-ABA] of 'treated' vines increased from Day 2 to Day 4 while control [X-ABA] remained relatively constant is important because it suggests that there was a real increase in ABA synthesis in 'dry' roots, ie. the relative increase of 'treated' [X-ABA] was not due to an actual decrease of [X-ABA] of control from some other cause. Because average daily [X-ABA] has been shown to set the maximum g_S achieved during that day (Wartinger *et al.* 1990; Correia *et al.* 1995), for period 8, this was plotted over time together with g_S (measured in the morning) and SWC (Figure 10.4a) ['treated' as a %

of 'control' for both [X-ABA] and g_S, actual values for SWC]. The changes in these variables over time shown in Figure 10.4 are very similar to the results of Khalil and Grace (1993) for half-dried sycamore, but grown in a controlled environment (Figure 10.6 shows their original data replotted on the same time scale and 'treated' calculated as a % of 'control'). In both cases, the increase in [X-ABA] either preceded or coincided with the

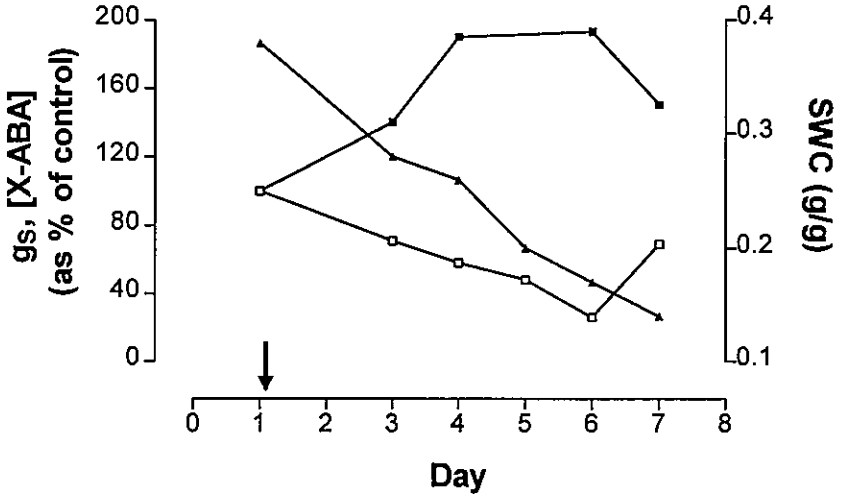


Figure 10.6. Effect of half-drying on xylem sap concentration ([X-ABA],) and stomatal conductance (g_S, ≤) of split-root sycamore: 'treated' (one pot irrigated) as % of 'control' (both pots irrigated). Gravimetric soil water content (SWC, g/g, ▲) of 'dry' pot; drying started on Day 1(arrow). This figure is entirely based on both actual and reworked data interpolated from figures in Khalil and Grace (1993).

decrease in stomatal conductance, and changes for both variables occurred as part of the root system dried over a similar time period. Furthermore, recovery of g_S coincided with the decrease in [X-ABA] after ca. five or six days of partial drying in both cases. It is unfortunate that no further samples were collected or g_S measurements were made after Day 6 in period 10 because it is possible that decrease of [X-ABA] and/or recovery of g_S may have taken place soon after Day 6. The high degree of similarity between these two experiments is surprising when one considers that ABA can interact with a large number of environmental variables to influence the effect on shoot function (Dodd *et al.* 1996); for example, leaf growth responses to ABA are temperature dependent (Dodd and Davies 1994). Khalil and Grace (1993) found strong negative correlations between SWC of the 'dried' pot and both g_S and [X-ABA] of half-dried plants during the initial drying period. Due to insufficient number of samples/measurements over this period, it was not possible

to calculate these correlations for the field experiment, but it is likely that the relationship was the same.

Relationship between stomatal conductance and [X-ABA]: Stomatal conductance and [X-ABA] of partially-dried grapevines were negatively correlated: this suggests that xylem sap ABA concentration has an effect on stomatal behaviour that is independent of shoot water status. [X-ABA] has been shown to correlate with g_S in experiments with the grapevine (Loveys 1984a,1991; Correia *et al.* 1995), other woody species (Fusseder *et al.* 1992; Khalil and Grace 1993; Jackson *et al.* 1995; Liang *et al.* 1996) and non-woody species (Zhang and Davies 1990a; Jokhan *et al.* 1996) where plants have been exposed to varying degrees of soil drying. Not only has this relationship been demonstrated under controlled conditions but also in the field despite plant-to plant variation, different soil conditions and changes over the drying cycle. Therefore, it seems reasonable to propose that g_S of partially-dried grapevines is regulated by xylem sap [ABA]. That such a relationship exists is not surprising because xylem sap appears to be directly in contact with the apoplast surrounding the guard cells (Dodd *et al.* 1996). By comparison, other studies have shown a relatively poor correlation between g_S and bulk leaf ABA (Khalil and Grace 1993; Dodd *et al.* 1996); this suggests that bulk leaf ABA is not a sensitive indicator of soil drying and this may be explained by the fact that most of the leaf ABA is isolated in the mesophyll away from the apoplast around the guard cells.

Correlations between ABA and shoot function are not sufficient to ascribe cause and effect (Tardieu and Davies 1993). Therefore, as Dodd *et al.* (1996) emphasized, experiments which follow the time-course of changes in function and growth regulators are critical to demonstrate that changes in the concentration of the regulator precede the physiological or developmental response of interest, or that the two variables change simultaneously. This criterion was satisfied in the field experiment.

Most studies on the response of [X-ABA] to soil drying have compared fully-droughted plants with well-watered controls and it may not be valid to compare results from these with studies involving partially-dried plants for several reasons:

a) Droughted plants typically have substantially lower transpiration rates than well-watered plants, particularly from midday onwards, which may affect the flux of ABA to leaves. On the other hand, transpiration rates of partially-dried plants are only slightly reduced relative to controls (Chapters 4,5).

b) Droughted plants typically have lower Ψ_L than controls whereas shoot water status does not appear to change in response to partial drying. This difference may be important because Tardieu and Davies (1992) and Trejo and Davies (1994) have shown that stomatal sensitivity to an ABA signal may increase as Ψ_L decreases. They have suggested that the high g_S of droughted plants early in the day, despite a relatively high [X-ABA], may be explained by a reduced sensitivity of stomata to the high [X-ABA] when Ψ_L is high. As Ψ_L declines to a minimum value at approximately midday, stomata are sensitised to the ABA signal (Davies *et al.* 1994) and low g_S is associated with high [X-ABA]. Although this phenomenon may explain the relationship between g_S and [X-ABA] of droughted plants on a diurnal basis, it cannot be invoked to explain the apparent anomalies in the same relationship between half-dried plants and controls, nor between UW and LW shoots in Exp. 8 (ie. same g_S but different [X-ABA]). Furthermore, Correia *et al.* (1995) were unable to account for the afternoon closure of stomata, typically observed in field-grown grapevines, in terms of an interaction between Ψ_L and [X-ABA].

c) After severe drought, stomata may remain closed after [X-ABA] has decreased (Wartinger *et al.*1990). By comparison, stomata of partially-dried plants appear to respond rapidly to decreased [X-ABA]: recovery of shoot function, with or without rehydration of the dried portion of the root system, occurs at a faster rate than for fully-droughted plants (Chapters 4,5). Correia *et al.* (1995) have suggested that, after prolonged exposure to drought, the relationship between [X-ABA] and g_S may be over-ridden by other factors which are more persistent and less reversible.

The potential source of ABA in xylem sap: Increased [X-ABA] in response to partial drying could have been the result of increased ABA synthesis in leaves and/or roots. Drying roots are known to produce ABA (Robertson *et al.* 1990; Davies and Zhang 1991) but there is also the possibility that increased [X-ABA] in response to soil drying is the consequence of a redistribution of ABA as a result of perturbed water relations in older leaves (Zhang and Davies 1989b). Loveys (1984b) showed that ABA could be transported from leaves to roots in well-watered grapevines grown under controlled conditions. However, for several reasons, the evidence weighs more strongly in support of root-sourced ABA in the experiments in this study. For a start, there was no significant change in shoot water status in response to partial drying and thus no stimulus for ABA synthesis in leaves. Secondly, an increase in [ABA] in drying roots may precede the decrease of g_S of droughted plants and a tight relationship may exist between root [ABA] and soil water content over a drying cycle (Zhang and Davies 1989a). Thirdly, in split-root experiments with other species, the half of the root system in drying soil contained significantly increased concentrations of ABA compared to the other (watered) half of the root system, and the increase in root [ABA] either preceded or occurred concurrently with decreased g_S (Zhang *et al.* 1987; Khalil and Grace 1993). The concentration of ABA in roots was not measured in the experiments described in this thesis. However, in a related study with split-root Sultana (*Vitis vinifera)* grapevines grown in pots, concentration of ABA (nmol/g fresh weight) in the 'dry' roots of half-dried plants increased five-fold coincidentally with the decrease in SWC and decrease in g_S; furthermore, g_S recovered after SWC of the 'dry' pot had reached its minimum level, coincidentally with a decrease in root ABA concentration (Dry *et al.* 1996; B.R.Loveys and P.R.Dry, unpublished data). Therefore, it is likely that the significant increases in [X-ABA] of partially-dried grapevines are caused by enhanced synthesis of ABA in drying roots.

Does [X-ABA] measured in pressurized sap accurately represent the concentration of ABA in the transpiration stream?: [X-ABA] of partially-dried and fully-droughted plants has been shown to be negatively correlated with g_S. However, it has been difficult to establish whether [ABA] in the xylem sap is a true measure of the concentration in the transpiration

stream, ie. [X-ABA] might not reflect the true concentration in the xylem vessels before destructive sampling (Dodd *et al.* 1996). The Scholander pressure chamber has been widely used for collection of xylem sap from leaves and shoots of intact plants for determination of ABA concentration; however, there have been concerns that application of pressure to the leaf or shoot may force symplastic sap into the xylem vessels thereby changing the concentration of ABA in xylem sap (Dodd *et al.* 1996). If the expressed sap shows the same [ABA] at various pressures, then it is likely that such contamination has been minimal; this has been shown for woody almond twigs (Wartinger *et al.* 1990) and large maize leaves (Dodd *et al.* 1996). In the latter case, over-pressure of up to 1.0 MPa had no effect on [X-ABA]. Evidence of contamination of the sample by cell sap (crushed cells or phloem exudation) has been obtained by analysing the sample for sugars in species such as the grapevine where sugar concentration in xylem sap is close to zero after shoot growth has commenced (Correia *et al.* 1995): in this way an estimate of [X-ABA] at zero sucrose can be obtained. In this present study, the following steps were taken to minimise potential contamination. Firstly, large leaves were sampled (as recommended by Dodd *et al.* 1996); this was more readily achieved with field vines than with potted. Secondly, over-pressurizing was minimised and only one over-pressure step was used for each leaf.

Does [X-ABA] indicate the actual amount of ABA moving from roots to shoots?: Increases in xylem sap concentration associated with soil drying have been interpreted as a measure of increased ABA passing to the shoots from drying roots. However, this approach may be misleading for several reasons: for example, as previously discussed, the sap sampled may not always reflect the true concentration in the xylem vessels before destructive sampling. Furthermore, [X-ABA] can vary, not only in response to changes in the amount of ABA entering the transpiration stream from the roots, but also in response to changes in sap flow rate. For example, increased transpiration rate will have a diluting effect. To overcome the problem of different sap flows in droughted and control plants, Jokhan *et al.* (1996) calculated ABA delivery rates in terms of μmol/s at the same notional sap flow rate: droughting for 24h or more increased ABA delivery from roots to shoots by five-fold. There is a further problem of interpretation of [X-ABA] in that it does not necessarily

indicate the amount of ABA which passes from the shoot xylem to the site of action, eg. guard cells, meristematic tissue. Flux is the concentration multiplied by the flow rate of the transpiration stream. However, the results of correlative studies reported in this chapter suggest that stomata, at least, respond to changes in [X-ABA]. Furthermore, Trejo *et al.* (1995) were able to change ABA flux at a given concentration of endogenous ABA by manipulating temperature and VPD. At a given concentration, a three-fold variation in flux had no effect on g_S, while the same flux achieved by altering the ABA concentration was able to decrease g_S further. Working with detached leaves, Gowing *et al.* (1993) found that the relationship between g_S and [X-ABA] was much closer than that between g_S and ABA flux. Dodd *et al.* (1996) state that once the stomata are open, then variation in flux will have little influence on stomatal behaviour. These and other results suggest that measurement of [X-ABA] is a useful indicator of soil water deficit, despite the potential problems with interpretation of data .

The relationship between [X-ABA] and shoot growth: The relationship between [X-ABA] and shoot growth over time was not directly examined in this study because shoot growth had almost ceased in 'treated' vines when the field experiment was conducted. However, a strong correlation between stomatal conductance and shoot growth rate has been consistently demonstrated in the experiments described in this thesis: in particular, changes in SGR over time in response to partial drying take place in concert with changes in g_S. Therefore, it is likely that shoot growth also responds to changes in [X-ABA]. ABA has been shown to have an inhibitory effect on leaf growth (van Volkenburgh and Davies 1983) and leaf growth rate was negatively correlated with endogenous ABA accumulation in leaves (Puliga *et al.* 1996). Application of ABA externally by root-feeding (Zhang and Davies 1990a) or stem injection (Tardieu *et al.* 1993) have demonstrated relationships between g_S, leaf growth and [X-ABA] that were closely comparable with those caused by roots in drying soil. In order to relate changes in shoot growth over a drying cycle, it would be appropriate to measure ABA content in cells that are actually growing. This has been attempted with the elongation zone of grasses; however, there are problems with this analysis (Dodd *et al.* 1996) and growth reductions of up to 35%, induced by soil drying,

have not been associated with any significant accumulation of ABA in the elongation zone (Dodd and Davies 1996). There do not appear to have been any reports on the measurement of ABA in the elongation zone in the field nor in the meristematic regions of non-grass species.

There is also the possibility that [X-ABA] affects shoot growth indirectly via an effect on whole-plant assimilation. Changes in Pn in response to half-drying were closely correlated with changes in g_S (Chapters 4,5) and it is likely that stomatal closure fully accounts for the effect of partial drying on Pn. Non-stomatal limitation of photosynthesis is more likely as a consequence of severe stress conditions (Düring and Loveys 1996). A reduction in carbon fixation on a whole plant basis in response to PRD may occur as a result of both a reduction in average Pn per unit leaf area and a reduction in leaf area per plant.

The relationship between soil water status and ABA: An average soil matric potential (Ψ_m) of the root-zone may be estimated from volumetric SWC by determination of water retention curves for samples of soil from different depths in the profile. However, the validity of this estimation may be confounded by errors associated with the various steps of the technique: these include the variability of the soil samples taken from the field, the accuracy of the water retention curve, and the interpolation of Ψ_m values from the curve. Furthermore, an average Ψ_m is often estimated for the whole root-zone by interpolation and root distribution is not taken into consideration (Stevens *et al.* 1995). Nevertheless, estimation of Ψ_m allows a comparison of the degree of soil water stress between experiments where different soil types have been used; but, for the reasons outlined, such comparisons should be made with caution.

In period 8 of Exp. 16, [X-ABA] and g_S of 'treated' vines increased and decreased respectively, relative to the 'controls', from Day 2 to Day 4. At the same time, volumetric SWC on the 'dry' side decreased from 29.0 to 23.0% at 20cm depth, and 23.5 to 19.8% at 40cm depth (measured by the capacitance probe sensors at those depths): this corresponds to a decrease of Ψ_m from -10kPa in both cases to -68 and -35kPa for 20 and 40cm

respectively, based on the soil water retention curves calculated for soil samples at those depths (Appendix V). It is likely that Ψ_m of the soil from 0 to 20cm depth (where roots are relatively plentiful as a result of drip irrigation) would have been lower than -100 kPa by Day 4. There are relatively few split-root experiments where Ψ_m has been measured: for example, Ebel *et al.* (1994) found that leaf growth of half-dried, split-root sorghum, grown under controlled conditions, was not significantly different to controls until the average Ψ_m in the 'dry' pot had decreased to between -10 and -100 kPa. Therefore, this is similar to the results from experiments with both field-grown, fruiting Cabernet Sauvignon vines (described in this chapter) and with potted, non-fruiting Shiraz vines (Chapter 5). In Exp. 13 (Chapter 8), SGR and g_S of non-fruiting vines appeared to respond (ie. 'treated' significantly less than 'control') when the SWC (0-50cm) of the 'dry' side of the vine had decreased to 19%: this corresponds to -354 and -78kPa at 20 and 40cm depths respectively. However, because the SWC measured for 0 to 50cm may not have been representative of the actual SWC at those depths, it is difficult to compare the results of Exps. 13 and 16.

There have been relatively few reports of experiments where there has been an attempt to determine a correlation between [ABA] of xylem sap or tissues and soil water status. Zhang and Davies (1989a) and Khalil and Grace (1993) found a strong negative correlation between root [ABA] and soil water content. However, it is likely that roots respond to changes in soil water potential rather than bulk water content: Zhang and Davies (1989a) found that as Ψ_m around maize roots decreased to -200 to -300 kPa, partial dehydration stimulated ABA production in those roots. It is possible that the Ψ_m of the surface layer of soil, eg. 0-20cm, may decrease to this level while the deeper layers of the root-zone are still at much higher values. Such relatively 'mild' drying of the surface roots may be sufficient to cause an increase in [X-ABA] and consequent change in shoot function.

Recovery of stomatal conductance and decrease in [X-ABA] in period 8 of Exp. 16 after Day 4 coincided with no further decrease in SWC on the 'dry' side: at 20 and 40cm soil depth, this corresponded to 22 and 19% volumetric SWC respectively, ie. soil matric potentials of -100 and -77 kPa respectively (Appendix V). Similarly, SWC of the dry pot

of half-dried plants (Chapters 4, 5, 7) did not decrease to less than 7%, ie. a soil matric potential of ca. -100kPa. This means that shoot function recovered when the soil of the 'dry' part of the root-zone had a relatively high matric potential, and before the plant-available water (ie. between -10 and -1500kPa) had been fully extracted by the 'dry' roots.

The evidence for the involvement of ABA in the response of grapevine shoot function to partial drying of the root system: In isolation, the results from the experiments reported in this chapter are not sufficient evidence to support an unequivocal case for the involvement of ABA in the response of grapevine shoot function to partial drying of the root system. However, these data should be considered together with the results from other studies. Jackson (1987) has proposed criteria for testing the physiological significance of hormone effects [reviewed by Dodd *et al.* (1996) in the context of ABA]. *Correlation* and *duplication* may indicate a potential regulatory role: in the case of the grapevine, these criteria have been met by the results of this and other studies. For example, Loveys (1984a) found that stomata of field-grown grapevine leaves responded to the application of synthetic ABA (supplied to the transpiration stream) at a concentration equivalent to that in xylem sap. Similarly, Correia *et al.* (1995) found that the introduction of ABA to the transpiration stream of detached leaves from well-watered grapevines mimicked the response obtained with a soil water deficit. *Deletion* and *reinstatement* are said to be more certain criteria to test the specificity of hormone action, achieved by manipulation of endogenous hormone levels. *Deletion* has been achieved in various ways by genetic (use of ABA-deficient mutants), chemical (use of inhibitors of ABA synthesis) or other means. For example, although ABA was not measured, it could be argued that the excision of drying roots in the split-root experiment of Gowing *et al.* (1990) removed the putative source of extra ABA. The *reinstatement* criterion is usually satisfied by external ABA treatment. The most specific type of *deletion* and *reinstatement* experiment is the use of a technique to remove ABA from xylem sap but the results from such experiments have been criticised on several grounds by Dodd *et al.* (1996). There do not appear to have been any *deletion* and *reinstatement* experiments conducted with the grapevine but the results of

experiments with other species tend to support the case for ABA as a signal produced by grapevine roots in response to changes in soil water status.

As noted in previous chapters, the initial decrease in g_S in response to drying of the soil around part of the root system, and the subsequent recovery of g_S when the rate of soil drying slows substantially, provides evidence in favour of 'positive' signal produced by drying roots rather than a 'negative' signal. Inhibitors such as ABA satisfy the criteria for a positive signal (Zhang and Davies 1991) because stomatal closure depends on an increased amount of inhibitor in the transpiration stream. When there is no further decrease in soil water content of the 'dried' part of the root-zone, the amount of inhibitor decreases and stomata open. A chemical signal such as ABA, as opposed to an hydraulic signal, provides a sensitive means of detecting small changes in water status of the soil in the vicinity of each individual root tip. ABA can be synthesised by root tips in response to loss of turgor (Robertson *et al.* 1990; Zhang and Davies 1987) and the turgor of root tips in drying soil may decrease while root tips on the same plant in wet soil will retain turgor (Davies *et al.* 1986; Zhang and Davies 1989b). As increasing numbers of root tips encounter drying soil, the intensity of a chemical signal would be expected to increase and this has been shown to be the case for [X-ABA] in the experiments described in this chapter and in other studies.

The evidence in favour of ABA in the regulation of shoot function appears to be relatively strong; however, the possible involvement of other anti-transpirant and growth-limiting factors (chemical or not), acting in concert with ABA or independently, cannot be ignored. There is a need for more research to determine if [X-ABA] truly indicates the 'strength' of the signal arriving at the site of action. Also, does the sensitivity of the tissue change in response to changes in [X-ABA], and how do the stomata and the sites of growth activity actually 'read' the ABA signal?

10.5 Conclusions

a. *Stomatal conductance of partially-dried grapevines was negatively correlated with concentration of ABA in xylem sap.*

b. *For grapevines grown in the field, g_S decreased coincidentally with the reduction in soil water content of the dried part of the root system and the increase in [X-ABA]; recovery of g_S coincided with no further soil drying and a decrease in [X-ABA].*

c. *Differences in g_S between different shoot types of half-dried, approached-grafted grapevines may be partly explained by differences in [X-ABA].*

d. *Changes in [X-ABA] were not associated with any significant effect of partial drying on shoot water status as indicated by Ψ_L.*

e. *The hypothesis that the reduction in stomatal conductance in response to partial drying of the root system of grapevines is associated with an increase in the concentration of ABA in xylem sap can be accepted.*

Chapter Eleven - General Discussion

11.1 The background to the study

This study had its origins in the observations of Gowing *et al.* (1990) and others that drying one half of the root system of split-root plants, while maintaining the other half wet, could reduce various components of shoot growth of woody plants, without any associated hydraulic change. Reduction of grapevine vigour had been successfully achieved by various deficit irrigation strategies but this was usually associated with a significant reduction in leaf water potential and a negative effect on fruit yield, mainly due to an effect on berry weight (Williams and Matthews 1990). Because of the possible nexus between the negative effect on yield and the perturbation of plant water status, it was thought that a 'partial drying' of the root system whereby there is a reduction in shoot growth, with no associated change in plant water status, may have potential for vigour control in grapevines without a detrimental effect on yield. At the same time, there was a possibility of an improvement in the quality of fruit for winemaking because vigour reduction should lead to more open canopy with better bunch exposure, and the relationship between canopy microclimate and wine quality had been well documented (Smart 1992). Loveys (1991) first proposed the potential of partial root-zone drying for vigour control in grapevines in the context of a discussion on root-derived chemical signals such as ABA.

The grapevine was a good candidate for study, not only for the reasons outlined above, but also because experiments with split-root grapevines had shown that half-drying of the root system caused a reduction in stomatal conductance without any associated hydraulic change (Düring 1990; H. Düring and B.R.Loveys, unpublished data). At the same time, an increase in the bulk leaf concentration of ABA in response to half-drying (B.R. Loveys and H. Düring, unpublished data) suggested the involvement of ABA as a putative root signal.

11.2 Development of the PRD strategy

The initial experiments successfully demonstrated, for the first time, that half-drying of the root system could significantly reduce shoot growth of grapevines growing under controlled conditions. Furthermore, changes in both shoot growth and stomatal conductance in response to half-drying occurred in the absence of any change in shoot water status suggesting the involvement of a non-hydraulic signal in mediating this response. Shoot growth of half-dried grapevines recovered without any change in soil water content of the dried half of the root system, coincidental with the point at which there was no further decrease in soil water content: this was confirmed by experiments with non-fruiting vines in pots (Chapter 5) and in the field (Chapter 8). It appears to be the first report of this phenomenon for any species.

A single brief period of shoot growth depression during the season is likely to have a marginal benefit for field vines. A significant and long-term reduction in shoot growth is possible if the recovery of shoot growth is minimised. Therefore, the strategy of alternating the irrigation from one half of the root system to the other was tested on both non-fruiting potted vines (Chapter 7) and fruiting field vines (Chapter 9) with split-root systems. This strategy was successful in both situations: for potted vines, alternation of irrigation from one half of the root system to the other every 11 days on average maintained shoot growth at a substantially lower rate than the well-watered control and reduced components of shoot growth by up to 40%. Similarly, with field vines, vegetative growth was substantially reduced. The alternation of irrigation from one side of the vine to the other was timed so that it coincided with the point at which there was no further decrease in SWC on the 'dry' side of the vine: this was successful in limiting the recovery of shoot growth. Furthermore, over three seasons, this strategy resulted in a substantial reduction (up to 50%) in the amount of irrigation applied, without any detrimental effect on fruit yield, but with the bonus of a significant improvement in fruit quality. The reduction in vegetative growth in response to this treatment significantly reduced canopy density and increased bunch exposure.

This strategy has been termed 'partial root-zone drying' rather than 'partial root-zone wetting' because it is the 'drying' which is the functional component of this strategy, ie. the plant appears to be responding to that part of the root system in drying soil. The aim of the strategy is to maintain part of the root system in a drying phase at all times in order to maximise production of the putative signal, so that recovery of shoot growth is minimised.

11.3 Leaf water potential

One of the most consistent observations in these experiments was that the changes in shoot growth and gas exchange in response to soil drying were not associated with any significant changes in shoot water status as indicated by Ψ_L. This was the case for both field vines and vines in a controlled environment. Leaf water potential is the most commonly used indicator of shoot water status and some authors have attempted to quantify the degree of water stress in terms of absolute values of Ψ_L (Hsiao 1973). Furthermore, the measurement of Ψ_L has been recommended for irrigation strategies which aim to manipulate grapevine shoot growth (Hardie and Martin 1990; Goodwin and Jerie 1992). However, substantial reductions in shoot growth were achieved with PRD in this study in the absence of any significant change in Ψ_L. Also, McCarthy (1997) found that Ψ_L and soil water status were not correlated in a deficit irrigation experiment. Therefore, the validity of the use of Ψ_L for irrigation scheduling must be questioned.

11.4 Symptoms of water stress

The substantial reduction in vegetative growth in response to partial drying was not associated with many of the characteristic symptoms of grapevine water stress, as described by Smart and Coombe (1983) and Williams and Matthews (1990). For example, shoot diameter, berry size and internode elongation were not greatly affected and these are known to be very sensitive to water deficits. Also, there was no tendril necrosis, abscission of mature leaves or change in leaf colour, nor was there any effect on Ψ_L. On the other hand, some of the responses to partial drying were characteristic of water-stressed vines;

however, the magnitude of the response and/or degree of expression of the symptoms were relatively less than with moderate to severe water stress. For example, stomatal conductance was only reduced by 20 to 35% with potted vines and by 12 to 30% with field vines in response to partial drying whereas stomata may almost fully close with severe water stress, eg. Exp. 1 (Chapter 4). The situation with shoot growth rate was similar with a reduction of 30 to 50% for potted vines and 18 to 30% for field vines in response to partial drying whereas severe stress may reduce SGR by 90% relative to the control, eg. Exp. 1 (Chapter 4). Furthermore, the reduction in SGR in response to partial drying was mainly due to a termination of node initiation rather than a reduction in the rate of internode elongation, whereas the latter component of SGR is more typically affected in response to water stress.

The fact that neither expansive growth nor shoot water status were affected significantly by PRD may be important in this context. Expansive growth is considered to be one of the most sensitive of plant processes to development of water deficits and directly affected by shoot water potential (Bradford and Hsiao 1982). Traditionally, effects of low water potential on expansive growth have been attributed to turgor loss but when measurements have been made in regions where cell elongation taking place, turgor often shows little change even though elongation is significantly reduced (Sharp and Davies 1989).

11.5 The evidence for ABA as the signal which mediates the response of shoot growth to PRD

The determination of the nature of the putative signal produced in response to partial drying of the root system, and the mechanism by which the signal regulates shoot development and stomatal behaviour, was not one of the main objectives of this study. However, sufficient evidence has been accumulated to suggest that: a) the signal is non-hydraulic; b) the signal is positive in nature; and c) ABA is a likely candidate as the signal.

A chemical signal such as ABA provides a sensitive means of detecting small changes in water status of the soil in the vicinity of each individual root tip (Zhang and Davies 1991). It appears that the grapevine is one of those plants for which the drying of a relatively small proportion of the root system is sufficient to generate a signal which will influence shoot function, even though the bulk of the root system may be in soil of high water content. This is not the case for all plant species: for example, Gallardo *et al.* (1994) found that it was necessary to dry out the whole of the root system of lupin in order to significantly reduce shoot growth rate.

It may be argued that, even if the signal is produced by drying roots, the flux of the signal from 'dry' roots to shoots will decrease in proportion to the reduced transpirational flow from 'dry' roots as the soil dries. However, transpirational flow from 'dry' roots may be possible due to rehydration of dry roots by 'wet' roots during the night (Saab and Sharp 1989) and this has been confirmed by the results of Exp. 11 (Chapter 5) in this study. It is likely that flow from 'dry' roots will be highest in the morning; Jackson *et al.* (1995) suggested that this was the explanation for the relatively high flux of ABA in the morning in droughted conifer seedlings, and they proposed that stomata respond to a 'morning dose' of ABA. Labelled ABA moved from 'dry' roots to the shoots of split-root, half-dried grapevines within 10 hours of application at a time when the water content of soil around the 'dry' roots was relatively low (B. R. Loveys, unpublished data).

11.6 Recovery of shoot function

Shoot function of half-dried grapevines recovered, relative to fully-watered control vines, without any change in soil water content of the dried half of the root system. This recovery coincided with the point at which there was no further decrease in soil water content, for vines grown in containers and in the field. Recovery of shoot function coincided with an reduction in [X-ABA] for both container and field vines (Chapter 10) and a reduction in root ABA concentration (Dry *et al.* 1996; B.R.Loveys and P.R.Dry, unpublished data).

The observation that shoot growth could recover without rehydration of the dried half of the root system was important in this study for several reasons. Firstly, it lead to the development of the PRD strategy (with alternation of drying from one part of the root system to the other) because it became necessary to find a means of preventing this recovery if there was to be a significant reduction of shoot growth in the long-term. Secondly, it provided additional evidence for a positive non-hydraulic signal produced by drying roots: if the signal was negative in nature, there would be no recovery because the supply of the signal would not change so long as the soil around the roots remained dry. Recovery can be explained in terms of a positive signal, ie. when there is no further decline in water content and/or matric potential of the soil around the drying roots, there is a decrease in flux of the signal from roots to shoots and thus shoot function rapidly recovers. However, it is more difficult to explain the recovery in terms of a negative signal such as cytokinins. One possibility is an increased transport of cytokinins to shoots as a result of a stimulation of root activity in the 'wet' part of the root-zone during the late stages of a drying cycle. Davies and Zhang (1991) suggested that "...positive and negative signals may combine and interact in their effects on shoot processes, perhaps with positive signals dominating in plants in moist soil and negative signals increasing in importance as the soil dries further."

The observation that [X-ABA] decreased coincidentally with recovery of shoot function (Chapter 10) supports the hypothesis that ABA is a positive signal. There are at least two possible explanations to account for this decrease in [X-ABA]:

a) Signal production is maintained in dry roots but transpirational flow from dry roots is low so the flux of the signal from roots to shoots is also low. Khalil and Grace (1993) favour this hypothesis because the 'dry' roots were still accumulating ABA on Day 7 in their experiment. However, ABA content of 'dry' grapevine roots has been found to decrease coincidentally with the recovery of shoot function (Dry *et al.* 1996; B. R. Loveys, unpublished data).
b) There are no more fine roots being dried; therefore, production of ABA by that part of the root system ceases. Transpirational flow of water from dry roots to shoots is

maintained: for example, day-time sap flow in the 'dry' half of the root system was reduced by no more than ca. 50% relative to the 'wet' half (Figure 9.5). Therefore, the net result is a diminished export of the signal from roots to shoots. Also, roots in a container of dry soil will remain alive for a relatively long time, so long as they are connected to roots in a container of wet soil (Chapter 7): this suggests that the 'dry' roots are supplied with water by the 'wet' roots.

11.7 Response to partial drying

The vegetative growth of field vines was substantially reduced in response to partial drying. Most indices of vegetative growth were reduced by up to 30 to 35% relative to the control but lateral shoot growth was the most responsive index of all. Total lateral shoot length per main shoot was reduced by 50 to 66% relative to the control in the Waite experiment, due to a combination of fewer (-28 to -47%) and shorter (-5 to -35%) lateral shoots. It is difficult to draw any firm conclusions about the relative effect of either timing or duration of PRD treatment on these components because there were differences in shoot number per vine, shoot positioning and shoot trimming between seasons, all of which would have influenced the degree of lateral shoot development. The regulation of lateral shoot growth has been a relatively neglected area of viticultural research and is worthy of greater attention, not only because it is an important determinant of canopy density (Smart 1992) but also because there is the possibility of a direct relationship between lateral shoot development and fruit composition (Jackson and Lombard 1993); however, the mechanism of such a relationship is not known. Paclobutrazol sprayed on hedged Riesling grapevines in the field significantly reduced lateral shoot development (Reynolds et al., 1992); fruit of treated vines had higher TSS than controls and the authors suggested that this response may have been due in part to a reduction in the proportion of photosynthate partitioned to lateral shoots.

In the context of this study, further research is required to determine the mechanism by which a non-hydraulic signal from drying roots may influence lateral shoot development.

A possible role for ABA as an inhibitor of lateral bud outgrowth was suggested by Phillips (1975). ABA levels were 50 to 250 times greater in non-growing than growing lateral buds of *Xanthium strumarium* (Tucker and Mansfield 1972, 1973; cited in Phillips 1975); these authors suggested that the high concentration of ABA in lateral buds prevented them from responding to the high levels of endogenous cytokinins in inhibited buds.

The ability of grapevines to regulate leaf area, in response to soil water status, by means of lateral shoot growth may have evolved as a drought-tolerating mechanism. The reduction in main leaf area in response to drought is not reversible in the case of the grapevine because it is associated with termination of main shoot growth; however, lateral shoots may commence or continue growth after main shoots have ceased to grow, in response to favourable soil conditions. Furthermore, because the grapevine has a large number of 'reserve' axillary buds (Mullins *et al.* 1992), several generations of lateral shoots are possible during a single growing season, thus allowing the plant to maximise its carbon-fixing capacity during favourable periods. Therefore, because lateral leaf area component is effectively reversible, it is logical that lateral shoot growth would be particularly responsive to a signal from drying roots.

The drought-tolerating strategy of the grapevine is not based entirely on leaf area control because stomata may also close partially in response to mild soil water deficits. However, shoot growth appeared to respond more rapidly and to a greater degree than stomatal conductance when grapevines were subjected to PRD in this study. The partial closure of stomata in response to PRD may be beneficial because plant water-use efficiency is increased (Düring 1992).

The magnitude of the reduction in vegetative growth of fruiting vines in the field in response to PRD was as great as that which has been achieved in deficit irrigation experiments with a similar reduction in the amount of water applied or similar difference in replacement of Class A Pan evaporation, eg. McCarthy and Staniford (1984); Matthews and Anderson (1988,1989); Goodwin and Jerie (1992); Poni *et al.* (1993); McCarthy

(1997). Also, the magnitude of the reduction in vegetative growth was sufficiently large so as to significantly alter the canopy microclimate and thus affect fruit composition. At the start of this study, a slight reduction in yield in response to a substantial decrease in amount of irrigation water was considered to be acceptable so long as there was a concomitant increase in fruit quality. However, I did not anticipate that the amount of irrigation could be halved without any yield penalty at all.

Why does PRD cause a substantial reduction in vegetative growth without any yield penalty? One obvious explanation is that as vegetative growth is inhibited, an increased proportion of carbohydrate is partitioned to reproductive growth. However, this assumes that the total amount of carbon fixed by PRD-treated vines was similar to control vines: it may have been less due to the reduction in leaf area (although the leaves on PRD vines may have been more efficient on average because there would have been a smaller proportion of shaded leaves). Also, the relatively small decrease in stomatal conductance (12 - 30% for field vines) would have had a some effect on carbon fixation. However, Grimes and Williams (1990) found that a reduction in midday g_S of the order of 20% relative to maximum g_S had no effect on the yield of field vines in an irrigation experiment. There is also the possibility that an increased proportion of carbohydrate is partitioned to the roots in response to a reduction in vegetative growth. Root biomass of plants in drying soil may increase relative to well-watered controls and roots may grow deeper (Sharp and Davies 1989). Therefore, one of the potential benefits of PRD may be a stimulation of root growth, resulting in a deeper, more exploitative root system; this may or may not be an advantage, depending on the viticultural situation. There is obviously a need for further research on the effect of PRD on carbon partitioning.

It is likely that one of the keys to the maintenance of fruit yield with PRD, despite the substantial reduction in the amount of irrigation, was the maintenance of berry weight. In deficit irrigation studies, berry weight is usually the most 'responsive' yield component (Williams and Matthews 1990). In this study, the amount of irrigation with PRD was halved; by comparison, a reduction of this magnitude with conventional irrigation would

typically result in much smaller berries, particularly because of the timing of the deficit. The timing of PRD in all seasons was such that it always covered the period between flowering and veraison: berry weight is most sensitive to water deficits during the period immediately after flowering and becomes increasingly resistant thereafter. McCarthy (1997) found that Shiraz berries were two and three times as susceptible to water stress during the post-flowering period as during the pre-veraison and post-veraison periods respectively. Why was there no change in berry weight in response to PRD? Based on the results from deficit irrigation studies, it appears that a relatively large degree of plant and soil water tension must develop before berry weight is affected (as indicated by Ψ_L and soil matric potential respectively). For example, Goodwin and Jerie (1992) found that Chardonnay berry weight was only reduced if the average matric potential of the soil in the root-zone decreased below -300 kPa post-flowering. Similarly, McCarthy (1997) suggested that Shiraz berry weight will only be reduced if the average soil matric potential of the root-zone decreases to -450 kPa or less in the post-flowering period and to -1000 kPa post-veraison. In view of this, the lack of effect of PRD on berry weight may be attributed to the fact that at least half of the root zone of the field vines, ie. the 'wet' side, was maintained at a relatively high level for the whole of the period of berry development.

The other yield components which could have been potentially affected in response to PRD were bunch number per vine and berry number per bunch (fruit set). The former is a consequence of both shoot number per vine and bunch number per shoot (fruitfulness). Shoot number per vine is mainly a function of pruning level and this was the same for both treatments. Both fruit set and fruitfulness are only responsive to severe water stress (Williams and Matthews 1990) and there was no detrimental effect of PRD treatment on these parameters. On the contrary, fruit set and fruitfulness of PRD-treated vines might reasonably be expected to increase in the long-term as a consequence of reduced shoot vigour and improved radiation microclimate of the renewal zone.

11.8 Shoot growth and stomatal function recover before the plant-available soil water on the 'dry' side of the vine is completely depleted

In this study, inhibition of shoot growth appeared to take place in response to a relatively small decrease in matric potential below that at field capacity. If this is the case, this observation would concur with those of Ebel *et al.* (1994) for plants in general, and with van Zyl and Kennedy (1983), Christensen (1975), Hardie and Martin (1990) and others with reference to grapevines. When the soil matric potential decreases below -60 kPa, it becomes increasingly difficult for roots in that soil to extract water (Mitchell and Goodwin 1996). For partially dried plants, it is likely that the total water requirement of the plant becomes increasingly supplied by the roots in 'wet' soil until a point is reached at which the plant becomes completely dependent on those 'wet' roots. This stage is reached before the plant-available water (between -10 and -1500kPa) has been fully extracted by the 'dry' roots, ie. when the soil of the 'dry' root-zone is at a relatively high matric potential; this was demonstrated in Exp. 12a (Chapter 7). For example, in the pot experiments conducted on the Waite campus (Chapters 4, 5,7), SWC of the 'dry' pot of half-dried plants did not ever decrease lower than 7% in the soil mix used for those experiments (see Ch. 3.2): this represents a matric potential of ca. -100 kPa. Similarly, for field vines (Chapters 9,10), the SWC at 20 and 40cm depths on the 'dry' side of the vine did not decrease lower than 22 and 19% respectively, ie. a matric potential range of -100 to -77 kPa. By comparison, much lower matric potentials (-1,000 kPa or less) have been recorded in irrigation experiments where the whole root-zone has been dried and vines have been severely stressed (McCarthy 1997). Therefore, recovery of shoot function appears to take place when the soil of the 'dry' part of the root-zone has a relatively high matric potential, which is probably not much lower than the matric potential at which shoot growth is inhibited in the first instance.

The previous discussion was written with the assumption that non-hydraulic signals are produced by drying roots in response to changes in soil water potential rather than soil water content. Although this may seem to be a reasonable assumption, it was not one of the aims of this study to investigate the nature of the relationship between soil water

potential and vine response. This is an area for future research, not only in the context of partial drying but also in terms of the general response of grapevines to water stress.

There is also a need for further research to determine the most appropriate indicators for irrigation scheduling when the PRD strategy is employed. If measurements of soil water status (either volumetric or matric potential) are used, which part of the root-zone is most appropriate? On the other hand, should the timing be based on plant indicators because they are able to integrate the effects of the 'wet' and 'dry' parts of the root systems? Hardie and Martin (1990) recommended the use of de-fruited shoots on field vines for the implementation of their irrigation strategy, presumably because such shoots are more sensitive to changes in soil water status than shoots with bunches. Measurements of the rate of shoot growth every two or three days on several de-fruited vines in a vineyard may provide an economical indication of the recovery of shoot growth and thus be used to schedule the alternation of drying from one side to the other.

11.9 Reduction in the amount of irrigation with PRD

The amount of irrigation water applied to PRD-treated vines was substantially reduced relative to the control vines in all field experiments. For example, during the 1995/96 season in the Waite experiment, the control vines received 1.34 ML/ha while the PRD-treated vines received 0.7 ML/ha, ie. the amount of irrigation was effectively halved. It could be argued that a reduction of similar magnitude, but applied by conventional means (ie. 0.7 ML/ha, with both sides irrigated as for the control), would have produced a similar reduction in vegetative growth as achieved in this study. While the results from other irrigation deficit experiments would tend to support that proposition with respect to vegetative growth, a reduction in the amount of irrigation water of this magnitude in such experiments have, to my knowledge, always been associated with significant reductions in fruit yield. For example, a 50% reduction in the amount of water applied resulted in a decrease in yield of the order of 15 to 50% (Freeman 1984; McCarthy and Staniford 1984; Matthews and Anderson 1988,1989; Grimes and Williams 1990; Goodwin and Jerie 1992; Poni *et al.* 1993; McCarthy 1997). It is difficult to make exact comparisons with other

experiments because it is not always possible to determine if the controls in those experiments had been irrigated at an optimal level: if the controls had been over-irrigated then a halving of the amount of water applied may have had no effect on yield. For example, Freeman (1984) examined the relationship between fruit yield and crop factor (= proportion of Class A Pan evaporation applied as irrigation, plus rainfall) in a long-term field experiment with Shiraz grapevines. There was no yield response for a change in crop factor over the range from 0.9 to 0.3; however, a reduction in irrigation amount equivalent to a change in crop factor from 0.3 to 0.15 resulted in a halving of yield. The control vines in the Waite experiment were certainly not over-irrigated: the actual amount of irrigation water applied in all seasons was less than that applied to commercial vineyards in a nearby region with similar growing season rainfall and comparable yield per hectare (Chapter 9). Furthermore, the calculated crop factors for the irrigation period in 1995/96 for the Waite experiment were 0.26 and 0.18 for control and PRD-treated vines respectively (Appendix VI). By comparison, in the Shiraz irrigation experiment reported in McCarthy and Staniford (1984), the crop factor calculated for the control vines was 0.36; a reduction in irrigation amount equivalent to a change in crop factor from 0.36 to 0.22 resulted in 22% lower yield.

Therefore, it is highly likely that, had a third treatment been included with the same amount of water per vine as PRD-treated vines, ie. 0.7 ML/ha in 1995/96, but applied both sides of the vine as for the control vines, the yield of that treatment would have been significantly reduced relative to both PRD-treated and control vines. This hypothesis is currently being tested in new field experiments.

This study was commenced with the main aim of achieving vine balance through control of shoot vigour. However, the water conservation potential of the partial root-zone drying technique may prove to be more important from an environmental perspective. Irrigated horticultural crops occupy approximately 250,000ha in Australia, and major problems are likely to be encountered in sustaining water supply to these crops in future, particularly

In the case of the grapevine, there is strong evidence that cytokinins play an important role in the regulation of reproductive development (Mullins et al. 1992), starting with the promotion of fruitfulness of latent buds (Srinivasan and Mullins 1981) and finishing with maintenance of the berries as a strong sink for sugar (Hoad et al. 1977). The xylem sap of the grapevine contains high cytokinin activity during budburst and flowering (Skene and Kerridge 1967). Therefore, a reduced supply of cytokinins caused by soil drying may be expected to have a negative effect on fruit yield and quality. On the other hand, it is also known that bud fruitfulness is strongly regulated by radiation and temperature, and the effect of these factors on fruitfulness is thought to be mediated by cytokinins (Srinivasan and Mullins 1981). PRD reduces shoot vigour and improves the radiation microclimate of the buds of the renewal zone (= the basal portion of the mature shoot which is retained at pruning). Therefore, the potential negative effect of PRD (via an effect of soil drying) on fruit yield may be counteracted by the positive effect (via an effect of microclimate).

11.11 Practical application of this research

The evaluation of the PRD technique has already commenced in commercial vineyards (Loveys et al. 1997). The technique will only be commercially viable if it can be applied to conventional grapevines, ie. without the use of plastic membrane and split-root plants. Therefore, there was a need to design irrigation systems which would result in spatially-separated 'wet' and 'dry' zones of the root system on a single vine at the same point in time. This has been achieved in several different ways:

a. Conventional drip irrigation: two dripper outlets per vine, one on each side of every vine in-line with the trunks (eg. 50 cm from the trunk) with each dripper of the pair connected to different laterals;
b. Sub-surface drip irrigation: two dripper tubes per vine row, one on each side of the row, spaced about 50cm from the trunks and at a depth of about 25cm (Figure 11.1);

c. Microjet sprinkler irrigation: half-circle microjets are used instead of the usual full-circle microjets; two sets per row on different laterals allows alternate inter-row spaces to be 'wet' or 'dry'.

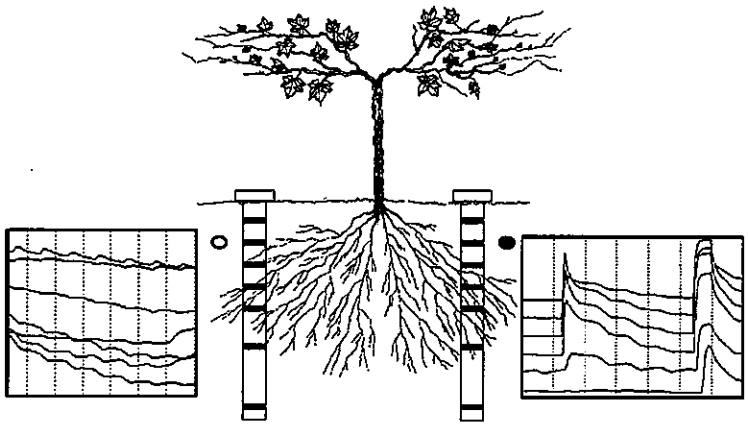


Figure 11.1 Diagrammatic representation of a grapevine irrigated using the PRD technique. Sub-surface drip lines supply water to one side or the other of the vine. In this figure, water is supplied through two irrigation cycles to the right hand line. The soil water content at various depths is shown as output from the Enviroscan® sensors: while the soil around the right hand sensor wets and dries in response to irrigation, there is a gradual decrease in soil water content on the other non-irrigated side (source = Loveys et al. 1997).

For established vineyards with existing drip irrigation, the system may be modified with minimal cost: the one-off cost of installing an additional lateral in each row and an additional sub-main is likely to be less than the value of a tonne of sub-premium grapes. The additional maintenance and operating costs associated with the application of PRD are likely to be negligible. For new vineyards, the installation of sub-surface drip irrigation may be an attractive proposition, not only because it will allow the adoption of PRD (if two lines are installed per row), but also because it should result in reduced evaporative loss and thus more efficient water use.

The opportunities for utilisation of PRD for vigour control may appear to be limited for those vineyards in which the soil remains relatively wet due to high spring rainfall and/or soil storage capacity. Fortunately, this is not the case for a large proportion of the vineyard

areas of Australia, California, South Africa etc. which experience a Mediterranean-type climate and thus soil water deficits tend to develop in spring. Nevertheless, it may be possible to 'dry' the soil by the use of deep-rooted cover crop species and subsequently apply PRD to reduce vigour. This approach is being tested with inter-row cover crops of ryegrass and chicory in conjunction with deficit irrigation in the Marlborough region of New Zealand (M. Trought, Lincoln University, pers. comm.). Such an approach would only be viable where supplies of irrigation water were both plentiful and cheap.

11.12 Possibilities for future research

Grapevine shoot vigour is strongly correlated with the supply of nitrogen (N) to the plant (Mullins et al. 1992): one of the most obvious symptoms of N deficiency is a reduction in shoot vigour. It is possible that the reduction in vegetative growth in response to PRD may be due, in part, to a reduction in the availability of N to shoots. In turn, this may be a consequence of either reduced uptake of N from the soil or changes in partitioning of N within the plant. Araujo et al. (1995) found that irrigation method affected the partitioning of N with *Vitis vinifera* cv. Thompson Seedless (syn. Sultana): N concentration of fruit and roots was significantly lower with drip irrigation than with furrow irrigation. The authors attributed this difference to the effect of irrigation method on relative size of the root system. Therefore, the effect of PRD on partitioning of N is worthy of future investigation.

Much of the water used for irrigation of horticultural crops in Australia has low to moderate salinity. Grapevines are classified as moderately sensitive to salinity based on yield and foliar symptoms (Williams and Matthews 1990). The effect of PRD on uptake of chloride should be investigated because both irrigation method and irrigation strategy may affect the concentration of chloride in leaves and fruit (Boland et al. 1993).

The field experiments of this study have focused on the effects of PRD on winegrapes. However, it is likely that this strategy will also be useful if applied to both drying and table-grape vineyards. Drying and table-grapes are mainly produced in intensively-

irrigated vineyards in southern Australia and California, and Sultana is the most important cultivar for both end-uses (Dry and Smart 1988). A large proportion of Sultana is grafted to the nematode-resistant rootstock cultivar Ramsey in Australia and this combination tends to be excessively vigorous under the growing conditions of the Murray-Darling basin. Lighter pruning, eg. nil or minimal pruning (Clingeleffer 1983) cannot be used to reduce shoot vigour with either end-use because this practice reduces berry size and this is an important quality parameter with these products. In fact, pruning tends to be relatively severe in order to maximise berry size and cultivars such as Sultana are generally cane-pruned because they have low fertility of basal buds. Therefore, PRD has the advantage that shoot vigour of drying and table-grapes may be reduced without any detrimental effect on berry size; furthermore, water may be conserved as with winegrapes.

Appendices

I. List of experiments
II. Climatic data
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IV Calculation of anthocyanins, total phenolics, total G-G and 'red-free' G-G of berry homogenate
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Appendix I. List of experiments

Season	Location	Details	No.
1991/92	Waite	Chardonnay / container-grown	1
1992/93	Waite	Shiraz /container-grown	2
	"	Ramsey / "	3
	"	Shiraz/Ramsey / "	4
1993	Geilweilerhof	5BB / container-grown	5
	"	110R / "	6
	"	5BB / "	7
1993/94	Waite	Shiraz / container-grown	8
	"	Ramsey / "	9
	"	Shiraz/Ramsey / "	10
	"	Tritiated water experiment	11
1994/95	Waite	Shiraz / container-grown	12
	"	Chardonnay / "	12a
1992/93	Waite	Cabernet Sauvignon field vines - non-fruiting	13
1993/94	"	Cabernet Sauvignon field vines - first harvest	14a
1994/95	"	" - second "	14b
1995/96	"	" - third "	14c
1994/95	Blewitt Springs	Cabernet Sauvignon field vines - second harvest	15
1995/96	Waite	Cabernet Sauvignon field vines - ABA measurement	16

Appendix II. Summary of climatic data for Waite Campus, The University of Adelaide; 1993/94, 1994/95 and 1995/96 growing seasons.

Parameter[a]	Season	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Max Temp	**1993/94**	19.3	19.9	24.2	24.1	25.1	27.1	26.3
Min Temp		9.6	11.4	13.4	14.8	14.8	16.1	15.4
Rainfall		66	78	23	76	44	16	0
Evaporation		87	133	159	178	179	144	186
SSH		6.7	7.6	8.9	7.9	7.8	7.2	8.5
Max Temp	**1994/95**	16.5	20.8	21.8	28.4	29.2	28.8	23.1
Min Temp		9.1	12.0	12.5	16.8	17.4	17.8	14.2
Rainfall		32.0	49	50	9	19	33	17
Evaporation		88	129	129	234	231	201	128
SSH								
Max Temp	**1995/96**	17.3	20.4	23.0	24.8	26.4	27.3	26.5
Min Temp		9.9	11.4	13.2	13.4	14.3	16.4	16.3
Rainfall		50	27	13	11	46	15	23
Evaporation		88	123	171	180	195	188	162
SSH		6.3	7.0	7.9	8.4	9.4	7.6	6.9

[a] Max Temp = maximum daily temperature (°C); Min Temp = minimum daily temperature (°C); Rainfall = total rainfall (mm); Evaporation = total Class A pan evaporation (mm); SSH = mean hrs of bright sunshine/day.

Appendix III. Characteristics of soil at Waite Campus site

The soils of the Waite campus of the University of Adelaide were mapped and described by Litchfield (1951). A large proportion of the arable land of the campus was classified as a 'red-brown earth'. Subsequently, this soil type was reclassified as 'Dr2.23 Hard Pedal Red Duplex' by Northcote et al. (1975; pp. 105-108). The Alverstoke vineyard is located on one of the large areas of this particular soil type.

Depth (cm)	Texture	pH	$CaCO_3$ (%)	TSS (%)	NaCl (%)
0 - 15	Loam	6.5	0	0.03	0.01
15 - 30	Clay loam	6.8	0	0.03	0.02
30 - 70	Medium clay	8.0	0	0.04	0.02
70 - 120	"	8.5	5	0.08	0.02

Appendix IV. Calculation of anthocyanins, total phenolics, total glycosyl-glucose (G-G) and 'red-free' G-G of berry homogenate.

$$\text{anthocyanins (mg/berry)} = \frac{A_{520} \,.\, DF \,.\, V \,.\, M_1}{2500 \,.\, M_2}$$

$$\text{anthocyanins (mg/g berry mass)} = \frac{A_{520} \,.\, DF \,.\, V}{2500 \,.\, M_2}$$

$$\text{total phenolics (absorbance units [OD}^{280}\text{]/berry)} = \frac{A_{280} \,.\, DF \,.\, V \,.\, M_1}{5000 \,.\, M_2}$$

$$\text{total phenolics (absorbance units [OD}^{280}\text{]/g berry mass)} = \frac{A_{280} \,.\, DF \,.\, V}{100 \,.\, M_2}$$

$$\text{total G-G per berry (µmol/berry)} = \frac{G - G_e \,.\, V \,.\, M_1}{50000 \,.\, M_2 \,.\, 0.93 \,.\, 0.94}$$

$$\text{total G-G (µmol/g berry mass)} = \frac{G - G_e \,.\, V}{1000 \,.\, M_2 \,.\, 0.93 \,.\, 0.94}$$

$$\text{anthocyanin-glucose (µmol/berry)} = \frac{0.00076 \,.\, A_{520} \,.\, DF \,.\, V \,.\, M_1}{M_2 \,.\, 0.93}$$

$$\text{anthocyanin-glucose (µmol/g berry mass)} = \frac{0.038 \,.\, A_{520} \,.\, DF \,.\, V}{M_2 \,.\, 0.93}$$

V = extract volume (mL); M_1 = mass of 50-berry sample (g); M_2 = mass of homogenate taken for extraction (g); A_{520} = absorbance at 520 nm; A_{280} = absorbance at 280 nm; DF = dilution factor for the dilution of portion of the extract into 1M HCl eg. 0.5 mL extract into 10 mL final volume; $G\text{-}G_e$ = glycosyl-glucose concentration (µM) of the extract.

Appendix V. Soil water retention curve for soil in the Waite experiment

Water retention curves were determined in the laboratory on three undisturbed soil cores sampled at 20 and 40 cm depths from the sides of a pit excavated 10 m away from the experiment in the Waite vineyard. Soil matric potentials of -1, -5, -10, -20, -30 and -60 kPa were applied using tension tables, and of -300 and -1500 kPa using a pressure membrane apparatus. Curves of the form $\Psi_m = a\theta^b$ were fitted to the data by least squares regression to allow estimation of soil matric potential (Ψ_m, kPa) at different soil water contents (θ, % v/v): $\Psi = 0.0023 \cdot \theta^{-8.58}$ ($r^2 = 0.97$; $p < 0.001$) and $\Psi_m = 0.00038 \cdot \theta^{-8.75}$ ($r^2 = 0.96$; $p < 0.001$) for 20 and 40 cm samples respectively . Data are summarised in Table 1. Readily available water was calculated as 54 and 44 mm/m respectively.

Table 1. Relationship between soil matric potential (Ψ_m, kPa) and volumetric soil water content (SWC, %) of soil at depths 20 and 40 cm from the Alverstoke vineyard, Waite campus.

Ψ_m	SWC	
	20 cm	**40 cm**
10[a]	29.0	24.0
60	23.5	19.5
100	22.0	18.5
200	20.3	17.0
1500[b]	16.0	13.5

[a] Field capacity; [b] wilting point

Appendix VI. Summary of irrigation, rainfall, evaporation and calculated crop factors for Waite and Blewitt Springs experiments

1. Irrigation

Irrigation amount (mm)[1] = vine number/ha . L/vine . 10^{-4}

Vine number/ha for Waite and Blewitt Springs = 1250 and 1388 respectively; for the latter, row spacing was assumed to be 3.6m (= mean of 3.0 and 4.2m)

2. Rainfall and Class A Pan evaporation

Data for Waite and Blewitt Springs experiments were sourced from Waite campus and Southcorp Wines 'Seaview' vineyard meteorological stations respectively.

3. Crop factor

Crop factor = [irrigation amount(mm) + rainfall(mm)]/evaporation(mm)

a. **Waite**

1993/94

	Time periods	Control (mm)	Treated (mm)
Irrigation			
Prior to PRD	8/11/93- 23/11/93	8	8
PRD period	24/11/93 - 2/3/94	70	35
Total for start of PRD to harvest	24/11/93 - 2/3/94	70	35
Total for budburst to harvest	1/9/93 - 2/3/94	78	43
Rainfall			
Prior to PRD	1/9/93 - 23/11/93	185	185
PRD period	24/11/93 - 2/3/94	131	131
Total for start of PRD to harvest	24/11/93 - 2/3/94	131	131
Total for budburst to harvest	1/9/93 - 2/3/94	316	316
Evaporation			
Prior to PRD	1/9/93 - 23/11/93	338	338
PRD period	24/11/93 - 2/3/94	632	632
Total for start of PRD to harvest	24/11/93 - 2/3/94	632	632
Total for budburst to harvest	1/9/93 - 2/3/94	970	970
Crop factor			
Prior to PRD	1/9/93 - 23/11/93	0.57	0.57
PRD period	24/11/93 - 2/3/94	0.32	0.26
Average for start of PRD to harvest	24/11/93 - 2/3/94	0.32	0.26
Average for budburst to harvest	1/9/93 - 2/3/94	0.41	0.37

[1] To convert to ML/ha, divide mm by 100

1994/95

	Time periods	Control (mm)	Treated (mm)
Irrigation			
Prior to PRD	15/9/94 - 5/11/94	31	31
PRD period	26/11/94 -14/2/95	76	38
Total for start of PRD to harvest	26/11/94 - 6/3/95	94	56
Total for budburst to harvest	3/9/94 - 6/3/95	125	87
Rainfall			
Prior to PRD	3/9/94 - 25/11/94	120	120
PRD period	26/11/94 - 14/2/95	47	47
Total for start of PRD to harvest	26/11/94 - 6/3/95	65	65
Total for budburst to harvest	3/9/94 - 6/3/95	185	185
Evaporation			
Prior to PRD	3/9/94 - 25/11/94	337	337
PRD period	26/11/94 - 14/2/95	564	564
Total for start of PRD to harvest	26/11/94 - 6/3/95	695	695
Total for budburst to harvest	3/9/94 - 6/3/95	1032	1032
Crop factor			
Prior to PRD	3/9/94 - 25/11/94	0.45	0.45
PRD period	26/11/94 - 14/2/95	0.22	0.15
Average for start of PRD to harvest	26/11/94 - 6/3/95	0.23	0.17
Average for budburst to harvest	3/9/94 - 6/3/95	0.30	0.26

1995/96

	Time periods	Control (mm)	Treated (mm)
Irrigation			
Prior to PRD	1/9/95 - 15/10/95	5	5
PRD period	16/10/95 - 27/2/96	129	65
Total for start of PRD to harvest	16/10/95 - 27/2/96	129	65
Total for budburst to harvest	1/9/96 - 27/2/96	134	70
Rainfall			
Prior to PRD	1/9/95 - 15/10/95	63	63
PRD period	16/10/95 - 27/2/96	82	82
Total for start of PRD to harvest	16/10/95 - 27/2/96	82	82
Total for budburst to harvest	1/9/96 - 27/2/96	145	145
Evaporation			
Prior to PRD	1/9/95 - 15/10/95	135	135
PRD period	16/10/95 - 27/2/96	812	812
Total for start of PRD to harvest	16/10/95 - 27/2/96	812	812
Total for budburst to harvest	1/9/96 - 27/2/96	947	947
Crop factor			
Prior to PRD	1/9/95 - 15/10/95	0.50	0.50
PRD period	16/10/95 - 27/2/96	0.26	0.18
Average for start of PRD to harvest	16/10/95 - 27/2/96	0.26	0.18
Average for budburst to harvest	1/9/96 - 27/2/96	0.29	0.23

b. Blewitt

1994/95

	Time period	Control (mm)	Treated (mm)
Irrigation			
Prior to PRD - estimated	1/9/94 - 27/11/94	30	30
- actual	28/11/94 - 8/12/94	11	11
PRD period	9/12/94 - 1/3/95	196	98
Total for start of PRD to harvest	9/12/94 - 26/4/95	238	140
Rainfall			
PRD period	9/12/94 - 1/3/95	30	30
Total for start of PRD to harvest	9/12/94 - 26/4/95	76	76
Evaporation			
PRD period	9/12/94 - 1/3/95	570	570
Total for start of PRD to harvest	9/12/94 - 26/4/95	710	710
Crop factor			
PRD period	9/12/94 - 1/3/95	0.40	0.22
Average for start of PRD to harvest	9/12/94 - 26/4/95	0.44	0.30

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