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
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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.

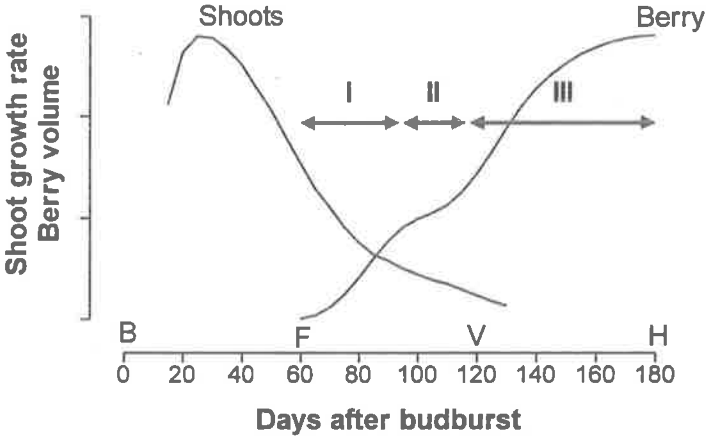

Figure 2.1 Schematic representation of shoot growth rate ('main' shoots) and berry volume of the grapevine (B, F, V, H indicate times of budburst, flowering, veraison and harvest; I, II, III indicate stages of berry development).

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 1990a) 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).

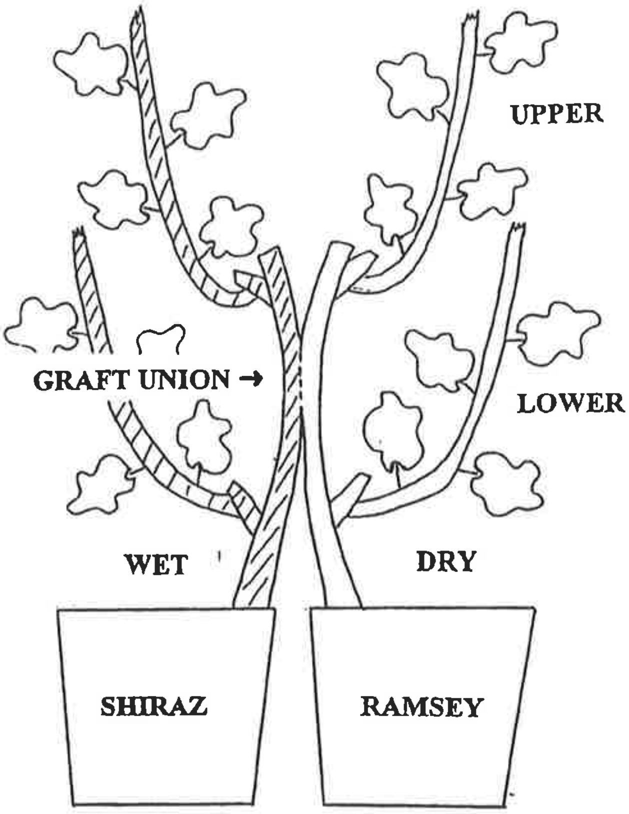

Figure 3.3 Diagrammatic representation of a half-dried, approached-grafted split-root grapevine (Shiraz/Ramsey combination) showing the different shoot types ie. ‘lower/wet’, ‘upper/wet’, ‘upper/dry’, ‘lower/dry’. In this example, the pot containing Shiraz roots is irrigated (‘wet’), the other pot is 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) = $N_m \cdot A_m$

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.

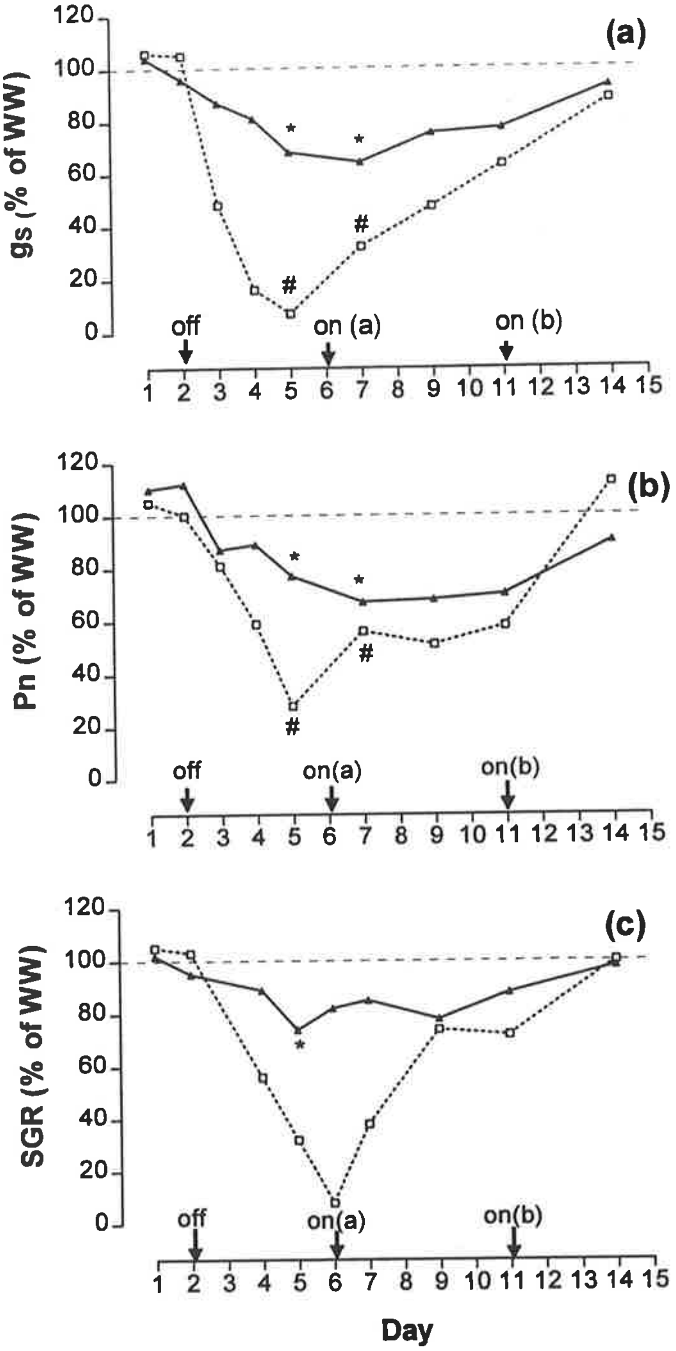

Figure 4.1 Effect of wet/dry combinations on gas exchange and shoot growth, Chardonnay, Exp. 1. WD (▲) and DD (☐) treatments expressed as % of WW; on(a): DD changed to WD; on(b): all pots irrigated. * indicates those days when WD significantly different ($p<0.05$) from WW and # those days when DD significantly 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

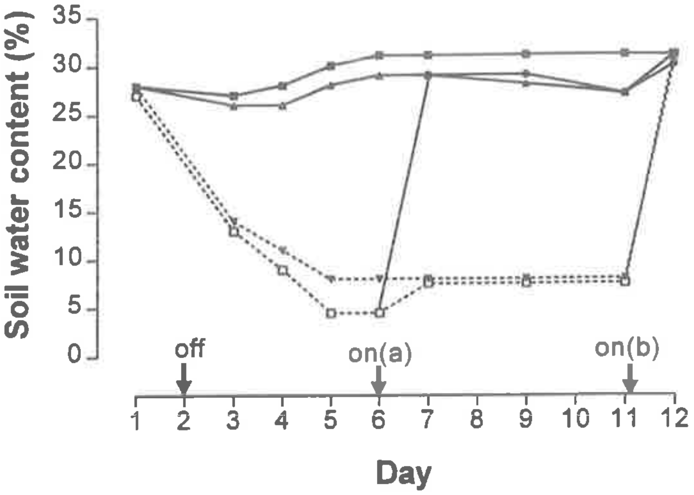

Figure 4.2 Volumetric soil water content (%) measured by TDR: average of both pots WW (■); irrigated pot of WD (▲); non-irrigated pot of WD (▼);average of both pots DD to day 6 and non-irrigated pot only thereafter (□); irrigated pot of DD from D7 (●); Chardonnay, Exp. 1. On(a): DD changed to WD; on(b):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

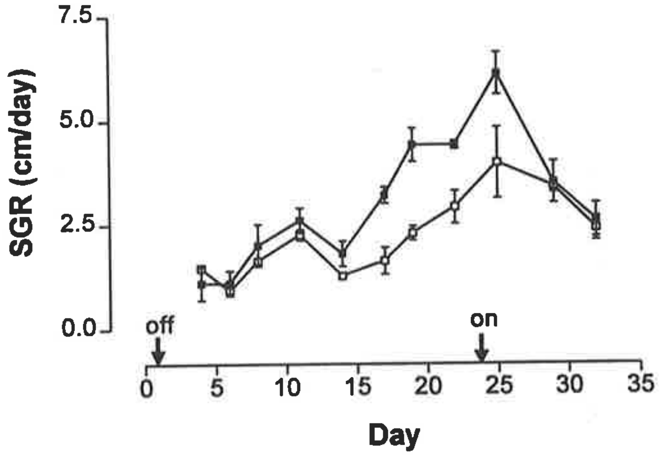

Figure 4.3 Effect of partial drying on shoot growth (SGR, mean ± se, cm/day) of Shiraz, Exp. 2: 'control' vines (C, ■) and 'treated' vines (T, mean of 'wet' and 'dry' shoots,☐). T significantly different ($p<0.05$) to C from D17 to D25. One pot of T not irrigated from D2 to D23.

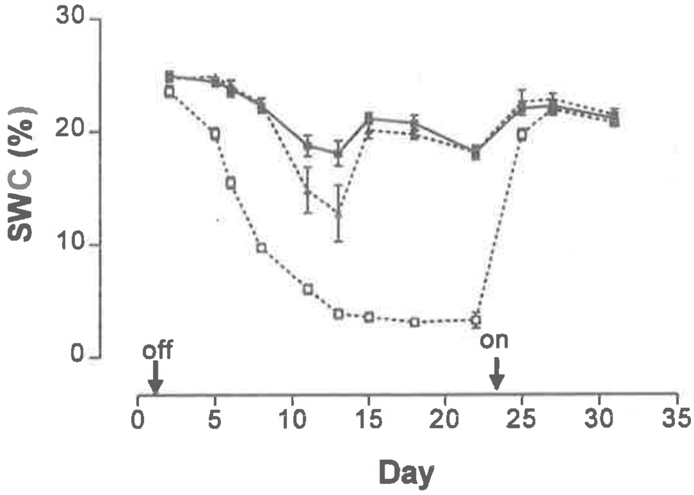

Figure 4.4 Volumetric soil water content (SCW, %) measured by TDR: average of both pots of 'control' (C, ■); irrigated pot of 'treated' (T, ▲); non-irrigated pot of T (□); Shiraz, Exp. 2 .One pot of T not irrigated from D2 to D23.

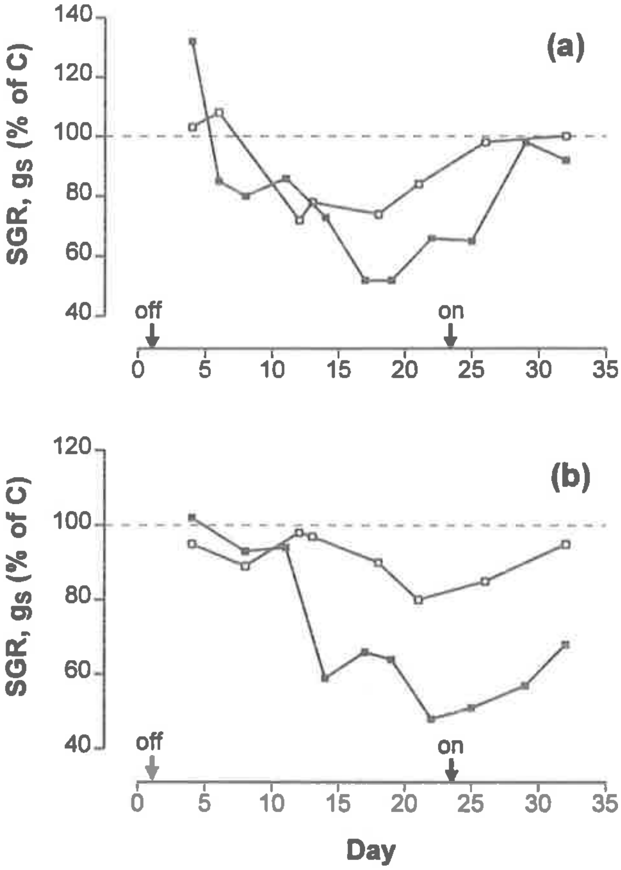

Figure 4.5 Effect of partial drying on shoot growth rate (SGR, ■) and stomatal conductance (g_S, □): 'treated' (T) as % of 'control' (C). One pot of T not irrigated from D2 to D23.

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

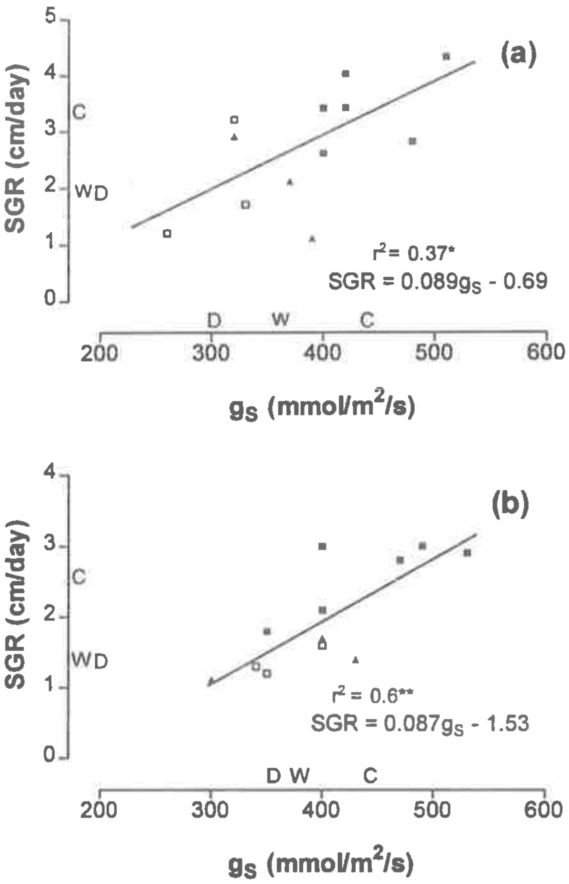

Figure 4.6 Relationship between shoot growth rate (SGR, mean per shoot, D11-D22, cm/d) and stomatal conductance (g_S; mean per shoot, D12-D21, mmol/m^2/s) for six control shoots(C, ■), three 'wet' (W, ▲) and three 'dry' (D, □) shoots of half-dried plants. Letters on axes represent mean values of all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

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[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/m^2/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 more roots being dried, will be tested in an experiment described in this chapter. The question of signal production is addressed in Chapter 10.

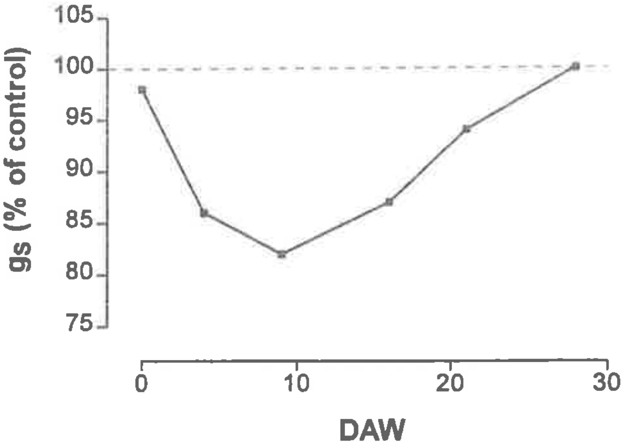

Figure 5.1 Effect of drying half the root system of split-root plants on stomatal conductance (g) (as % of control with the whole root system watered); mean of apple, peach, pear and grape. Time scale = days after water 'off' (DAW). Data reworked from Poni *et al.* (1992).

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

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[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.

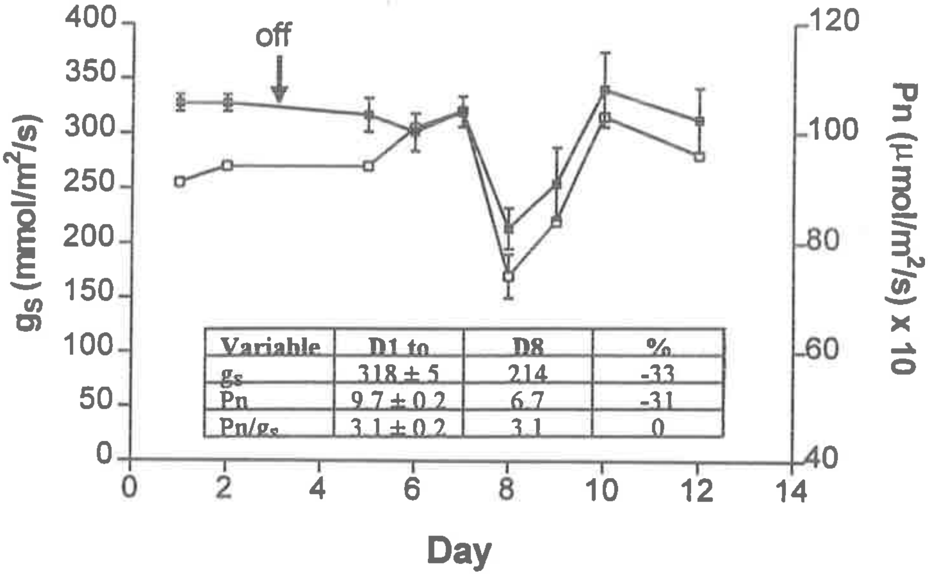
5.3 Results

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



Variable	D1 to	D8	%
g_S	318 ± 5	214	-33
Pn	9.7 ± 0.2	6.7	-31
Pn/g_S	3.1 ± 0.2	3.1	0

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.

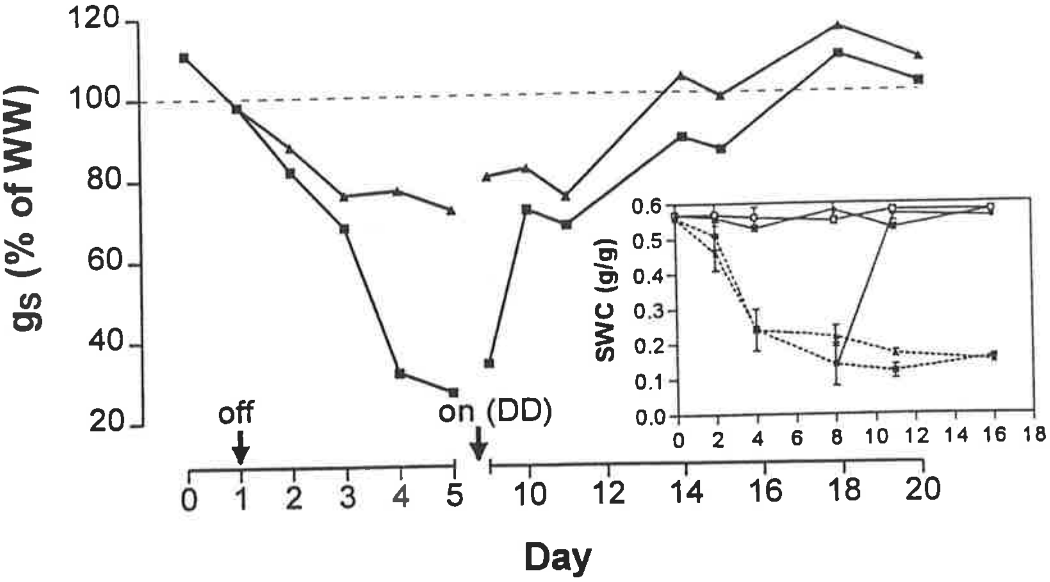


Figure 5.3 Effect of wet/dry combinations of 5BB split-root vines on stomatal conductance (g_S, mmol /m^2/s) of WD (▲) and DD (■) treatments expressed as % of WW; Exp. 7. Off: one pot of WD and both pots of DD not irrigated from D1; on: DD changed to WD. g_S measured in pm (mean of two to three measurements 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.

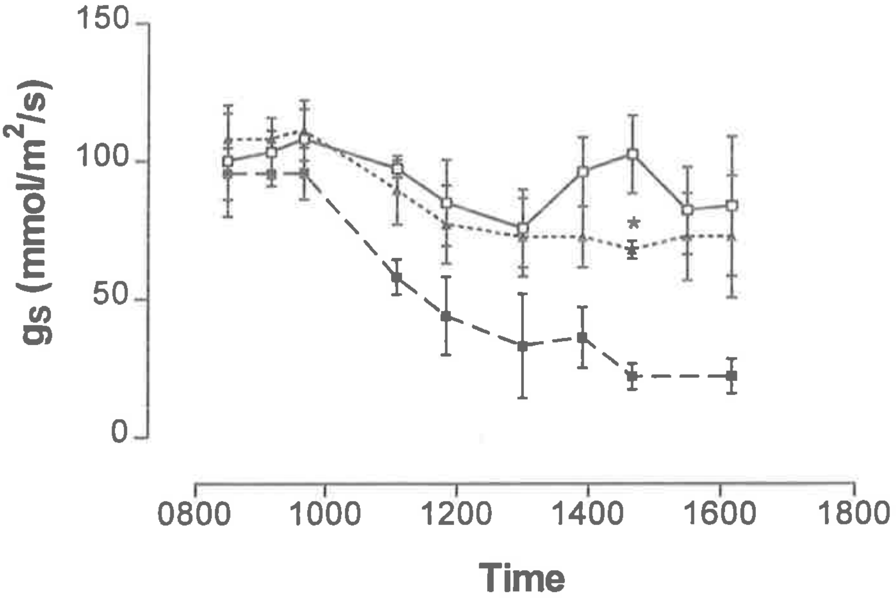


Figure 5.4 Effect of wet/dry combinations of 5BB split-root vines on diurnal change on D4 of stomatal conductance (g_S, mean ± se, mmol /m^2/s); WW (□), WD (▲) and DD (■), Exp. 7.
Off: one pot of WD and both pots of DD not irrigated from D1; on: DD changed to WD. * indicates WD significantly different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

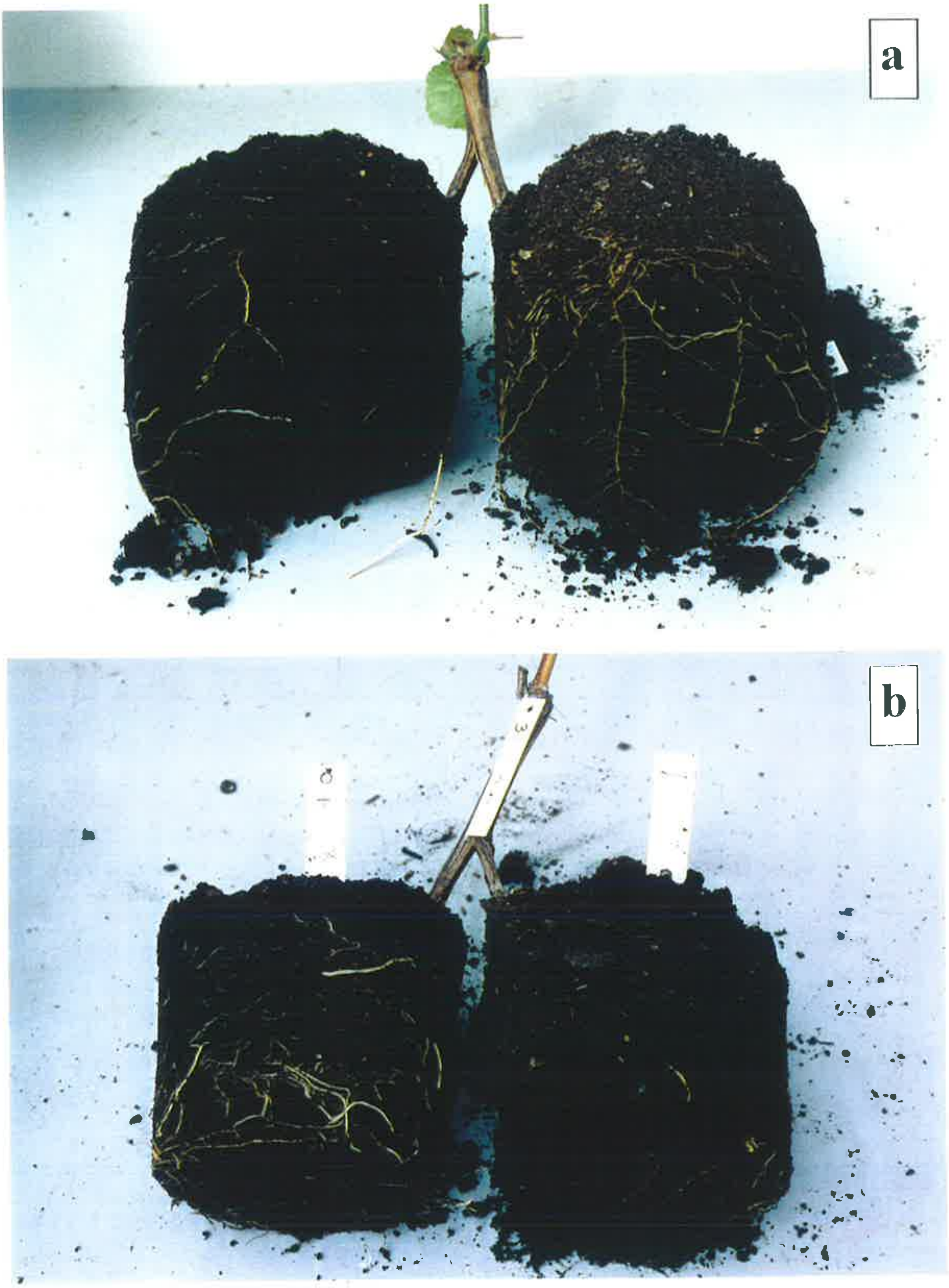


Figure 5.5 Effect of wet/dry combinations on root development of 5BB split-root vines, Exp. 7. Irrigated ('+', left) and non-irrigated ('-', right).
(a) WD plant on D22; ' -' pot not irrigated from D1.
(b) DD plant on D22; '+' pot irrigated on D8, '-' pot not irrigated from D1 to D21.

c. Experiment 8

The reduction of SGR and g_s of 'treated' plants relative to 'control' coincided with a decrease in soil water content of the 'dry' pot from D3 (Figure 5.6). 'Treated' SGR and g_s had decreased by ca. 35% relative to the 'control' by D9 and D10 respectively and soil water content of the 'dry' pot also decreased to the minimum of ca. 7% at the same time. The lowest value of g_s on 'treated' plants relative to the control, on D10, was not associated with any significant effect of treatment on Ψ_L (-1.17 and -1.14 MPa for 'control' and 'treated' respectively). Both g_s and SGR of 'treated' plants recovered after D10 and were complete by about D14 and D17 respectively while the soil water content of the 'dry' pot remained at ca. 7% (Figure 5.6).

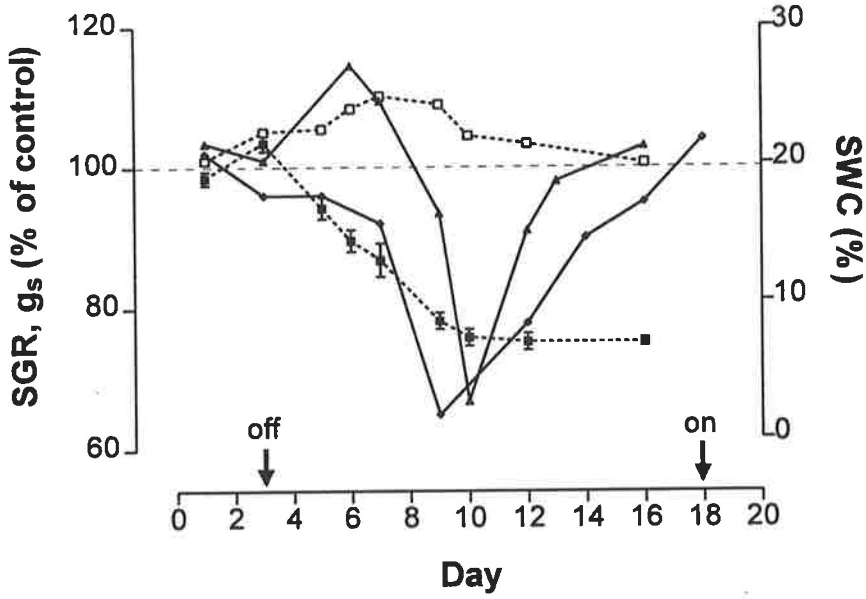


Figure 5.6 Effect of half-drying split-root Shiraz on shoot growth rate (SGR,◆) and stomatal conductance (g_s, ▲); mean of upper shoots; 'treated' (T) as % of 'control' (C), Exp. 8. One pot of T not irrigated from D3 ('off') to D18 ('on'). T significantly different ($p<0.05$) to C on D9 and D12 for SGR, D10 for g_s. Volumetric soil water content (SWC, dotted lines) measured by TDR (mean ± se, %): 'wet' pot (□) and 'dry' pot (■) of T plants.

Stomatal conductance of different shoot types on 'treated' plants, as a percentage of the upper shoots of 'control' plants, was plotted over time (Figure 5.7). On D10, when the average g_S of the upper shoots of 'treated' was lowest relative to 'control', g_S of LW shoots was significantly higher than the three other shoot types, but it was not significantly different to 'control' g_S. Also, g_S of UD and LD shoots was significantly lower than g_S of 'control' shoots (Figure 5.7, Table 10.1). Recovery of g_S of UW shoots, relative to the 'control', started before the recovery on either of the 'dry' shoots; the latter recovered at a similar rate (Figure 5.7). Ψ_L of different shoot types was also measured on D10 (pm) (Table 10.1): LD shoots had a higher Ψ_L than all other 'treated' and 'control' shoots (but the difference was only statistically significant for LW).

d. Experiment 10

For those treatments with one 'dry' pot not irrigated from D2, ie. S-R+ and S+R-, the reduction in g_S relative to the control coincided with the decrease in soil water content of

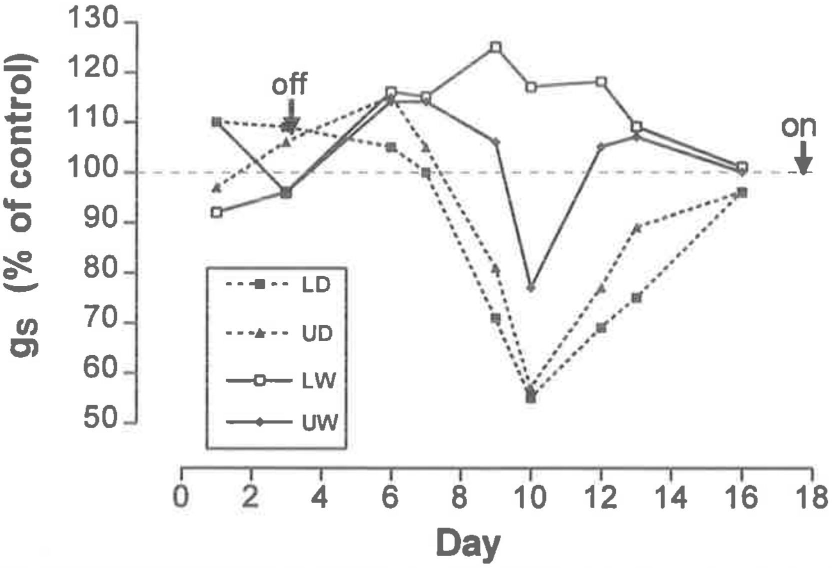


Figure 5.7 Effect of half-drying split-root Shiraz on stomatal conductance (g_s, mmol/m^2/s) of four shoot types (upper/wet UW (◆), upper/dry UD (▲), lower/wet LW (□), lower/dry LD (■) of 'treated' (T) as % of 'control' (C, mean of upper shoots); Shiraz, Exp. 8. One pot of T not irrigated from D3 ('off') to D18 ('on'). LW significantly different ($p<0.05$) to LD and UD on D9 to D13 inclusively; LW sig. diff. to UW on D10; LD sig. diff. to UW on D13 ($p<0.05$).

the 'dry' pot (Figure 5.8; data only shown for S-R+); g_s had decreased to 54% of the 'control' by D15. Recovery of g_s relative to the 'control' started after D15, ca. 4 days after soil water content reached the minimum value, and was completed by D21 without rewatering of the 'dry' pot. The differences in g_s between treatments were not associated with any difference in Ψ_L : on D15, Ψ_L of S+R+ (mean of Shiraz shoots) and S-R+ (mean of 'dry' Shiraz shoots) was -0.83 and -0.82 respectively.

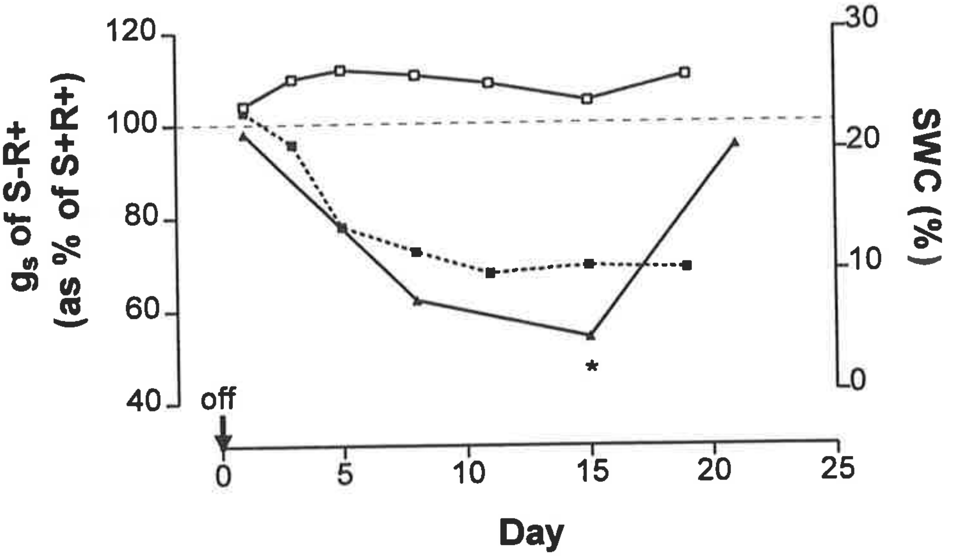
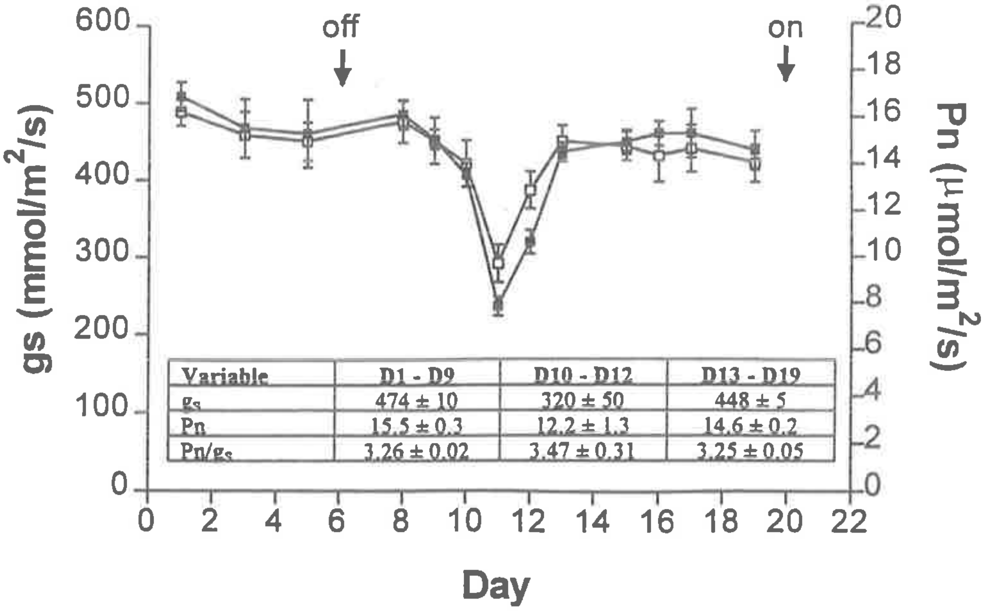


Figure 5.8 Effect of half-drying split-root Shiraz/Ramsey on stomatal conductance (g_s, mean of Shiraz shoots, mmol/m^2/s); S-R+ (▲) as % of S+R+, Exp. 10. Volumetric soil water content (SWC, %): mean of 'wet' (□) and 'dry' (■) pots of all treatments; 'dry' pot not irrigated from D2 ('off'). * indicates g_s of S-R+ significantly different ($p<0.05$) to S+R+.

e. Experiment 6

Stomatal conductance decreased in response to drying of one pot: average g_s for the period from D10 to D12, relative to periods immediately before and after, was 68 and 71% respectively (Figure 5.9). The response of Pn was similar to that of g_s. Water-use efficiency (estimated by Pn/g_s) was highest from D10 to D12. Actual values of g_s and Pn were least on D11, after five days of half-drying (5DAW). Both g_s and Pn started to recover from D11 and recovery was complete by D15 [9DAW] (Figure 5.9).



Variable	D1 - D9	D10 - D12	D13 - D19
g_s	474 ± 10	320 ± 50	448 ± 5
Pn	15.5 ± 0.3	12.2 ± 1.3	14.6 ± 0.2
Pn/g_s	3.26 ± 0.02	3.47 ± 0.31	3.25 ± 0.05

Figure 5.9 Effect of half-drying 110R split-root vines on stomatal conductance (g_s, mean ± se, mmol /m^2/s, ■) and assimilation rate (Pn, mean ± se, μmol/m^2/s, □); Exp. 6. 'Dry' container not irrigated from D6 ('off') to D20 ('on'). Insert: mean values (± se) of g_s, Pn and Pn/g_s (μmol /mmol x10^2) for D1-D9, D10-D12, D13-D19.

The large decrease of both g_S and Pn from D10 to D11 coincided with the slowing in the rate of soil drying (as indicated by the depth of the wet/dry margin; Figure 5.10a). Soil water content data from Exp. 7 (Figure 5.3) was included for comparison in Figure 5.10a (plotted on the same time scale relative to the onset of soil drying) because the same soil mix was used. The percentage of 'new' root area dried almost reached its maximum value at the same time, i.e D11 (Figure 5.10b). Recovery of g_S and Pn after D11 coincided with the maximum depth of the wet/dry margin (Figure 5.10a) and the highest percentage of 'new' root area dried (Figure 5.10b). Leaf water potential on D12 (when g_S was ca. 66% of the D1 to D9 average) was -0.22 MPa at 0730h. The rate of change in root area per day (RRA; Figure 5.10c) was positively correlated with root dry weight. RRA peaked from D8 to D10 in both 'wet' and 'dry' containers with no significant effect of container treatment on the changes over time. The rate of growth of roots on the glass in the 'dry' container was significantly greater than that in 'wet' container from D9 to D10, and again from D16 to D19 (Figure 5.10d).

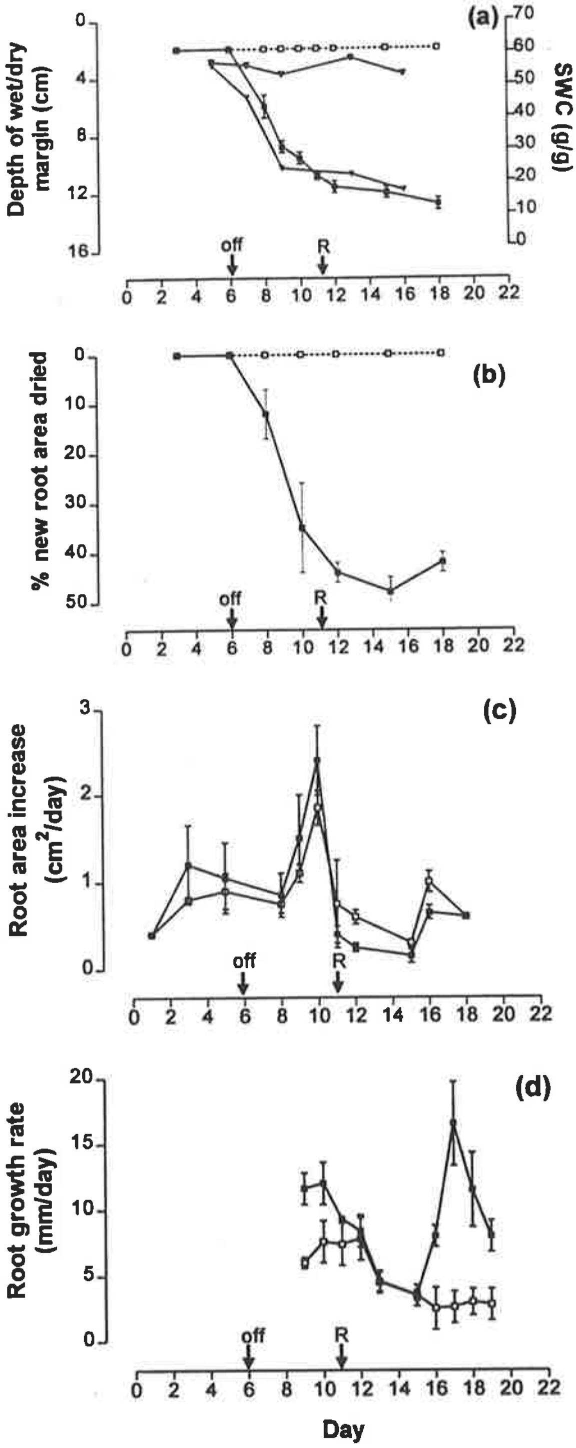


Figure 5.10 Effect of half-drying 110R split-root vines on root development in 'wet'(□) and 'dry' (■) containers, Exp. 6. 'Dry' container not irrigated from D6 ('off') to D20; R indicates day when recovery started (from Fig.5.9).

(a) Depth (mean ± se, cm) of wet/dry margin below soil surface on glass wall of containers.
Gravimetric soil water content (SWC, g/g): data from Figure 5.3 adjusted to same time scale relative to cessation of irrigation of 'dry' container; 'wet' (∇) and 'dry' (▼) containers.

(b) Percentage of 'new' root area dried (mean ± se).

(c) Rate of increase in root area in contact with glass wall (mean ± se, cm^2/day).

(d) Growth rate (mean ± se, cm/d) of individual roots on glass wall.

There was a significantly larger root area for >15 cm depth in 'dry' than 'wet' containers on D22, particularly deeper than 20cm (Figure 5.11), but no difference in the total amount per container. The average depth of roots on the glass on D22 was 22.6 ± 1.5 and 34.0 ± 2.5 for 'wet and 'dry' containers respectively.

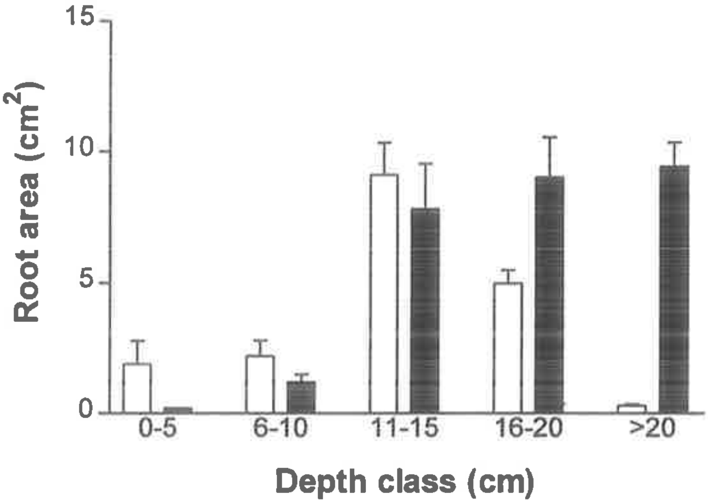


Figure 5.11 Effect of half-drying 110R split-root vines on the area (cm^2) of white and suberised roots on the glass surface (mean ± se of 3 plants) of 'wet'(☐) and 'dry' (■)containers, Exp. 6. 'Dry' container not irrigated from D6 to D20.

By D12, there were no fine, white roots in the 'dry' container between 0 and 5cm and very few between 5 and 10cm depth; many of the roots between 5 and 15cm depth were suberised ('brown'). By comparison, there were many white roots in the 'wet' container between 5 and 15cm. A photograph taken on D19 (Figure 5.12) shows relatively thick, white, descending roots in the 'dry' container which had grown to ca. 30cm depth and a few brown roots above the wet/dry margin; by comparison, the roots in the 'wet' container of the same plant were not growing on the glass wall below ca. 16cm depth. Excavation of soil from containers after the experiment revealed that there were many roots in the 'wet' container deeper than 20cm on the side away from the glass wall. Nevertheless, dry weight of roots in the whole container and the area of roots on the glass on D21 were positively correlated: dry weight = 0.124.area - 0.03; $r^2 = 0.78$**.

Figure 5.12 Effect of half-drying 110R split-root vines on root development, Exp. 6. 'Dry' container not irrigated from D6 to D20. Photograph on D19 of 'wet' (left) and 'dry' (right) containers of the same split-root plant. The wet/dry margin in the 'dry' container is indicated by the black line (average depth below surface = 13.5cm) and the base of the original root ball in both containers is ca. 11cm below the surface.

f. Experiment 11

Label from ^3H_2O was detected in leaves of UW, UD and LD shoots within two hours of application, with maximum counts measured between five and seven hours after application to the 'wet' roots (Figure 5.13). This indicates that water moved from the 'wet' roots to the shoots on the 'dry' side of the plant. By comparison, a relatively small amount of ^3H_2O was detected in leaves of the LW shoot.

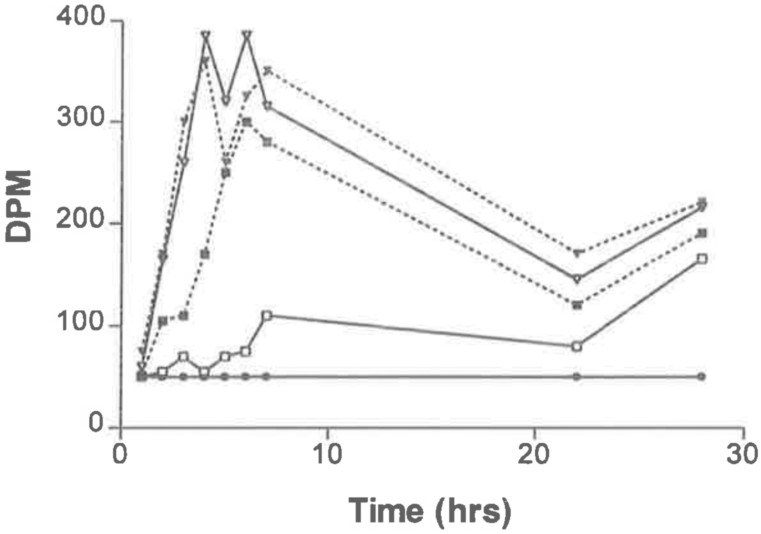


Figure 5.13 Uptake of ^3H_2O over time from 'wet' roots of Ramsey split-root, half-dried plants; disintegrations per minute per disk (DPM) measured in leaves from lower/dry LD (■), upper/dry UD (▼), upper/wet UW (σ) and lower/wet LW (□) shoots and of background (●). ^3H_2O applied to roots at 0 hours.

5.4 Discussion

Recovery coincided with decrease in rate of soil drying: Recovery of shoot function of half-dried grapevines was observed to take place without any change in soil water content of the dried half of the root system. Therefore, it is possible to accept the first hypothesis without reservation. There were some differences between shoot growth rate and gas exchange with respect to both the time at which recovery commenced and the rate of recovery in the experiments described in this chapter and in Chapter 4, but there were no consistent trends. This suggests SGR and gas exchange were affected to a similar degree by influences originating in the roots in contact with dry soil.

Recovery started between five and 15 days after the onset of soil drying in these experiments, which is comparable with the results of both Khalil and Grace (1993) and Poni *et al.* (1992). The rate of recovery, ie. the time from onset to completion, varied from two to ca. 10 days in my experiments with both *Vitis* hybrids and *Vitis vinifera*; this is less than the 18 days or so calculated from the data of Poni *et al.* (1992) for a cultivar of *Vitis* *vinifera*. The differences between experiments may be a function of the soil type, size of the root system and the number of root tips dried, the growth stage of the plants or differences in genotype (see Chapter 6 for the effect of the latter on response to half-drying). Recovery occurred in two days in Exp. 5 and there were few new roots in either the 'wet' or 'dry' pot at the end of the experiment; these plants had been growing outside at relatively low temperatures prior to use and may not have developed many new roots. In the experiment of Poni *et al.* (1992), treatment was not imposed until ca. 50 days after budburst when shoot growth rate was starting to slow. There did not appear to be any relationship between the magnitude of depression of SGR or g_s and the rate of recovery. For example, SGR was inhibited by ca. 35% relative to the control but recovery occurred in eight days (Exp. 8); for g_s, 20 to 30 % inhibition was associated with recovery in two days (Exps. 5,6), four days (Exp. 8), ca. 10 days (Exp. 7) or 18 days (Poni *et al.* 1992).

Recovery of shoot function started about the time when there was no further decrease in soil water content of the dried half of the root system. Differences in timing of recovery relative to the soil water status of the 'dry' container may be due to differences between experiments with respect to rate of soil drying of the different soil mixes, methods of soil water determination and/or differences between species. For Khalil and Grace (1993), soil water content of 'dry' pot was still decreasing when recovery started, recovery was only partial and it was only observed for one day. Therefore, this thesis appears to be the first report of : a) a recovery of shoot function coincidentally with no further decrease in soil water content of the dried half of the root system; and b) complete recovery of shoot function without any change in water status of the soil containing the dried roots. Recovery of g_s started after the soil water content of the 'dry' pots had decreased to 0.16 g/g with half-dried plants of sycamore (Khalil and Grace 1993) and this is almost identical to the gravimetric SWC at recovery with 5BB in Exp. 7. However, it is difficult to draw any useful conclusion from this similarity because it is likely that the matric potential would have been different at the same SWC. The soil water status at the time of recovery is examined in more detail in Chapter 10.

Response to half-drying of the root system: The initial reduction of shoot growth rate and gas exchange coincided with the decrease in soil water content of the dried half of the root system as in Chapter 4. SGR and/or g_S values of half-dried plants were statistically different to the controls once the soil water content of the dried pot had decreased to 0.25 g/g (Exp. 7), between 13 and 7% (Exp. 8), 8% (Exp. 1, Chapter 4) and 0.25 g/g (sycamore; Khalil and Grace 1993). However, because different soil mixes were used in these experiments, the roots in the 'dry' containers in each case may have responded at a similar value of soil water potential. It is possible that roots respond to soil water potential rather than bulk water content. The matric potential at 7% volumetric SWC for the soil mix used in Exps. 1 and 8 was estimated to be approximately -100 kPa: therefore, g_S and SGR of half-dried plants decreased relative to controls when the matric potential of the root-zone of the 'dry' container decreased from field capacity (-10kPa) to -100kPa. This was a similar result to Ebel *et al.* (1994) who found that leaf expansion rate of half-dried sorghum plants was not significantly different to controls until the soil in the 'dry' container had decreased to ca. -100 kPa.

The magnitude of the reduction of SGR and g_S, ie. 20-30%, was similar to that measured in Chapter 4 and in some other studies (Tan and Buttery 1982; Poni *et al.* 1992; Khalil and Grace 1993). That the half-drying treatment induces only partial stomatal closure before recovery provides some evidence for a non-hydraulic signal because experiments where the whole of the root system is dried usually produce complete closure over the same time period.

It is not clear why there was no response of shoot growth rate to half-drying of 5BB in Exp. 7. It is possible that shoot growth is less responsive to root signals than stomatal conductance with this cultivar. On the other hand, shoot growth of 5BB may have responded had the experiment been conducted in the open under conditions of higher VPD and irradiance.

There was no effect of half-drying on Ψ_L at the times of greatest inhibition of SGR and g_S relative to the control, as in Chapter 4. This provides additional evidence in favour of a non-hydraulic signal originating from the roots in contact with drying soil. On some occasions, Ψ_L of half-dried plants was slightly lower than controls eg. Table 5.1; although differences were not statistically significant, this may have been the result of an inadequate water supply to the 'wet pot, ie. irrigation was not frequent enough meet the entire needs of the plant. Khalil and Grace (1993) made the same observation and concluded that, because the real differences in Ψ_L were small, they were unlikely to induce any important perturbation in shoot function.

Drying of the whole root system of plants in Exp. 7 almost caused complete stomatal closure by D7, similar to the results of Exp. 1 (Chapter 4). The response of these fully-dried plants is an indication that a significant reduction in shoot water potential may override a non-hydraulic signal produced by drying roots. Such a direct control of stomatal function by leaf water status may play an important role when the soil dries to such an extent that bulk water relations are perturbed (Gowing *et al.* 1990). A combination of decreased leaf area (as a result of decreased rate of shoot growth and thus leaf initiation, plus reduction in size of expanding leaves) and decreased transpiration rate would allow the plant to extend its growing season given a finite water supply.

After the DD plants in Exp. 7 were converted to half-dried on D7 by watering one of the dry pots, g_S recovered to the WD level within three days; thereafter, the DD plants behaved in a similar way to WD plants, ie. they completely recovered over the next seven days or so while one pot remained unwatered. This was similar to the results of Exp. 1 (Chapter 4).

There were no 'control' plants used in Exps. 5 and 6. Nevertheless, the response to half-drying was estimated by comparison of the mean values of g_S and Pn during the 'response period' with mean values of the periods immediately before and after the 'response period'. This was valid for gas exchange because measurements were conducted under constant conditions on each occasion ie. control of input humidity, leaf temperature, PAR etc. (see Chapter 3) and sufficient time was allowed for the leaf to equilibrate to conditions inside the chamber, and for the stomata to respond to those conditions. Therefore, any differences in gas exchange from one day to the next are likely to be the result of changes in soil conditions, rather than ambient conditions. This contrasts with measurement of stomatal conductance in Exps. 8 and 10 in which the input conditions were not controlled and the values of this parameter for both control and treated plants varied from one day to the next in response to both atmospheric conditions and soil factors.

Changes in stomatal conductance in response to half-drying were strongly correlated with shoot growth rate (Figure 4.6). For this reason, and the fact that g_S is easier to measure in the field than Pn (more leaves can be measured in the same time with more portable equipment), stomatal conductance only was measured in subsequent experiments in this study to provide an estimate of the effect of partial drying on total assimilation. Besides, Pn is closely correlated with g_S for grapevines growing in the field under a wide range of temperature and humidity conditions (Jacobs *et al.* 1996). Changes in Pn in response to half-drying were closely correlated with changes in g_S in Exps. 1, 5 and 7 and it is possible that stomatal closure may fully account for the effect of treatment on photosynthesis, ie. there was no effect on carboxylation efficiency in response to half-drying. Poni *et al.* (1992) came to the same conclusion in their half-drying experiment. The ratio of Pn to partial pressure of intercellular CO_2 (Ci) could have been used in these experiments to estimate non-stomatal limitation of photosynthesis, as originally proposed by Downton *et al.* (1987). However, more recently, Downton *et al.* (1988a,b) and Düring (1992) have shown that Ci data derived from gas exchange does not necessarily indicate the actual average leaf value of intercellular CO_2 due to the effect of non-uniform stomatal behaviour ('patchiness'). Using different techniques, these authors have shown that, under conditions of water stress, low air humidity, salinity or after application of ABA, photosynthesis of *Vitis* leaves is restricted to small areas. Thus, reduced CO_2 fixation under these conditions cannot be attributed to non-stomatal limitation of photosynthesis unless severe stress conditions occur (Düring and Loveys 1996).

The ratio of Pn to both g_S and transpiration (E) has been used to indicate water-use efficiency of grapevines (Düring 1987, 1990, 1991): water stress increases the ratio of Pn to g_S because partial stomatal closure has a greater effect on g_S than Pn. Drought-tolerant cultivars tend to have higher Pn/g_S values under the same environmental conditions as drought-sensitive cultivars (Düring 1990). For non-*Vitis vinifera* cultivars in my study, although the ratio of Pn to g_S was not increased during the brief period of relative stomatal closure in response to half-drying in Exp. 5 (Figure 5.2), it was 20% higher during the equivalent period in Exp. 6 (Figure 5.9) and slightly increased in Exp. 7 (Table 5.2). In the case of *Vitis vinifera* (cv. Chardonnay) in Exp. 1 (Figure 4.1a,b), the maximum reduction in g_S of half-dried plants relative to controls (36% decrease) was associated with a 15% increase in Pn/g_S ; for the fully-dried treatment, the effect on Pn/g_S was even greater during the early stages of soil drying and a 50% reduction in g_S relative to the control was associated with a 68% increase in Pn/g_S . These results lend support to the proposal that the plant's first line of defence against drought is an ability to sense changes in available soil water and induce partial stomatal closure (Düring 1992).

Partitioning of the signal from drying roots: The response of stomatal conductance of the lower/wet (LW) shoot to half-drying in Exp. 8 suggests that the LW shoot behaved independently of the other shoot types (Figure 5.7). On the other hand, the upper/wet (UW) shoot responded similarly to the two 'dry' (LD, UD) shoots, but to a lesser degree. This suggests that the putative signal moves from the 'dry' roots to the 'dry' trunk where it affects the gas exchange of both upper and lower 'dry' shoots. The signal can move from the 'dry' trunk to the 'wet' trunk via the graft union but, once in the 'wet' trunk, the signal strength is weakened, particularly in the case of the LW shoot. This proposal is further discussed in Chapter 10. There was no significant difference in Ψ_L between either of the 'wet' shoots and the UD shoot (as in Exp. 2), confirming that the stomatal response was not a consequence of perturbation of shoot water status. Ψ_L of the LD shoot was statistically higher than the other shoots on D10; however, the actual difference was small and the possibility of a direct effect of Ψ_L on stomatal conductance can probably be ruled out because the decrease in g_S relative to the control on that day was the same as for the UD shoot. It is possible that stomatal closure caused the relatively higher Ψ_L of the LD and UD shoots relative to the LW shoot.

Root development in 'wet' and 'dry' soil: There was no significant difference between 'wet' and 'dry' containers for either the rate of root area (dry weight) increase over time or total root area (dry weight) at D21 (root area and dry weight were positively correlated). However, the root growth rate of individual roots on the glass wall was significantly greater in the 'dry' containers from D9 to D10, and again from D16 to D19. The latter may not be a true indication of the growth rate of all new roots in the containers because the roots which were generally available for measurement, by virtue of their location on the glass wall, were mainly the descending roots at the lower extremity of the root system and these were more plentiful in the 'dry' container (Figure 5.11). There appeared to be no difference in the total root dry weight between wet and dry containers; however, there was a major difference with respect to the distribution of roots. The dry container had a larger proportion of white roots in the lower half and roots grew to a greater depth (Figures 5.5a, 5.11). This may have been due in part to the relatively better aeration of the soil at the bottom of the 'dry' containers. In other experiments with woody split-root plants, root growth in the 'wet' container of half-dried plants increased relative to the 'dry' such that ca. 60% of total 'new' root dry weight per plant was in the 'wet' container (Tan and Buttery 1982; Poni *et al.* 1992). However, these experiments were conducted over a longer time period than my experiments where the plants may not have had sufficient time to increase the size of the root system in the 'wet' container to compensate for the 'loss' of the root system in the 'dry' container. Furthermore, the root distribution with respect to depth was not examined in the other experiments.

Root development increased in moist soil layers: in the case of the half-dried plants, this could have been in either the 'wet' container or in the lower part of the 'dry' container. This may be a consequence of increased availability of carbohydrate as shoot growth is inhibited. Rühl and Alleweldt (1990) reported an increased accumulation of carbohydrate in grapevine roots in response to water stress. In turn, some of the 'spare' carbohydrate not required for growth may be used for osmotic adjustment of roots, thus assisting in maintenance of turgor under conditions of decreasing soil matric potential. Osmotic adjustment of grapevine roots in response to water stress has been reported by Düring and Dry (1995).

There was a large increase in the rate of root development in both containers from D8 to D11 (Figure 5.10c): this may have been a response by the whole root system to drying of part of the root system. If it is assumed that drying roots produce a non-hydraulic signal, then this would have been the period of maximum root signal production. ABA has been implicated as a possible signal and Zhang and Davies (1989b) proposed the concept of a 'front' of ABA accumulation in the root system which moves deeper as the topsoil dries, promoting root development at deeper layers. In a split-root system, root growth would be stimulated in the 'wet' container in addition to the lower part of the 'dry' container, because the plant is not likely to 'discriminate' between roots in wet soil in either container. It is more difficult to suggest an explanation for the second peak in individual root growth rate in the 'dry' container from D15 which occurred without any corresponding change in the 'wet' container; this coincided with the completion of recovery of gas exchange.

A possible scenario regarding root development of split-root plants under half-drying conditions is as follows: as the soil water content of the upper layer of soil decreases in the dry container, the development of descending roots increases to permit exploitation of the moister soil beneath. Stress is known to promote root elongation (Robertson *et al.* 1990; Zhang and Davies 1989b). At the same time, some of the new, white roots in the upper/dry layer die but others survive in the short-term by becoming suberised. Maintenance of the connection between the absorbing roots in the moist soil at depth and the shoot system is most likely to be the role of roots which have undergone secondary thickening and have developed periderm. However, it is possible that a long fine root may be suberised in that part of the soil which is dry, but the apical portion of that root in moist soil may be white. There appeared to be some examples of the latter in 'dry' containers, but, because the whole of the root system was not visible on the glass it is not possible to state this with certainty. Suberisation of new roots appears to be possible in a relatively short time under conditions of water stress (Mohr 1996). In this study, the oldest of the roots which became suberised had only been in existence for 21 days. In the 'wet' container, root development is also stimulated but development (branching) is largely confined to the upper part of the container and the production of descending roots is limited.

Recovery occurs because no more roots are being dried: Recovery of gas exchange of 5BB vines in Exp. 6, which started on D11 (five days after the onset of soil drying of the 'dry' container), coincided with no further decrease in soil moisture in the 'dry' container as indicated by the depth of the 'drying margin' on the glass wall (which correlated strongly with actual measurement of soil water content in the same soil mixture in Exp. 5). As a result, there was no substantial increase in area of new roots that had dried from D12. If the root development on the glass wall was representative of the situation in the rest of the container (this was likely to be the case for at least the top 15cm or so of the container, but not necessarily for the lower part), then it is reasonable to accept the second hypothesis that *recovery occurs because there are no more roots being dried.*

It therefore follows that the next hypothesis which needs to be tested is that *recovery occurs because there are no more roots being dried and therefore no further export of a signal from those roots.* Using split-root plants, several authors have suggested that the amount of roots in drying soil determines the degree of response to half-drying and some have implicated the actual number and/or proportion of root tips in contact with dry soil as the most important factor (Tan and Buttery 1982 ; Saab and Sharp 1989; Jensen *et al.* 1989; Zhang and Davies 1989b; Ebel *et al.* 1994). For example, Ebel *et al.* (1994) found that leaf expansion rate decreased by 15% relative to the control when one container was dried whereas the decrease was ca. 25% when two were dried. Using a similar system with peach seedlings, Tan and Buttery (1982) found decreases of 4, 12 and 25% in shoot weight with one, two or three out of four containers dried respectively. As increasing numbers of roots encounter drying soil, the intensity of the signal increases (Zhang and Davies 1989b); therefore, it should follow that as decreasing numbers of roots encounter drying soil, the intensity of the signal decreases. With regard to potential decrease in the flux of the signal from roots to shoots, and thus recovery of shoot function, there are several possibilities. Firstly, transpirational flow of water from dry roots to shoots may be maintained (as a result of rehydration of 'dry' roots by 'wet' roots during the night; Saab and Sharp 1989);

however, there is a reduction in signal production as drying of new roots slows and thus there is diminished export of the signal. Secondly, signal production may be maintained in dry roots but transpirational flow from dry roots is reduced: as a result, the flux of the signal from roots to shoots is also reduced. Khalil and Grace (1993) favour the second possibility and the evidence in support of all possibilities is discussed in Chapter 10. Drying roots are known to produce ABA (eg. Robertson *et al.* 1990) and the possible role of ABA in the response of grapevines to partial drying is also examined in Chapter 10.

If recovery of shoot growth can take place without rewatering of the soil occupied by 'dried' half of the root system, then this suggests that the 'wet' roots may supply water to the 'dry' roots, thus maintaining the water-absorbing capacity in case of rewatering, allowing the plant to resume root growth under favourable soil conditions. Turner *et al.* (1996) found that water uptake by the 'wet' roots of half-dried plants increased relative to those of the 'control' plants.

<u>Movement of water from 'wet' roots to 'dry' roots</u>: Saab and Sharp (1989), Poni *et al.* (1992) and Glenn and Welker (1993) have proposed that the plant can equilibrate with the wettest part of the root system and that roots in drying soil are rehydrated during the night. The results of the ^3H_2O experiment support the hypothesis that *the roots in dried soil can survive because water moves from 'wet' roots to 'dry' roots.* Although ^3H_2O was not actually measured in the roots of the 'dry' pot, the fact that it was detected in the LD shoot within a few hours of application to 'wet' roots indicates that water can move across the graft union towards the 'dry' root system. The relatively small amount of ^3H_2O initially detected in the LW shoot, and the relative delay in movement of ^3H_2O into that shoot, was unexpected: because this shoot was closest to the 'wet' roots, one might predict that it would have been the first to show detectable amounts of ^3H_2O. The ^3H_2O was 'fed' to a very small proportion of the root system in the 'wet' pot and it is possible that, due to the sectorial nature of the xylem tissue of the grapevine stem (Shani *et al.* 1993), the LW shoot was not supplied with water from that part of the root system; as a result, the ^3H_2O measured in the LW shoot was the result of redistribution from the other shoots. Another

possibility is that the dilution of ^3H_2O with unlabelled H_2O was relatively higher for the LW shoot than the other shoot types.

Practical implications of recovery: If partial drying is to be used for vigour control in the field, the benefits of a single brief period of shoot growth depression during the season are likely to be marginal. This is probably the actual situation in many drip-irrigated vineyards with significant winter rainfall and summer drought: drying of the soil between the rows in spring may produce a signal from that part of the root system in the mid-rows which results in reduced shoot growth. However, recovery is likely to occur within a relatively short period, because the water requirements of the vines are supplied by the roots underneath the drippers, and shoot growth is restored to its previous rate. This is not to say that this one-off depression is not beneficial because if it happens at the right time, there may be some reduction in leaf area. Furthermore, it may be responsible in part for the characteristic 'switching-off' of shoot growth commonly observed in vineyards in Mediterranean-type climates. However, it is not possible to manipulate this phenomenon under such circumstances.

Therefore, significant and long-term reduction in shoot growth will only be possible if the recovery is minimised. It is unlikely that this will be achieved by a strategy whereby one half of the root system is permanently irrigated while the other half is subjected to a succession of wet and dry cycles because recovery will take place at the end of each drying period and a wetting period of appropriate length would be required to regenerate the root system on that wet/dry side so that it could respond to the next drying cycle. Although this strategy has not been tested in the field, it is unlikely to produce the desired result because it is analogous to the upper and lower parts of the profile of conventionally-irrigated vineyards, ie. the roots of the surface soil go through a series of wet/dry cycles during the season whereas the lower part of the root system may be in permanently moist soil.

Therefore, the following strategy was tested on potted vines (Chapter 7) and on field vines (Chapters 8,9) with split root systems: the drying of half the root system is alternated from one pot to the other pot, or from one side of the vine to the other, ie. while one half of the root system (called 'west' here for the purpose of explanation) is irrigated, the other half ('east') is allowed to dry, and after an appropriate period, the irrigation is switched to the east side while the west side is allowed to dry, and so on during the season. Recovery at the end of each drying period may be hypothetically minimised or even prevented by timing the switch so that it occurs at, or just before, the start of recovery: for potted vines, this would be at the time when soil water content of the dried pot approaches its minimum value.

If it is assumed that the west side is wet and the east is dry, then as SWC approaches the minimum at the end of the period, irrigation is switched to the east side and turned-off on the west. Because the west side should have many healthy root tips, drying should generate a signal from those roots which replaces the declining signal from the east roots, thus minimising the recovery of shoot growth. Even though the east side has been previously dried, water uptake should take place immediately on that side: experience with split-root plants with both pots dried to wilting point has shown that the roots are capable of absorption immediately after rewatering such that hydrature is restored within 24 hours or less). As the west roots dry, the root system on the east side, which was maintained by water supplied by the west roots during the previous cycle, undergoes regeneration and produces new roots in the upper part of the profile (the roots in the lower part of the profile have remained in a healthy state during the drying cycle). So, by the time that the irrigation is switched back to the west side, there are sufficient east roots to provide the signal.

5.5 Conclusions

a. *The initial decrease in both shoot growth rate and gas exchange in response to half-drying coincided with the decrease in soil water content of the dried half of the root system.*

b. *Recovery of shoot function of half-dried grapevines occurred without any change in soil water content of the dried half of the root system, and coincided with the point at which there was no further decrease in soil water content.*

c. *There was no effect of half-drying on* Ψ_L *at the times of greatest inhibition of SGR and* g_S *relative to control; this suggests the involvement of a non-hydraulic signal originating from the roots in drying soil.*

d. *Changes in stomatal conductance in response to half-drying were strongly correlated with shoot growth rate.*

e. *For half-dried plants, there was a relative increase in root development in moist soil layers, both in the 'wet' pot as a whole or in the lower part of the 'dry' pot.*

f. *Recovery of gas exchange of half-dried plants occurred at the time when there were no more roots dried in the 'dry' container.*

g. *For half-dried plants, that part of the root system in dry soil can survive because water moves from 'wet' roots to 'dry' roots.*

Chapter Six - The effect of genotype on response to half-drying of the root system: comparison of Shiraz and Ramsey.

6.1 Introduction

Grapevine species appeared to respond differently to half-drying of the root system in the experiments described in Chapters 4 and 5. 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 rootstocks differ with respect to both vigour and drought tolerance (May 1994).

The effect of grapevine genotype on physiology and growth has been examined in various ways. For example, self-rooted vines of different genotypes have been compared (Düring 1987, 1990; Rühl and Alleweldt 1990); in these cases, any measurable differences in physiology and/or growth may be entirely attributed to a genotype effect, either at the root level or at the shoot level, or to a combination of these. Another example would be those rootstock experiments in which the effect of different rootstock cultivars on the performance of a particular scion cultivar has been tested; in this case, any measurable differences in physiology and/or growth is usually attributed to an effect of the genotype of the root system. It may also be possible to test the effect of rootstock by comparing the effect of one or more rootstocks on the performance of a particular scion cultivar. The scion cultivar may also be included, either as a self-rooted or a homo-grafted plant (in the latter case, to exclude any possible effect of the grafting process); in this case, it is difficult to separate the different effects of scion and rootstock genotype.

Rootstock genotype can affect the performance of the scion with respect to shoot physiology, growth, fruit yield and composition (May 1994). Leaf area per vine was up to ca. 90% more when the same *Vitis vinifera* scion, grown on a vigorous rootstock, was compared with self-rooted vines (Williams and Smith 1991; Di Vaio and Boselli 1993; Sommer *et al.* 1993). Grapevine rootstock genotype may have a significant effect on gas exchange of the *Vitis vinifera* scion (Williams and Smith 1991; Sommer *et al.* 1993; Di

Vaio and Boselli 1993) but, in some cases, there has been no effect (Scienza *et al.* 1980; Natali *et al.* 1985). For example, stomatal conductance of Cabernet Sauvignon (*Vitis vinifera*) averaged 33% higher over the whole growing season when grafted to Ramsey (*Vitis champini*) than when self-rooted (Sommer *et al.* 1993). Differences due to rootstock were not associated with any change in Ψ_L but rather attributed to better water uptake and supply by the rootstock (Sommer *et al.* 1993) or higher root density (Williams and Smith 1991). Similarly, gas exchange can also differ between cultivars of the same species (Düring 1987) or between different species. For example, field vines of Carignan (*Vitis vinifera*) had higher average gas exchange values than either *Vitis riparia* or Carignan grafted to *Vitis riparia* (M. Padgett-Johnson, University of California, pers. comm.).

Genotype can also affect the response to water stress: shoot growth was reduced more for 5BB (*Vitis berlandieri* x *Vitis riparia*) than for Riesling (*Vitis vinifera*) [Rühl and Alleweldt 1990]. Under drought conditions, gas exchange values of Carignan grafted to *Vitis riparia* more closely resembled those of self-rooted *Vitis riparia* than self-rooted Carignan (M. Padgett-Johnson, University of California, pers. comm.). The stomata of drought-tolerant grapevine cultivars may be more responsive to root signals than drought-sensitive cultivars (Düring 1990).

Genotype can also affect root development, although it has been suggested that edaphic factors determine root distribution whereas genetic factors determine root density (Southey and Archer 1988; Williams and Smith 1991). Ramsey (with Sultana as scion) had both a deeper root system and greater root density than self-rooted Sultana (*Vitis vinifera*) [Nagarajah 1987].

In the experiments described in this chapter, the effect of genotype on the response to half-drying is assessed by comparing Ramsey (*Vitis champini)* with Shiraz (*Vitis vinifera)*; the former is used as a rootstock, primarily for nematode resistance but it is also known to be relatively drought-tolerant and to be very vigorous (May 1994), particularly under irrigated conditions (Hardie and Cirami 1988). Relative to Ramsey, Shiraz is more drought-sensitive and much less vigorous.

The experiments described in this chapter were conducted to test the following hypothesis: *that genotype determines the response of shoots to half-drying of the root system of potted vines.*

6.2 Materials and methods

a. Experiments 2,3

Two-year-old *Vitis vinifera* cv. Shiraz (Exp. 2) and *Vitis champini* cv. Ramsey (Exp. 3) split-root vines (approach grafts) were grown under identical conditions for 32 days from February 26. Treatments for both genotypes were: a) both pots irrigated daily (C); b) one pot not irrigated from D1 until D23, the other pot irrigated daily (T); see Chapter 4 for more details.

b. Experiment 4

Two-year-old Shiraz/Shiraz and Shiraz/Ramsey approach graft combinations (for the latter, each plant had both Shiraz and Ramsey roots and shoots; refer to Chapter 3.1 for further explanation) were grown in two, seven L pots per plant in a shade-house 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 low benches in 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.

Treatments were: a) both of Shiraz/Shiraz pots irrigated daily ('S+S+'); b) one pot of Shiraz/Shiraz not irrigated from D1 until D23, the other pot irrigated daily (S+S-); c) both of Shiraz/Ramsey pots irrigated daily ('S+R+'); d) one pot of Shiraz/Ramsey containing Ramsey roots not irrigated from D1 until D23, the other pot with the Shiraz roots irrigated daily (S+R-). Plants were blocked on the basis of stomatal conductance and treatments allocated at random (three replicates per treatment). 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 shoot growth rate calculated as cm/day since the previous measurement. The increase in node number per shoot was determined at intervals from four to six days. For treatments S+S- and S+R- , 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'. Only the Shiraz shoots were measured. 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+S- were pooled with that of S+S+ and S+R+ and data from 'dry' pots of S+R- and S+S- were pooled. Stomatal conductance was measured on four leaves per shoot on D18 between 1030 and 1200h with a Delta T porometer. Leaf water potential was measured on two leaves per shoot between 1300 and 1430h on D18 and D21.

c. Experiment 10: see Chapter 10 for details

6.3 Results

a. Experiments 2,3

The shoot growth rate (SGR) on Shiraz plants was greater than that on Ramsey (Figure 6.1a), particularly from D15 onwards: average SGR for the whole period was 3.17 ± 0.47 and 1.76 ± 0.22 cm/day respectively. By comparison, there was little difference in g_S over the same period (Figure 6.1b): average g_S was 420 ± 14 and 403 ± 22 mmol/m^2/s for Shiraz and Ramsey respectively. The response of SGR to half-drying was similar but the recovery was slightly delayed for Ramsey relative to Shiraz (Figure 6.2). However, for g_S (Figure 6.2), the response was different: the lowest value of g_S relative to the control occurred approximately 9 days later for Ramsey than Shiraz. Furthermore, the magnitude of the reduction relative to control was less for Ramsey than Shiraz, and the recovery after rewatering was delayed.

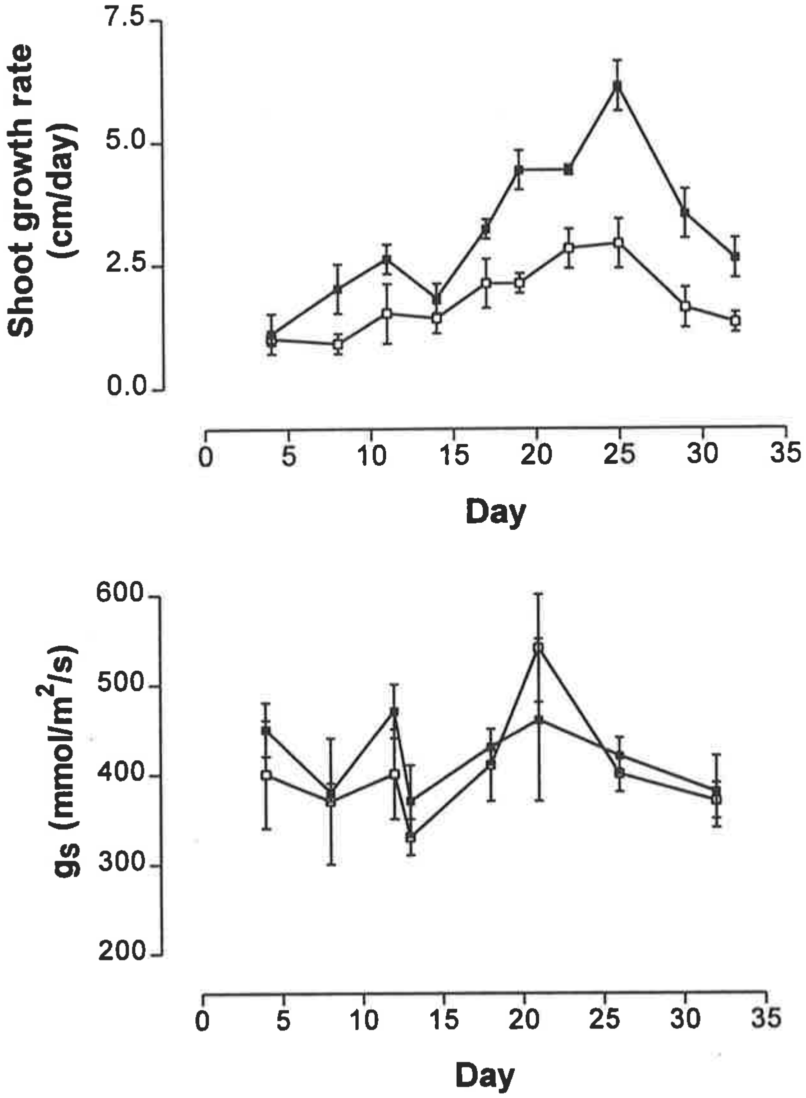


Figure 6.1 Effect of genotype on shoot growth rate (SGR) and stomatal conductance (g_S): 'control' Shiraz, Exp. 2 (■) and 'control' Ramsey, Exp. 3 (□).
(a) SGR(mean ± se, cm/day)
(b) g_S (mean ± se, mmol/m^2/s)

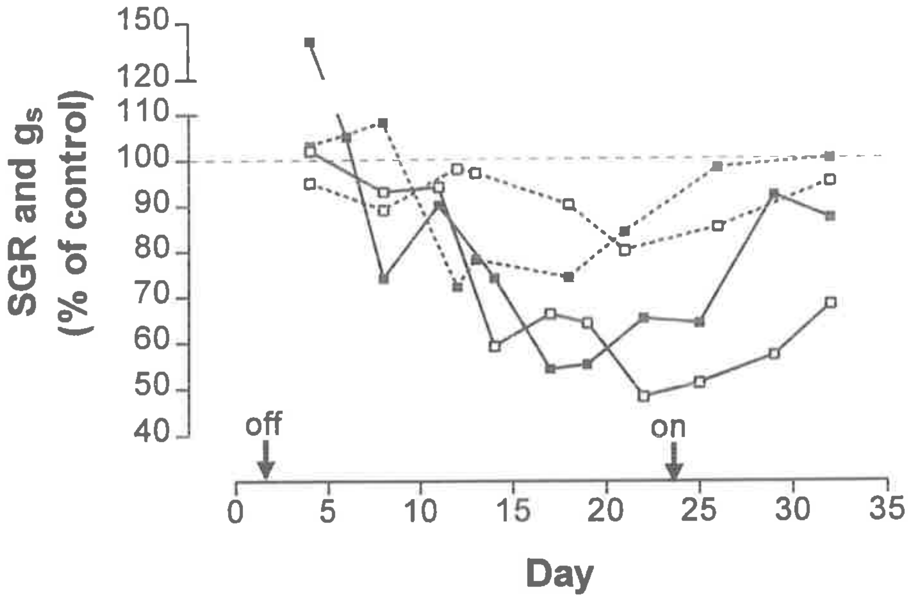


Figure 6.2 Effect of genotype on response to half-drying: shoot growth rate (SGR; solid lines) and stomatal conductance (g_S; dotted lines) of 'treated' as % of 'control' Shiraz, Exp. 2 (■) and Ramsey, Exp. 3 (□). 'Dry' pot of 'treated' not irrigated from D2 to D23.

c. Experiment 4

There was no difference in SGR of Shiraz shoots between plants with all roots as Shiraz (S+S+) and those with half the roots as Shiraz and the other half as Ramsey (S+R+) when both pots were irrigated; similarly for final shoot length increment (Table 6.1). On the other hand, there were differences in both SGR and g_S of Shiraz (S+) shoots when the dried half of the root system was Ramsey (S+R-) rather than Shiraz (S+S-). From D13 to D18 inclusively, shoot growth rate of S+R- was significantly greater than S+S- (Figure 6.3).

Table 6.1 Effect of genotype of root system on final shoot length increment (mean ± se, cm) on D32, and leaf water potential Ψ_L (MPa) and stomatal conductance g_S (mmol/m^2/s) on D18, Exp. 4. Refer to text for details of treatments. Means in columns followed by different letters are significantly different ($p < 0.05$).

	Shoot length	g_S	Ψ_L
S+S+	100 ± 6	430 c	-0.85 a
S+S-	77 ± 10	280 a	-0.90 a
S+R+	99 ± 12	-	-0.93 a
S+R-	87 ± 10	380 b	-0.88 a

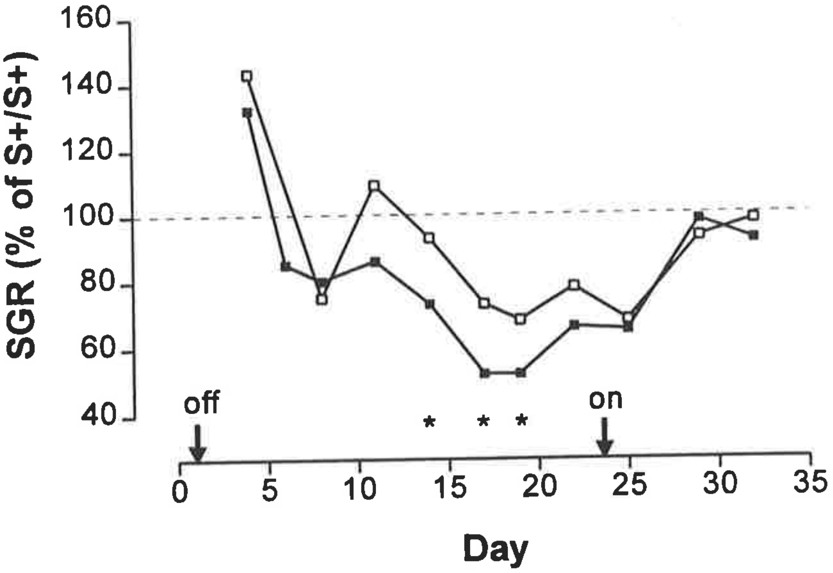


Figure 6.3 Effect of genotype of root system on shoot growth rate (SGR) in response to half-drying, Exp. 4. S+ shoots of S+S- (■) and of S+R- (□) as % of S+S+. 'Dry' pot of S+S- and of S+R- not irrigated from D2 to D23. S+S- and S+R- are both significantly different ($p<0.05$) to S+S+ on D13 to D25 inclusively; * indicates those days when S+R- significantly different ($p<0.05$) to S+S-.

SGR of S+S- was significantly less than S+S+ from D13 to D22 but SGR of S+R- was not significantly lower than S+S+ until D18. Stomatal conductance of both S+R- and S+S- was significantly less than that of S+S+ on D18 but S+R- was significantly higher than S+S- (Table 6.1). By comparison, there were no differences between any treatments for Ψ_L on either D18 (Table 6.1) or D21.

c. Experiment 10

Stomatal conductance of Shiraz shoots of S-R+ was lower relative to Shiraz shoots of S+R+ in response to drying of the roots in the Shiraz pot (Figure 6.4). However, when the dried half of the root system was Ramsey (S+R-) rather than Shiraz (S-R+), the response was delayed by ca.10 days and the magnitude of the response was much less for S+R- than for S-R+ (minimum levels of 54 and 84% relative to S+R+ respectively). There were no differences between treatments for Ψ_L of Shiraz shoots on D15.

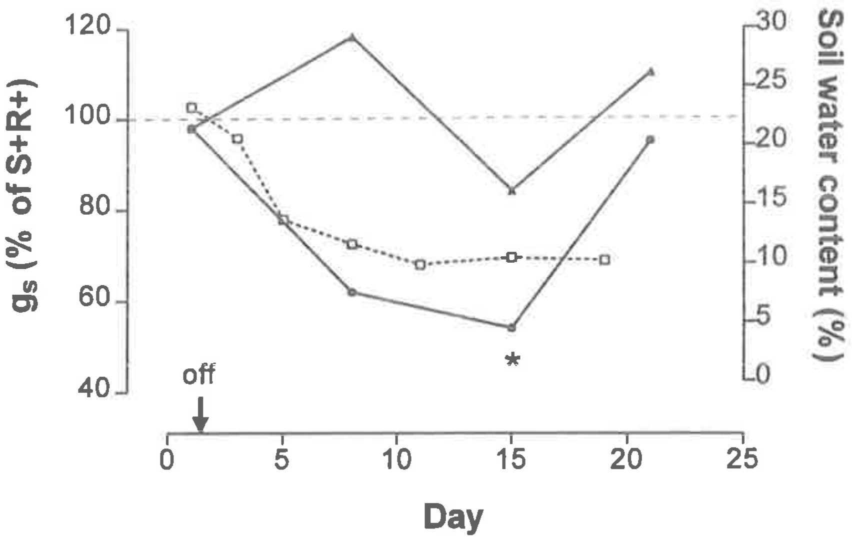


Figure 6.4 Effect of genotype root system on stomatal conductance (g_S; mmol/m^2/s) in response to half-drying, Exp. 10. Mean of Shiraz shoots of S-R+ (●) and S+R- (▲) as % of S+R+. Soil water content: mean of 'dry' (□) pots of all treatments; 'dry' pot not irrigated from D2 ('off'). * indicates g_S of S-R+ significantly different ($p<0.05$) to S+R-; S-R+ significantly different ($p<0.05$) to S+R+ on D15.

6.4 Discussion

Comparison of *Vitis vinifera* (Shiraz) with *Vitis champini* (Ramsey): The shoot growth rate of self-rooted Shiraz was greater than that of self-rooted Ramsey when grown under the same well-watered conditions. However, in these experiments, root volume was restricted by the size of the pots and there was no opportunity for the potentially more exploratory root system of the Ramsey to be expressed. Therefore, differences in shoot growth may be due to more production of assimilate by *Vitis vinifera* but, if this is the case, it is not due to greater g_S because there was no difference between species. Therefore, *Vitis vinifera* leaves may have greater carboxylation efficiency than *Vitis champini*. There are no known reports on the gas exchange properties of *Vitis champini* cultivars. On the other hand, under fully-watered conditions, there was no effect of rootstock genotype on the growth rate of Shiraz scion shoots, ie. S+S+ or S+R+. This is something of a paradox because it is known that if the whole root system is made up of Ramsey (eg. when *Vitis vinifera* cultivars are grafted to Ramsey), shoot vigour of the scion is greatly increased relative to self-rooted plants (ie. where the whole root system is *Vitis vinifera*) [Sommer *et al.* 1993]. However, this is the case with field vines where there may be differences in the size of the root system: *Vitis champini* has a more exploratory root system than *Vitis vinifera* (Nagarajah 1987). These results suggest that the excess vigour of *Vitis vinifera* scions, that is commonly observed when Ramsey is used as a rootstock in well-watered situations in the field, is a function of the size of the root system. It is possible that this vigour will not be 'expressed' if the size of the root system is restricted by soil factors or by cultural practices such as root pruning or irrigation technique.

Response to half-drying: The two species responded in a different manner to half-drying: Ramsey was slower to respond, the magnitude of inhibition relative to the control was less and recovery was delayed relative to Shiraz (Figure 6.2). But, unlike the comparison of well-watered plants, in this case, the difference in response was greater for g_S than for SGR.

When the dried half of the root system was Ramsey (S+R-), the response to half-drying (expressed by the Shiraz scion shoots) was different to those plants where the dried half comprised Shiraz (S+S-) [with Shiraz roots as the 'wet' half in both cases]. Shoot growth rate of S+R- was slower to respond than S+S- and the magnitude of the response was less (Figure 6.3). Similarly, g_S of S+R- was significantly higher than S+S- on D18 (Table 6.1), the time of maximum inhibition of SGR relative to the control. Likewise, when the dried half of the root system was Ramsey (S+R-), the response to half-drying (expressed by the stomatal conductance of Shiraz scion shoots) was different to those plants where the dried half comprised Shiraz (S-R+): relative to the S+R+ control, S+R- was slower to respond than S-R+ and the magnitude of the response was less (Figure 6.4). As shown by others (Williams and Smith 1991; Sommer *et al.* 1993), differences in shoot growth and stomatal conductance in this study were not the result of any hydraulic effects.

These results suggest that Ramsey roots are less responsive to soil drying than Shiraz. Commercial experience with *Vitis vinifera* grafted to Ramsey has led to the conclusion that

Ramsey is a drought-tolerant rootstock (May 1994). If a characteristic of drought tolerance is an ability to rapidly respond to soil drying by the production of root signals, as suggested by Düring (1990), then Ramsey does not appear to satisfy that criterion when grown under conditions where the size of the root system is limited. Therefore, the apparent drought tolerance of Ramsey under field conditions may be a function of its vigorous root system which, under drought stress, can extract limited supplies of water from the soil more efficiently than *Vitis vinifera.*

Although the hypothesis *that genotype affects the response of shoot function to half- drying of the root system of potted vines* may be accepted, there are a number of unanswered questions arising from the experiments described in this chapter: for example, is the response to partial drying of *Vitis vinifera* grafted to Ramsey different under field conditions to self-rooted *Vitis vinifera*? It is worth mentioning that the field vines used in the experiments described in Chapters 8, 9 and 10 were *Vitis vinifera* (cv. Cabernet Sauvignon) grafted to Ramsey and these vines were most responsive to partial drying. There is obviously a need for more research on the mechanism of drought tolerance in *Vitis* species. However, the use of conventional rootstock experiments may not be appropriate for this purpose because it is difficult to separate the effects of the scion and the rootstock and their interaction.

6.5 Conclusions

a. *Genotype may affect the response of shoot function to half-drying of the root system*

b. *Ramsey (Vitis champini) roots appeared to be less responsive to soil drying than Shiraz (Vitis vinifera)*

c. *The genotype of the root system had more effect on leaf gas exchange under half-drying conditions than under well-watered conditions*

d. *The apparent vigour and drought tolerance of Ramsey as a rootstock under field conditions may be a function of its extensive root system*

Chapter Seven - Alternation of drying of half of the root system of container-grown grapevines maintains the inhibition of shoot growth.

7.1 Introduction

Significant and long-term reduction of shoot growth using partial drying of the root system will only be possible if the recovery described in previous chapters is minimised. It is unlikely that this will be achieved by a strategy whereby one half of the root system is permanently irrigated while the other half is subjected to a succession of wet and dry cycles (see 5.4). Therefore, the following strategy was tested on container-grown vines with split-root systems in this chapter (and on field vines in Chapters 8 and 9): drying of half of the root system was alternated from one half to the other half. Recovery at the end of each drying period should be hypothetically minimised, or even prevented, by appropriate timing of the alternation.

There have been many pot experiments with grapevines where the effect of various levels and timing of stress on shoot growth, gas exchange and water relations have been examined e.g Hardie and Considine (1976), Poni *et al.* (1992), Reynolds and Naylor (1994). In general, increasing severity of water stress reduces vegetative growth and stomatal conductance, often in association with a decrease in Ψ_L. Reynolds and Naylor (1994) found that water stress particularly reduced number and length of lateral shoots. There have been relatively few long-term (eg. > 20 days) experiments with half-drying of woody plant species. For example, Tan and Buttery (1982) found that dry weight of shoots of peach seedlings was reduced by 12% at the end of the half-drying period. Similarly, Poni *et al.* (1992) showed that the shoot fresh weight (average of four woody species) of half-dried plants was reduced by 17% relative to the fully-watered controls. However, in these cases, there was no alternation of drying from one side to the other.

Water stress usually affects shoot growth to a greater extent than root growth (Sharp and Davies 1989; Setter 1990; Saab *et al.* 1990; Munns and Sharp 1993). Root growth may continue when shoot growth has been entirely inhibited, resulting in a decreased ratio of shoots to roots [S/R ratio] (Munns and Sharp 1993). ABA has often been implicated in the mediation of this differential root and shoot growth response (Sharp *et al.* 1994). Endogenous ABA accumulation at low soil water potential both maintains primary root elongation and inhibits shoot elongation (Saab *et al.* 1990; Sharp *et al.* 1994). The S/R ratio is used to provide a quantitative measure of the relationship between below- and above-ground growth. The S/R ratio was not changed in response to half-drying of the root system of some species (Tan and Buttery 1982; Poni *et al.* 1992; Ebel *et al.* 1994).

Experiment 12 described in this chapter was conducted to test the hypothesis: *that alternation of drying of half of the root system of container-grown vines maintains the long-term inhibition of shoot growth.*

The results of the experiments described in Chapters 4, 5 and 6 clearly indicated that, during half-drying, the rate of decrease of soil water content of the 'dry' container would eventually slow. The particular value of SWC at which this 'levelling-off' occurred was higher than anticipated from experiments where the whole of the soil was allowed to dry (for example, in Exp. 1, the SWC of the 'dry' pot of the WD treatment levelled off at 8.0% whereas the average SWC of both pots of the DD treatment decreased to 4.8%; Figure 4.2). This suggests that the 'wet' roots are able to take up sufficient water to meet the needs of the plant before all of the available water in the soil of the 'dry' container is depleted, while the soil matric potential remains relatively high. Therefore, if the soil in the 'wet' container was allowed to dry once the SWC of the 'dry' container had levelled-off, then the 'dry' roots may resume their water-absorbing activities (having been maintained by water transferred from 'wet' roots) and further deplete the 'dry' container. This was unintentionally tested during the course of Exp. 12: over a period of three days, the 'wet' pots of both treatments were not irrigated due to failure of the automated irrigation system. As a result of the observations made as a consequence of this event, a controlled experiment (Exp. 12a) was conducted to test the hypothesis that: *if the soil in the 'wet' container is allowed to dry after the SWC of the 'dry' container of half-dried plants has levelled off, the SWC of the 'dry' container will further decrease.*

7.2 Materials and methods

a. Experiment 12

Four-year-old *Vitis vinifera* cv. Shiraz (clone 12) split-root vines (approach grafts) were grown in two, seven L pots per plant. Plants were grown in the open for the whole of the growing season (Figure 3.3). Two weeks prior to the start of the experiment on December 8 (D1), all vines were thinned back to four shoots per plant (two 'upper' and two 'lower'; see Chapter 3.1 for further explanation); those shoots were topped, reduced to six mature leaves per shoot and all bunches and lateral shoots removed except for one terminal lateral, usually at the most distal node (at the time, the terminal lateral had two to four leaves and was less than 15cm long). The main shoot and the terminal lateral were trained vertically upwards. Each pot was irrigated with two, 2 L/h drippers and, prior to the start of the experiment, all pots were irrigated four times daily. The vines were de-fruited because it is known that crop load can affect both water-use (Loveys and Kriedemann 1974) and response to water stress (Poni *et al.* 1993) of potted vines and it was considered desirable to remove the possible confounding influence of this factor.

Treatments were: a) both pots irrigated four times daily (C); b) one 'dry' pot not irrigated from D4 until D70, the other 'wet' pot irrigated four times daily (T). The 'dry' pot was switched from one side to the other every 10 days on average (days 12, 22, 33, 43, 54) resulting in six 'cycles'. The schedule was determined in the following way: when these vines were two years old and were growing in these pots for the first season in the same soil mix, at least 10 days were required for the soil water content to reach a minimum level in the 'dry' pots (Figure 4.4) and 18 days before shoot growth rate (SGR) of T plants started to recover relative to C (Figure 4.5). When the plants were one year older, and the soil mix was fully explored by roots at the start of the experiment, seven to 10 days (Figures 5.5, 5.7) were required for the soil water content to reach a minimum level and recovery of stomatal conductance started from seven to 13 days after the start of drying of the 'dry' pots (Figures 5.5, 5.7). In this experiment, the vines were four years old and had not been re-potted for two years; therefore, the soil was fully explored by roots at the start of the experiment. For the initial drying cycle starting at D4, measurements of soil water content showed that the minimum was reached after eight days of drying. Therefore, a 10 day cycle was chosen for this experiment so that the switch would take place before the start of recovery of SGR. In practice, two of the cycles were 11 days due to unavoidable circumstances. The last cycle was deliberately prolonged (16 days) in order to examine possible differences between roots of 'dry' and 'wet' pots of the T vines, and to determine if recovery of shoot growth could take place on T vines. Treatments were chosen at random with four T and four C vines. For the T plants, the shoots on the side when the pot was irrigated were designated as 'wet', and those on the side when the pot was non-irrigated as 'dry'.

The increase in the length of the terminal lateral shoot (reference node = most basal node) was measured every five days on average and the shoot growth rate calculated as cm/day since the previous measurement (mean of four shoots per plant). The total shoot length and node number per shoot of all four terminal laterals per plant were measured on D70 and the average internode length (=shoot length/node number) calculated. 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'. Stomatal conductance (g_S) was measured on D3 and subsequently on six other occasions, usually in the middle of a cycle in sunny conditions, between 1030 and 1230h, with a Delta T porometer. Four leaves of similar maturity on the two upper shoots were used on each occasion (basal leaves were replaced by more distal leaves during the course of the experiment). Soil water content was measured weekly by time domain reflectometry. Leaf water potential was measured at 1300h on D27, 39 and 50 (mid-cycle) under sunny conditions on two leaves per plant on upper shoots.

On D70, three replicates were harvested. The shoot system ('top') was detached from the root system by cutting through the trunk at soil level and separated into the following components: a) trunk; b) stems and leaves present at the start of the experiment ('old'); c) stems and leaves which had grown since the start of the experiment ('new'). In turn, these were divided into stems, leaves. After weighing ('fresh weight'), they were dried at 60°C for 6 days, then reweighed ('dry weight'). Soil was carefully washed from the root system, weighed ('fresh weight') and stored in plastic bags at 2°C for three months; the root system from the pot which had not been irrigated for the previous 16 days was labelled. Roots were sub-divided into the following diameter (mm) classes: a) < 2; b) 2 to 3.9; c) 4 to 8; d) > 8. All components were dried at 60°C for 6 days, then reweighed ('dry weight').

b. Experiment 12a

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. From Day 1, one pot of each of six plants was not irrigated while the other pot of each plant was well-irrigated. On Day 17 (after the SWC of the 'dry' pots had been relatively constant for the previous five days), the irrigation was turned off on the 'wet' pots of the three plants with the highest SWC values (= 'Treatment A'), ie. neither the 'wet' pot nor the 'dry' pot of this treatment were irrigated from Day 17 onwards. The other three plants were kept as half-dried (= 'Treatment B'). Soil water content was measured daily by time domain reflectometry.

7.3 Results

a. Experiment 12

Soil water content of the 'dry' pot of T plants averaged 9.1 ± 0.4 % from D9 to D70 compared to 26.2 ± 0.9 % for the 'wet' pots of both T and C plants. In response to the alternated drying of one pot, shoot growth rate of the T plant initially decreased within one week to a level which was approximately 50% of the C rate. SGR stayed at that level until the last extended period when there was a slight recovery (Figure 7.1, Table 7.1).

SGR of 'wet' and 'dry' shoots (1.05 ± 0.21 and 1.01 ± 0.21 cm/day respectively) was not significantly different. As a result of the significantly lower SGR of T plants, the final shoot length was 42% less relative to C, mainly due to a significant reduction in node number (-36%).

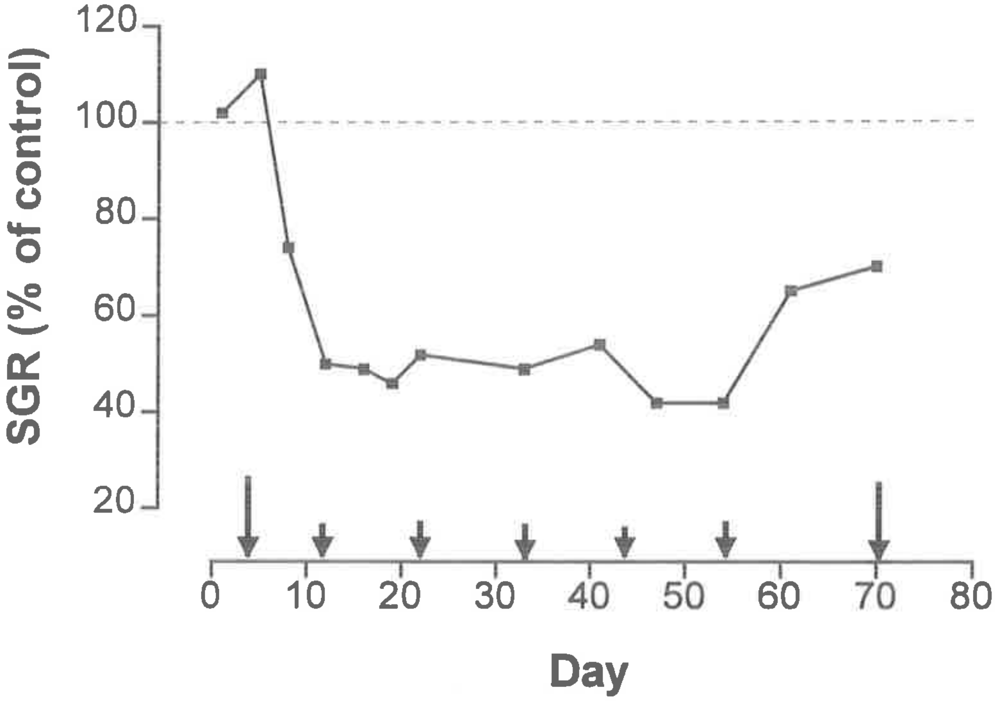


Figure 7.1 Response of shoot growth rate (SGR, cm/day) to alternation of drying of split-root Shiraz, Exp. 12; 'treated' (T, mean of 'wet' and 'dry' shoots) as % of 'control' (C). At any time, one pot of T plants not irrigated from D4 to D70 (long arrows); the other pot of T plants and both pots of C plants irrigated from D1 to D70; drying of one pot of T plants alternated five times (short arrows indicate days). T significantly different ($p<0.05$) to C from D12.

There was a lesser effect on average internode length (-12%). Average g_S of T plants was also significantly reduced (-33% ; Table 7.1) but there was no significant effect of treatment on Ψ_L (Table 7.1). Dry weight of new shoot growth (stems and leaves) was reduced by 34% in response to treatment due to a combination of a reduction in new leaf and new stem weight. The reduction in new leaf weight was due to a combination of fewer leaves and decreased mean leaf weight (Table 7.2). New leaf area was 29% lower on T plants due to a combination of reduced leaf number (-20%) and smaller leaves (-14%)[Table 7.2]. The total weight of the top, excluding the trunk, was reduced by 25% in response to treatment.

Table 7.1 Response of shoot growth, stomatal conductance (g_S) and leaf water potential (Ψ_L) to alternation of drying of split-root Shiraz, Exp.12. At any time, one pot of 'treated' (T) plants not irrigated from D4 to D70; the other pot of T and both pots of 'control' (C) plants irrigated from D1 to D70; drying of one pot of T plants alternated five times.

Variable	C	T	% diff.	Signif.
SGR [a], average: D6-D70 (cm/day)	1.78 ± 0.15	1.03 ± 0.18	-42	<0.01
Shoot length (cm)[b]	114 ± 10	66 ± 11	-42	<0.01
Node number [b]	20.4 ± 1.5	13.2 ± 1.1	-36	<0.01
Internode length (cm)[b,c]	5.6 ± 0.3	5.0 ± 0.2	-12	ns
g_S, average: D9-D67 (mmol/m^2/s)	460 ± 41	313 ± 52	-33	<0.01
Ψ_L, average: D27, D39, D50 (MPa)	-0.79 ± 0.08	-0.83 ± 0.11	-5	ns

[a] Mean of 4 terminal lateral shoots/plant; [b] Terminal lateral shoot at D70; [c] = shoot length/node number

There was less effect on root weight than top weight (in part because it was not possible to discriminate between 'new' and 'old' roots): total root weight excluding the trunk was 11% less for T plants. The ratio of total top/total roots was reduced by only 7% but the ratio of new shoots/roots (excluding the trunk) was 26% less (Table 7.2). The main effect of treatment on the root system was on the 4 to 8mm class (-30%) with less effect on the < 2 mm class (-11%). Approximately half of the total root dry weight comprised roots < 2 mm (Table 7.2). The roots in the 'dry' pots had not been watered for 16 days prior to harvest: there was no component of dry weight for which there was a significant difference between 'dry' and 'wet' root weight (Table 7.3), nor was there any difference in fresh weight/dry weight ratio. 'Dry' roots (excluding trunk) weighed 17% less with the biggest effect on the 2 to 3.9mm category (-39%). Visually, there was no obvious difference between 'wet' and 'dry' pots, nor between those of the T and C plants.

Between D16 and D19, the 'wet' pots of both treatments were not irrigated for part of this period due to failure of the automated irrigation system: as a result, the mean SWC of the 'wet' pots of T vines decreased from 27.5% (average of the previous two measurements) to

14.2%; at the same time, mean SWC of 'dry' pots of T vines decreased from 9.1% (average of the previous two measurements) to 5.3%.

b. Experiment 12a

SWC of the 'dry' pots of both treatments decreased from ca. 27% on D1 to level off at ca. 8-10% between D11 and D16 (Table 7.4). The 'wet' pots of Treatment A plants were not irrigated from D17: as a result, SWC decreased from ca. 27% to 5.2% by D27,

Table 7.2 Effect of alternation of drying of split-root Shiraz on shoot and root components (mean ± se), Exp.12; treatments as for Table 7.1.

Variable	**C**	**T**	**% diff.**	**Signif.**
New leaf number	269 ± 10	216 ± 10	-20	<0.05
New leaf area (m^2)	1.12 ± 0.07	0.79 ± 0.06	-29	<0.05
Mean area/new leaf (cm^2)	42 ± 1	36 ± 2	-14	ns
New leaf density ($g/cm^2 \times 10^2$)	2.17 ± 0.06	2.15 ± 0.05	-1	ns
Mean dry wt /new leaf (g)	0.30 ± 0.03	0.27 ± 0.02	-10	ns
Dry weight/plant (g)				
New leaves	82 ± 7	59 ± 3	-28	<0.05
Total leaves	112 ± 18	84 ± 12	-25	ns
New stem	72 ± 13	42 ± 6	-41	ns
Total stem	133 ± 29	100 ± 12	-26	ns
New leaf + stem	1545 ± 20	101 ± 9	-34	<0.07
Total top - trunk	244 ± 42	184 ± 23	-25	ns
Trunk	133 ± 9	127 ± 3	-5	ns
Total top	377 ± 51	311 ± 21	-18	ns
Roots - trunk	218 ± 23	194 ± 9	-11	ns
Total bottom	287 ± 26	255 ± 9	-11	ns
Roots > 8 mm diameter	3.0 ± 1.6	3.3 ± 3.3	+ 11	ns
4 -8 mm	41 ± 4	29 ± 6	-30	ns
2 -3.9 mm	56 ± 20	57 ± 6	+ 2	ns
< 2 mm	118 ± 6	105 ± 10	-11	ns
Top/bottom	1.31 ± 0.09	1.22 ± 0.04	-7	ns
New stem + leaves/roots	0.70 ± 0.04	0.52 ± 0.03	-26	<0.05

Table 7.3 Dry weight (g) and fresh weight/dry weight ratio[a] of 'wet' and 'dry' roots of different class diameters (<2, 2-3.9, 4-8, >8 mm) of partially-dried Shiraz split-root vines, harvested on D70; Exp.12. The 'dry' pot had not been irrigated for the 16 days prior to sampling.

Category	Wet	Dry	% diff.	Signif.
Dry weight				
> 8	3.3	0	0	ns
4 - 8	13.0	15.7	+21	ns
2 - 3.9	35.4	21.8	-39	ns
< 2	54.8	50.6	-8	ns
Total	**106.5**	**88.1**	**-17**	**ns**

concurrently with a further decrease in SWC of the 'dry' pots from 9.6% to 6.8%. By comparison, the 'wet' and 'dry' pots of Treatment B plants remained at ca. 27% and 8% respectively over the same period.

Table 7.4 Volumetric soil water content (mean ± se, %) of 'wet' and 'dry' pots of half-dried, split-root plants, Exp. 12a. 'Dry' pot of both treatments not irrigated from D1; 'wet' pot of both treatments irrigated from D1, but not irrigated from D18 for Treatment A only.

Period	**Treatment A**		**Treatment B**	
	'Wet'	**'Dry'**	**'Wet'**	**'Dry'**
D11 - D16	27.0 ± 0.2	9.6 ± 0.2	27.5 ± 0.3	8.4 ± 0.1
D22 - D27	5.2 ± 0.3	6.8 ± 0.3	27.0 ± 0.4	8.0 ± 0.3

7.4 Discussion

By alternation of the drying from one side to the other, it was possible to maintain the SGR of half-dried plants at 58% of the control rate over a period of 60 days. Over the same period, average g_S was reduced by 33%. The choice of a 10 day schedule for the second, third, fourth and fifth cycles was somewhat arbitrary and, in hindsight, it is likely that a weekly cycle would have been as effective. In any event, SGR showed no signs of recovering at any stage, with the exception of the last period which was deliberately prolonged. Also, the fact that SGR only averaged ca. 50% of the control rate over the whole period is an indication of the success of this treatment. The effect on vegetative growth in response to treatment was substantially greater than that reported by Poni *et al.* (1992) where drying was maintained on the same half of the root system, for a similar time period (ca. 65 days). For example, a reduction in leaf area of 41% was measured in this study whereas Poni *et al.* (1992) reported a decrease of 11% in response to half-drying.

Similarly to the experiments described in Chapter 4, the reduction in shoot length in this experiment was mainly due to an effect on node production rather than mean internode length. However, average internode length was calculated for the whole shoot length increment and this does not differentiate between internodes which elongated at different stages of shoot development. Nevertheless, Gowing *et al.* (1990) also 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. Reduced rate of node initiation resulted in decreased leaf number and thus smaller leaf area per plant; there was a lesser effect on the rate of leaf expansion as indicated by leaf weight and mean area per leaf. The effect of partial drying on the components of vegetative growth is discussed in detail in Chapter 9.

There was no significant effect of treatment on total plant root dry weight: this was a similar result to Poni *et al.* (1992) and Kosola and Eissenstat (1994). On the other hand, Tan and Buttery (1982) found that total root weight of half-dried peach seedlings increased by 20% relative to the controls after 21 days and that two-thirds of this total root dry weight was in the 'wet' container, ie. growth of the 'wet' half of the root system was stimulated in response to drying of the other half. The relative stimulation of root growth in the wet container of half-dried plants was also reported by Poni *et al.* (1992) and Kosola and Eissenstat (1994). The difference in response to half-drying between these pot experiments with respect to total plant root weight may be a result of differences in the volume of soil available for exploitation by new roots. The result of Tan and Buttery (1982) is similar to the results from field experiments where total root biomass of droughted plants may increase relative to that of well-watered plants (Sharp and Davies 1989) because growth of roots in the wetter zones is enhanced. In the grapevine experiment reported in this chapter, the soil in the pots was fully-exploited by roots before the start of the experiment. Perhaps if this experiment had been conducted with larger containers and the roots had been trimmed before the start of the experiment, there may have been an increase in total root weight in response to half-drying. The potential for root development in the lower, moist soil in 'dry' containers, in response to drying of the upper part, was demonstrated in Chapter 5.

Disregarding the roots >8mm diameter, which represent less than 2% of total root dry weight, the major difference between treatments was for the 4 to 8mm diameter category which weighed 30% less for the half-dried treatment (but this was not statistically significant). This category made up 15 to 20% of total root dry weight. The fact that there was no significant difference for the <2mm category suggests that new roots were produced on the treated plants during each 'wet' cycle. After 16 days of drying, there was no statistically-significant difference between 'wet' and 'dry' root systems for any category; however, the weight of <4mm diameter roots (which comprised 85% of the total root dry weight) was 20% lower for 'dry' than 'wet', which suggests that small roots grew preferentially in the upper part of the 'wet' pot during each cycle. It is possible that the expenditure of carbohydrate for growth of small roots, as the root system in the 'wet' pot regenerated every cycle, reduced availability of carbohydrate for maintenance of the larger (4 to 8mm) roots. Carbon allocation to roots in dry soil decreased at the expense of roots in wet soil as the intensity of drying increased with *Citrus* seedlings (Kosola and Eissenstat 1994); as a result, there was some shedding of larger roots in both pots of the half-dried plant in the long-term as the plant attempted to reduce this maintenance cost. Shedding of roots, particularly in surface soil, is known to increase in response to drought with some species. When the costs of maintaining roots in a region of dry soil exceed the costs of constructing new roots in moister soil, the roots in the dry region of soil should theoretically be shed (Kosola and Eissenstat 1994). However, it is not known if this occurs with the grapevine.

The actual values of the ratio of shoots to roots for the control vines are similar to those reported for field vines by Mullins *et al.* (1992). Because there was a relatively smaller effect on root dry weight than shoot dry weight in response to treatment, the ratio of new shoot growth/total root was 26% higher on treated plants. By comparison, Tan and Buttery

(1982) and Poni *et al.* (1992) found that half-drying had no effect on shoot/root ratio of woody plants; in the latter case, this was calculated as shoot fresh weight /new root fresh weight so it is difficult to exactly compare this with a S/R ratio based on total root weight. On the other hand, the data of Kosola and Eissenstat (1994), from an experiment where the roots of seedlings of several *Citrus* species were horizontally divided between two pots, indicated that half-drying may reduce S/R ratio. Shoot dry weight /total root dry weight (calculated from their data) was reduced by 10% and 24% for trifoliate orange and citrange respectively. It is important to point out that the other half-drying experiments cited here did not alternate the drying from one pot to the other. Therefore, the plants in those other studies may not have responded in a similar fashion to the half-dried grapevines in this experiment. It is possible that partial drying of field vines will increase S/R ratio because those roots have more opportunity for growth than in containers. ABA is thought to be involved in mediating this differential root and shoot growth response by stimulating root growth and inhibiting shoot elongation (Sharp *et al.* 1994). There was no significant effect of treatment on Ψ_L in this experiment, confirming that the response was most likely mediated by a non-hydraulic signal produced by the roots in drying soil. Evidence in favour of the involvement of ABA in response to partial drying of the root system is discussed in Chapter 10.

Shoot growth was more responsive to alternated half-drying than stomatal conductance: this is a desirable situation for the application of this technique to vines in the field. It appears from the results described in Chapters 4 to 7 that a partial reduction in stomatal conductance (and thus a concomitant reduction in assimilation) will be unavoidably associated with any substantial reduction in shoot growth in response to half-drying of grapevines. However, because of the nature of the relationship between g_S and Pn, a partial reduction of g_S (of the order measured in this experiment or less) will have a relatively small effect on Pn, and, at the same time, water-use efficiency will be increased (Düring 1987; Düring *et al.* 1996).

It may be argued that a third treatment, ie. maintenance of one pot dried without alternation, could have been included in this experiment. The addition of this treatment was considered at the time of planning the experiment; however, it was thought to be an unnecessary use of resources because the results of the experiments described in Chapter 5 had shown that shoot growth would recover if plants were maintained in a non-alternated, half-dried state. Furthermore, the relative recovery of the half-dried plants in this experiment during the last, deliberately-prolonged period (Figure 7.1) vindicated the decision to exclude that potential treatment.

The results of this experiment allow the hypothesis *that alternation of drying of half of the root system of potted vines maintains the long-term inhibition of shoot growth* to be accepted. The response of field vines to this technique will be tested in experiments described in Chapter 9. Hereafter, the term 'partial root-zone drying' (PRD) will be used to describe the technique whereby the drying is alternated from one part of the root system to the other, to distinguish it from those experiments where there is no alternation (the term 'half-drying' has been used in those cases).

The results of the experiments described in previous chapters indicated that shoot function of half-dried plants may recover before all of the plant-available water had been extracted from the 'dry' pot. This was confirmed by the results of Exp. 12a, together with the observations made in Exp. 12. In Exp. 12a, the SWC of the 'dry' pots decreased from 9.6% to 6.8% as the water supply from the 'wet' pots was depleted: although this is a small amount in terms of volumetric water content, it represents a relatively large decrease in terms of matric potential in this particular soil mix over that range of SWC. It is reasonable to assume that the SWC of the 'dry' pots was further reduced because the 'dry' roots resumed their water-absorbing activities (having been maintained by water transferred from 'wet' roots).

7.5 Conclusions

a. *Alternation of drying (six cycles in 66 days) of half of the root system of potted grapevines maintained shoot growth at a substantially lower rate than the well-watered control.*

b. *Components of shoot growth were reduced by up to 40% in response to half-drying over a period of 66 days while average stomatal conductance was reduced by ca. 30%.*

c. *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.*

d. *The rate of node initiation was affected more than the elongation of internodes or leaves in response to treatment.*

e. *There was no significant effect of treatment on* Ψ_L*, confirming that the response to partial drying is most likely mediated by a non-hydraulic signal produced by the roots in drying soil*

f. *If the soil in the 'wet' container was allowed to dry after the SWC of the 'dry' container of half-dried plants had levelled off, the SWC of the 'dry' container decreased even more, suggesting that the 'dry' roots resumed their water-absorbing activities (having been maintained by water transferred from 'wet' roots)*

Chapter Eight - Drying of one half of the root system of field vines reduces shoot growth.

8.1 Introduction

The results of the experiments described in Chapters 4, 5 and 8 have clearly shown that shoot growth of grapevines grown in containers may be significantly reduced by half-drying of the root system of split-root plants. This response may be achieved with different cultivars of *Vitis vinifera* and with different species of *Vitis,* in both controlled environments and in the open air. Stomatal conductance is also reduced in response to half-drying and the impact on shoot function is not associated with any effect on shoot water status. Furthermore, reversion to pre-treatment values of stomatal conductance and shoot growth rate may occur without any change in the water content of the soil surrounding the dried roots, and the start of recovery coincides with the point of maximum depletion of soil water.

The aim of the experiment described in this chapter was to determine if the same results could be achieved with non-fruiting field vines as with container-grown vines and to test the hypothesis *that drying of one half of the root system of field vines reduces shoot growth and stomatal conductance, and that shoot function may recover in the absence of rewatering of the dried half of the root system.* The vines were de-fruited because it is known that crop load can affect response to soil water deficits of grapevines growing in containers (Poni *et al.* 1993) and in the field (Hardie and Martin 1990). Also, bunch removal in the second growth season is a standard viticultural practice to encourage the development of the woody framework.

8.2 Materials and methods

One-year-old *Vitis vinifera* cv. Cabernet Sauvignon (clone LC14) [grafted to *Vitis champini* cv. Ramsey rootstock] split-root vines (from rootlings) were planted in two, north-south rows in the Alverstoke vineyard on the Waite Campus of the University of Adelaide in November, 1991. Cabernet Sauvignon grafted to Ramsey was used in preference to self-rooted vines because it is known that the latter are more vigorous: maximum leaf area per vine of Cabernet Sauvignon grafted to Ramsey was double that of own roots, and pruning weight was more than doubled, for vines grown under intensive irrigation in a hot climate (Sommer and Clingeleffer 1996). The vines were planted such that each half of the root system was separated by a membrane of black polythene (200μm) to 1.5m depth (Figure 8.1). The membrane was installed several days prior to planting by excavating a trench (70cm wide) with a backhoe and replacing the soil on either side of the membrane by hand; trellis posts were installed at the same time, before the soil was replaced.

The rows were 4m apart and the vines spaced at 2m along the rows. In the first growing season, irrigation was applied frequently by using two, 2 L/h drippers per vine, one dripper on either side of the vine (and thus either side of the plastic membrane), 30cm from the vine-line, ie. 60cm apart. The vines grew vigorously in the first season and in winter 1992, were pruned such that they almost filled the fruiting wire at 130cm. In the 1992/93 season, irrigation commenced in late September and was frequently applied on both sides until the end of January. All vines were irrigated on both sides for the last time on February 3 (D2).

There were three treatments for Experiment 13:
a) no irrigation on the east side from D4 until D37 (March 10) while the west side was irrigated on D4 and every 2 to 5 days thereafter ('S1');
b) no irrigation on the east side from D11 until D 37 while the west side was irrigated on D11 every 2 to 5 days thereafter ('S2');
c) both sides irrigated from D4 until D37 at the same time as the west sides of S1 and S2 ('Control', C).
The irrigation on the west sides of S1 and S2 treatments was scheduled so as to maintain the soil water content (0-50cm) above 21 to 22%.

Figure 8.1 Cabernet Sauvignon/Ramsey split-root vines in the Waite campus vineyard. The top of the plastic membrane and the split at the base of the trunk are visible. The tops of the capacitance probes used for soil water monitoring in Exp.14 can also be seen.

Strictly speaking, S1 and S2 could be said to have started on D2 and D9 respectively when the east side was irrigated for the last time; however, the treatments were considered to have started on the days when the west side only was irrigated for the first time. There were four replicates per treatment; treatments were allocated at random in one row with single vine plots and no buffer vines. Irrigation was stopped on the east sides of S1 and S2 by removing the drippers and replacing with plugs.

Two actively-growing shoots were selected on each vine on D2 and used for determination of shoot growth rate SGR by measuring the increase in shoot length (reference node = seven nodes below shoot tip) every three to five days and expressing as cm/d (average per vine) since the previous measurement; node number was also counted at the same time. All bunches were removed from vines earlier in the season. Gas exchange measurements were conducted, every 4.5 days on average, between 1300 and 1500h on four leaves per vine (leaves of similar maturity) using a LiCor photosynthesis system or a Delta T porometer; on D18, there were four measurements from 0830 and 1440h to examine the effect of treatment on diurnal changes. During gas exchange measurements, leaf temperature ranged from 22 to 35°C, relative humidity from 29 to 47% and VPD from 1.7 to 4.8 kPa. Leaf water potential was measured on D16 (1400-1500h) and D18 (pre-dawn, 0830, 1045 and 1430h). Soil water content was measured in the afternoon, every 3.5 days on average, by time domain reflectometry using 50cm probes permanently installed 10cm from the dripper on both sides of one vine of each treatment. Meteorological data was recorded daily at the campus weather station, 800m from the experimental site.

The relationship between stomatal conductance and SGR from D1 to D23 for both S1 and S2 was analysed by correlating g_S on a particular day with the closest corresponding SGR value; because S1 and S2 were started on different days and therefore their decrease relative to control occurred under different atmospheric conditions, g_S and SGR as % of control were used for the analysis instead of actual values.

8.3 Results

The soil water content (0-50cm) on the non-irrigated side of S1 vines decreased from 26% on D2 to 17% by D17 and levelled off from that time. For the non-irrigated side of the S2 vines, the rate of soil drying was similar, reaching a minimum of 17% by D25 (Figures 8.2, 8.4c). At the same time, frequent irrigation up to D26 maintained the SWC of the irrigated sides of S1 and S2 vines, and both sides of C vines, between 21 and 26%. Heavy rain on D26 (23mm) and D27 (9mm) re-wet the soil on the non-irrigated sides of S1 and S2 vines so that SWC was the same on both sides. No irrigation from D28 until D36 resulted in a decrease to 19-22% for both sides of all treatments (Figure 8.2).

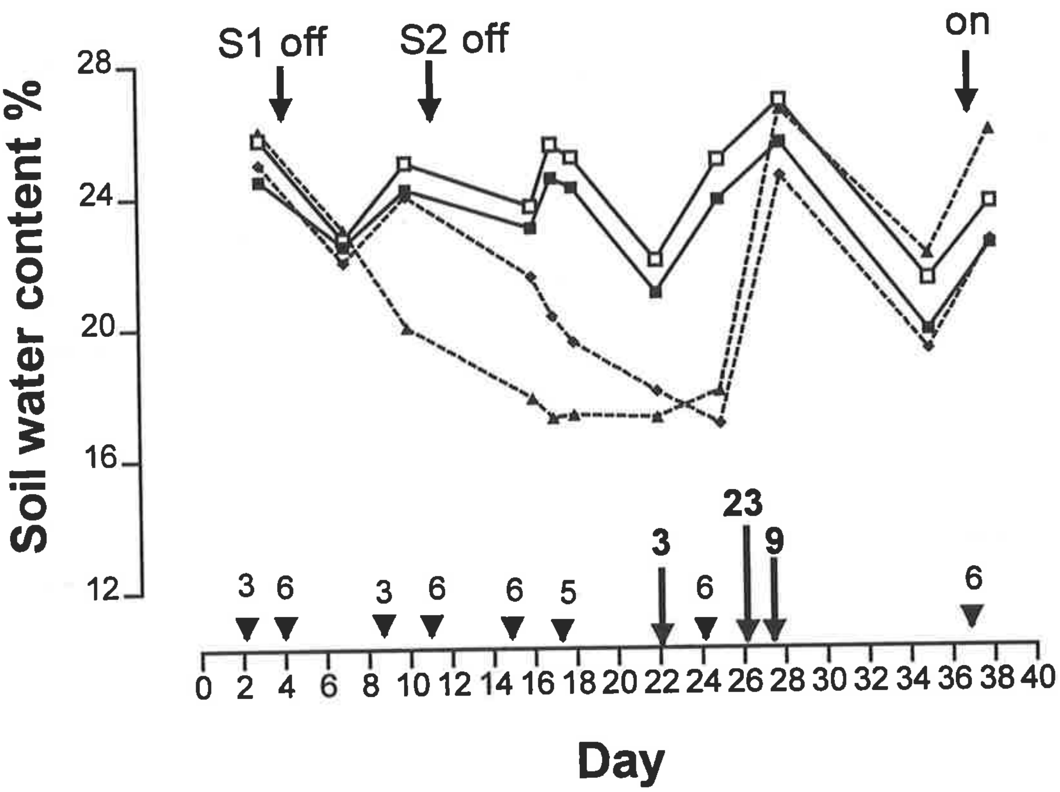


Figure 8.2 Volumetric soil water content (%): control (mean of both sides of vine, ■); mean of irrigated side of S1 and S2 (□); non-irrigated side of S1 (▲) and S2 (◆). Long and short arrows indicate rainfall and irrigation events (mm) respectively.

Shoot growth rate of the control vines increased to D17 and generally decreased thereafter (Figure 8.3a). The decrease from D17 was associated with a period of high temperature and high VPD. SGR of S1 and S2 vines was significantly lower than the controls by D14 and D17 respectively, ie. 10 and 6 days after irrigation ceased on one side (Figure 8.3a,b).

Relative to the control, SGR of S1 was 83% less by D21 and that of S2 67% less on the same day (Figure 8.3b). If S1 and S2 are plotted on the same time-scale, ie. D4 and D11 are changed to D0 (Figure 8.3c), the rate of decrease in SGR relative to the control was very similar up to D10 to 12. Thereafter, SGR of S2 started to recover but SGR of S1 continued to decrease for another 7 days or so before recovery. SGR of S1 vines recovered from D21 or later: this was approximately 4 days after the soil water content started to level off at 17%. On the other hand, SGR of S2 vines recovered after D24, but in this case, soil water content had only just reached the minimum level (Figures 8.2, 8.3b,c). The rate of soil drying was similar on the non-irrigated side for both S1 and S2 vines (Figure 8.4c) but SGR of S2 vines started to recover, relatively, up to 4 days earlier. Overall, the shoot length increment, from D4 to D31, for S1 and S2 vines was significantly reduced by 54 and 35% relative to the control respectively. This was mainly due to a significant decrease in node number (47 and 26% respectively) rather than a change in average internode length (14 and 11% decrease respectively; Table 8.1).

Table 8.1 Effect on shoot length components and stomatal conductance (g_S, mean ± se, D1 to D35) of drying one side of Cabernet Sauvignon/Ramsey split-root vines in the field, Exp.13. Both sides of vine irrigated (C); one side of vine not irrigated from D4 to D37 (S1); one side of vine not irrigated from D11 to D37 (S2). Means followed by different letters are significantly different ($p<0.05$).

Variable	**C**	**S1**	**S2**	**S1 relative to C %**	**S2 relative to C %**
Shoot length (cm)	31.0 c	14.3 a	20.3 b	- 54	- 35
Node number	8.7 b	4.6 a	6.4 a	- 47	- 26
Internode length (cm)[a]	3.6 a	3.1 a	3.2 a	- 14	- 11
g_S (mmol/m^2/s)	430 ± 50	313 ± 43	358 ± 59	- 27	- 17

[a] = shoot length/node number

The changes in stomatal conductance of all treatments showed a similar pattern over time (Figure 8.4a) and were associated with corresponding changes in atmospheric conditions. As for SGR, both S1 and S2 had lower average g_S than the control over the whole period of the experiment ie. 27 and 17% lower respectively (Table 8.1). Stomatal conductance of S1 vines was significantly lower than the controls by D11, ie. 7 days after the start of half-drying, and g_S of S2 vines was significantly lower than controls by D14, ie. 3 days after the start of half-drying (Figure 8.4b). Relative to the control, g_S of S1 and S2 was 46% and

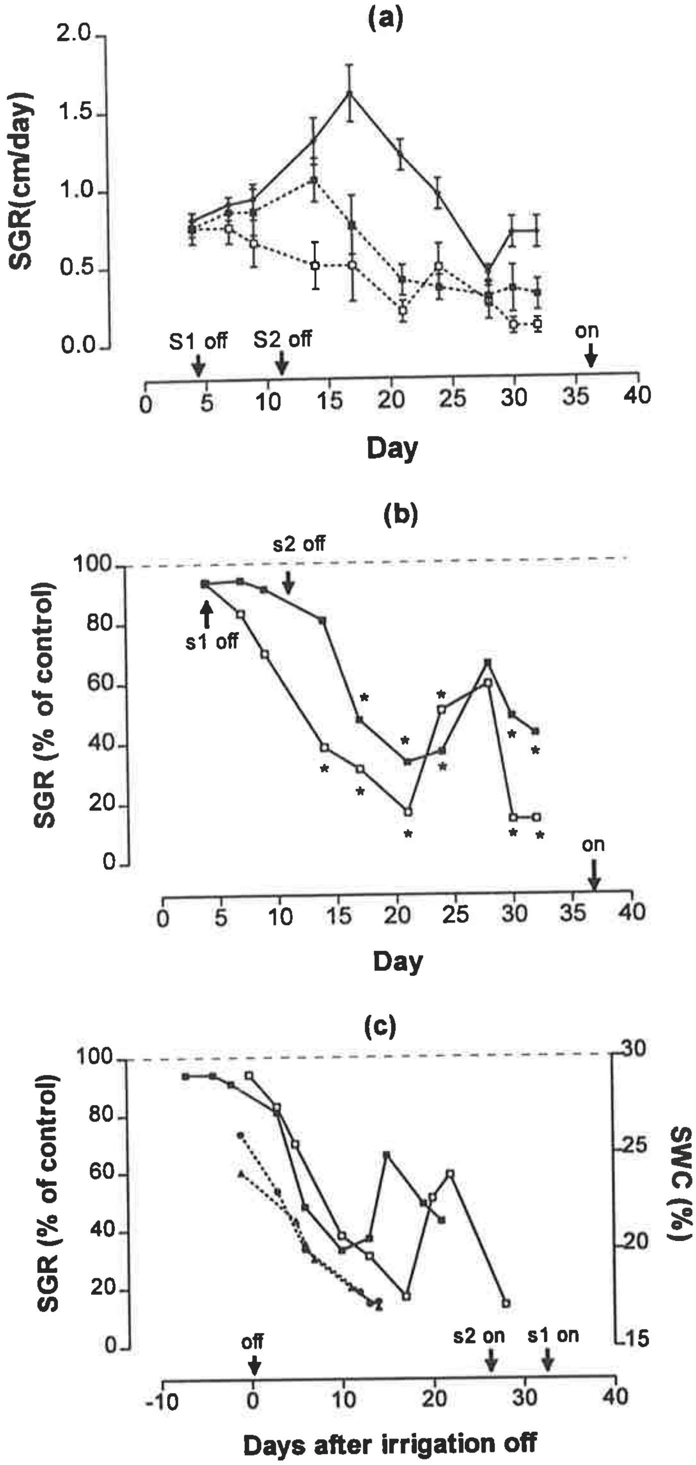


Figure 8.3 Effect on shoot growth rate (SGR) of drying one side of Cabernet Sauvignon/Ramsey split-root vines in the field, Exp.13. Both sides of vine irrigated (C, ◆); one side of vine not irrigated from D4 to D37 (S1, ☐); one side of vine not irrigated from D11 to D37 (S2, ■).

(a) SGR (mean ± se, cm/day)

(b) SGR (as % of C); * indicates those days when S1 or S2 significantly different ($p<0.05$) to control; S1 significantly different ($p<0.05$) to S2 on D14.

(c) SGR (as % of C) and volumetric soil water content (SWC, %) [non-irrigated side of S1 (●) and S2 (▲)]; time scale = days after irrigation on one side off (D0).

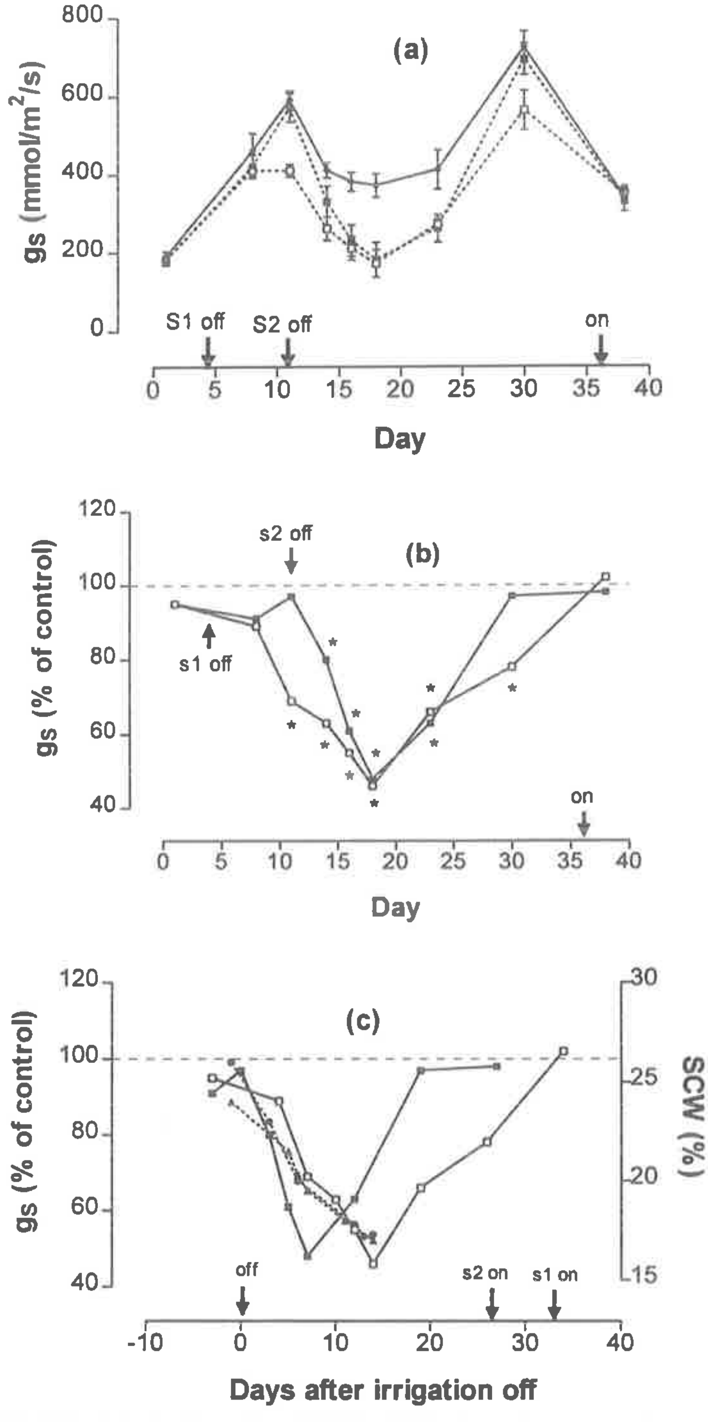


Figure 8.4 Effect on stomatal conductance (g_S) of drying one side of Cabernet Sauvignon/Ramsey split-root vines in the field, Exp.13. Both sides of vine irrigated (C, ◆); one side of vine not irrigated from D4 to D37 (S1, □); one side of vine not irrigated from D11 to D37 (S2, ■).

(a) g_S (mean ± se,cm/day)

(b) g_S (as % of C); * indicates those days when S1 or S2 significantly different ($p<0.05$) to control; S1 significantly different ($p<0.05$) to S2 on D11.

(c) g_S (as % of C) and volumetric soil water content (SWC, %) [non-irrigated side of S1 (●) and S2 (π)]; time scale = days after irrigation on one side off (D0).

48% lower respectively by D18 (Figure 8.4b). The reduction in g_S for both S1 and S2, relative to the control, coincided with decrease in soil water content on the non-irrigated side (Figure 8.4c).

If g_S of S1 and S2 vines is plotted on the same time scale, ie. D4 and D11 are changed to D0, the rate of decrease of S2 (relative to the control) was greater than that of S1; furthermore, g_S of S2 started to recover relatively earlier, ie. by approximately 7 days (Figure 8.4c). Once recovery had started, however, the rate was similar.

Recovery of g_S of S1 vines between D18 and D23 coincided with no further decrease in SWC (0-50cm) on the non-irrigated side whereas g_S of S2 vines started to recover between D18 and D23, a few days before the minimum SWC was reached, even though the SWC on the non-irrigated side decreased at similar rates for S1 and S2 vines (Figure 8.4c). Stomatal conductance of S2 vines had completely recovered by D30 but for S1 vines, this did not take place until D30 or later (Figure 8.4b). The response of photosynthetic rate (Pn) to the treatment was similar to that for stomatal conductance (data not shown).

On D18, when stomatal conductance of both S1 and S2 had decreased to the lowest values relative to the control, air temperature reached a maximum of 37°C by midday then decreased in the afternoon to 26°C by 1600h, as a result of a change in wind direction bringing cooler, moister air. VPD showed a similar pattern to temperature whereas radiation peaked at 1400h (Figure 8.5a). Stomatal conductance of the control vines increased during the morning, then levelled off from 1000h until midday, then increased further in the afternoon (Figure 8.5b). By comparison, g_S of S1 vines was slightly lower at 0830h than either S2 or control when the temperature was ca. 31°C and VPD was ca. 2.5 kPa, but there was no difference between S2 and control. Stomatal conductance of S1 and S2 vines decreased until midday then increased slightly in the afternoon but at a lesser rate than the control. Changes in Pn paralleled those of g_S. At midday, Pn and g_S of S1 and S2 were reduced by approximately 50 and 60% respectively relative to the control (Figure 8.5b). The differences between treatments for g_S and Pn over the course of D18 were not

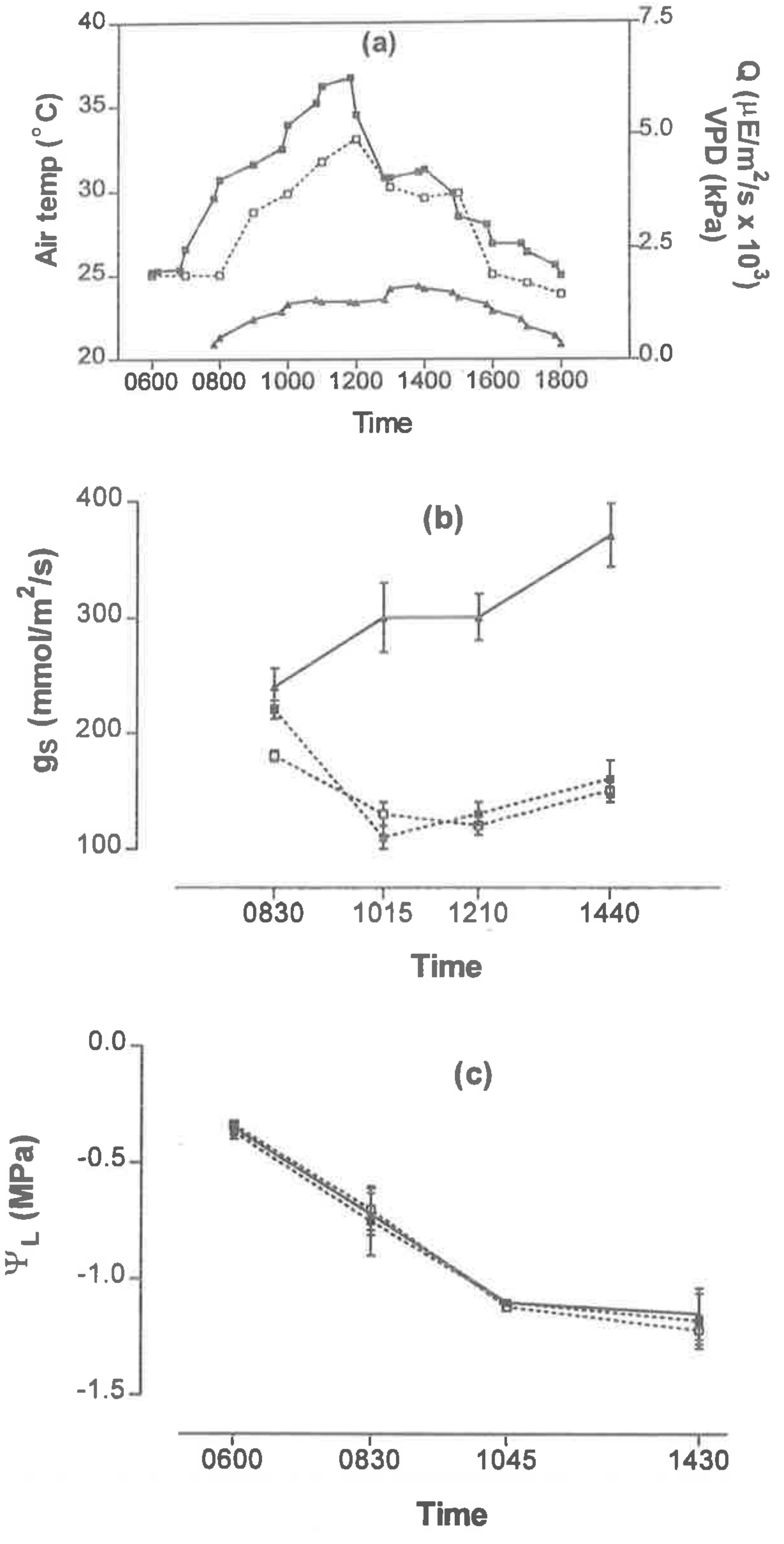


Figure 8.5 Diurnal changes on D18 in response to drying one side of Cabernet Sauvignon/Ramsey split-root vines in the field, Exp.13.

(a) Ambient air temperature (°C, ■), radiation (Q, μmol/m^2/s, ▲) and VPD (kPa, □).

(b) Stomatal conductance (g_S, mean ± se, mmol/m^2/s); both sides of vine irrigated (C, ▲); one side of vine not irrigated from D4 to D37 (S1, ≤); one side of vine not irrigated from D11 to D37 (S2, ■).

(c) Leaf water potential (Ψ_L, MPa); as for (b).

associated with any differences in Ψ_L (Figure 8.5c): Ψ_L of all treatments decreased until mid-morning then stayed about the same until mid-afternoon. Also, there was no difference in Ψ_L between treatments on D16.

There was a strong positive linear relationship between SGR and g_S (both expressed as % of control) from D1 to D23 for both S1 and S2 (Figure 8.6). For the initial drying cycle, g_S (as % of control; S1 only) and SGR (as % of control; S2 only) were plotted against soil water content of the dry side of the vine: because of the nature of the curves, different regression lines were calculated for the pairs of variables with three lowest values and the three highest values of soil water and the point of inflection indicated on the x-axis with an arrow (Figure 8.7). Both g_S and SGR appeared to respond once the soil (0-50cm) dried to approximately 19%; this level of SWC was reached eight days after the start of soil drying for both S1 and S2, ie. on D12 and D19 respectively. Using the water release curves calculated for this soil at 20 and 40cm (Appendix V), 19% SWC corresponds to soil matric potentials of -354 and -78kPa respectively.

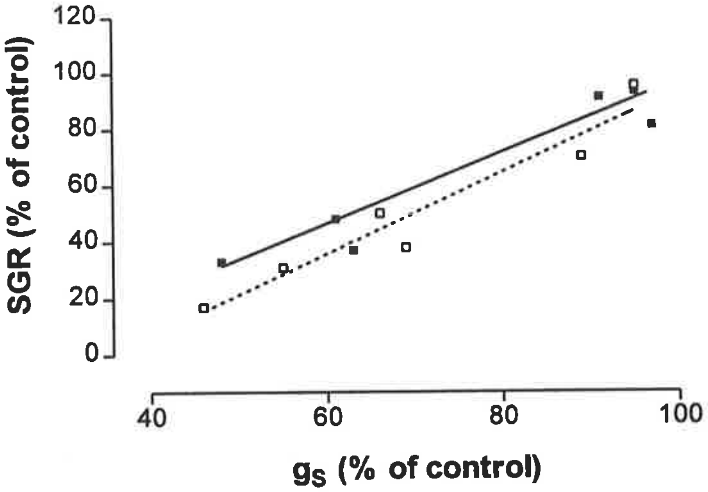


Figure 8.6 Response to drying one side of Cabernet Sauvignon/Ramsey split-root vines in the field, Exp.13: relationship between shoot growth rate (SGR) and stomatal conductance (g_S) [both as % of control from D1 to D23] for S1 ($r^2 = 0.93^{**}$; □) and S2 ($r^2 = 0.93^{**}$; ■).

8.4 Discussion

The results were similar to those of the experiments described in previous chapters: shoot growth rate and stomatal conductance decreased relative to the control in response to drying of the soil on one side of the vine, recovery coincided with the slowing in the rate of soil drying on the non-irrigated side, there was no change in leaf water potential associated with treatment and shoot growth rate was inhibited to a greater degree than stomatal conductance.

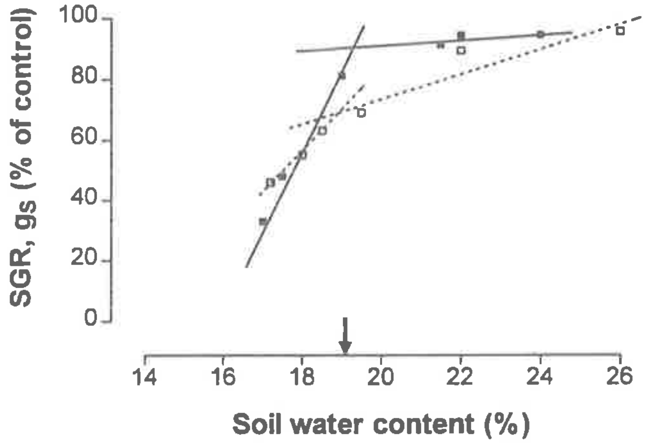


Figure 8.7 Response to drying one side of Cabernet Sauvignon/Ramsey split-root vines in the field, Exp.13: shoot growth rate [SGR] (S2, D4-D21, ■) and stomatal conductance [g_S] (S1, D1-D16, ☐) [both as % of control] plotted against volumetric soil water content (%). Different regression lines calculated for three lowest and three highest SWC values respectively. Arrow indicates estimated point of inflection.

There were some differences in the response of the different treatments, relative to the control, with respect to the initial rate of decrease and the timing of the recovery. This may be partly explained in terms of the actual changes in SGR and g_S of the control vines during the experimental period. For example, stomatal conductance of the control was decreasing at the start of the S2 treatment whereas it increased for the first seven days of the S1 treatment. Taking into consideration that the S1 and S2 treatments experienced different environmental conditions, it is possible to conclude that the initial rate of decrease of both SGR and g_S (relative to the control) was similar for each treatment and strongly correlated with the rate of decrease of soil water content on the non-irrigated side of the vine. The timing of the start of recovery was similar within treatments although g_S tended to start recovering three to five days earlier than SGR. The difference between S1 and S2 treatments was greater: recovery of both variables started ca. seven days earlier with S2 than S1, even though the rate of soil drying was similar. In both cases, shoot function recovered from about the time when the minimum value of SWC was reached. It is not possible to conclude that full recovery of shoot function would have taken place in the absence of rewatering of the 'dry' sides of the half-dried vines due to the significant rainfall on D26 which re-wet the dry sides and effectively terminated the experiment.

The shoot growth rate for S1 and S2 vines decreased again, relative to the control, after D28 (Figure 8.3b): it is likely that this relative decrease was a result of an actual increase in the rate of shoot growth of the control due to favourable climatic and soil conditions following the periods of rainfall from D26 to D28 (Figure 8.3a), without any significant change in SGR of S1 or S2 (by this stage, most shoots of both treatments had stopped growing).

The decrease in shoot length increment in response to half-drying was mainly due to an effect on the rate of node initiation rather than internode elongation. This is very similar to the results from pot experiments described in previous chapters. Gowing *et al.* (1990) found that the rate of leaf initiation of apple was more responsive to half-drying than the rate of leaf elongation. The effect of partial drying on the components of vegetative growth is discussed in detail in Chapter 9. The magnitude of the response, ie. 35 to 54% reduction in shoot length relative to the control, was slightly greater than that measured with the self-rooted *Vitis vinifera* cultivars in the pot experiments described in Chapters 4 to 6 where there was a single period of half-drying, and similar to the degree of inhibition reported in Chapter 7 where half-drying was alternated. Shoot growth appeared to be more responsive to half-drying than stomatal conductance; this was also observed in the pot experiments described in previous chapters and has been reported for other species (Passioura 1988; Davies *et al.* 1987).

Shoot growth rate, and particularly the rate of node initiation, affects the rate of leaf area development. Whole-plant transpiration is a function of both leaf area and average stomatal conductance of leaves. The mechanism of regulation of plant transpiration may be based on stomatal or leaf area responses or a combination of both. The relative sensitivity, therefore, of stomatal conductance and shoot growth in response to partial drying of the root-zone may reflect the general drought resistance strategy of the plant. If a plant's strategy is largely based on stomatal control, then this has the advantage that it is readily reversible in the event of more favourable conditions. One might predict that this would be a characteristic of drought-avoiding species which respond quickly to changes in soil water status, e.g cowpea (Ebel *et al.* 1994), thus sacrificing short-term carbon gain for long-term survival. Such plants may be well-adapted to environments where water deficits are transient. Stomatal closure in response to drying of the surface soil occurs much faster with drought-sensitive cultivars of wheat than with drought-resistant cultivars (Blum and Johnson 1993). This characteristic was correlated with the proportion of roots in the surface soil and the consequent strength of the signal produced by those roots. If the stomata are too sensitive to root signals, this may be a disadvantage because the supply of water from roots in wet zones in the soil may be adequate to meet the needs of the plant: thus the reduced assimilation, as a result of stomatal closure, may be unnecessary. At the other extreme, a relative insensitivity of stomata to water deficits may be an adaptive advantage because this allows transpirational cooling and maintenance of leaves within an optimal temperature range under elevated ambient temperature conditions (Sadras *et al.* 1993).

A strategy based on control of leaf area would seem to be more likely for drought-tolerating plants and/or situations where long-term water deficits are likely. Reduction in the rate of leaf area development may be the result of reduced rate of leaf initiation (ie. node initiation in grapevines), early cessation of shoot growth and reduced rate of leaf expansion (more likely with species with large leaves such as sunflower). In addition, actual decrease in leaf area may result from leaf abscission. Species with one or more of these strategies may have little or no response by stomata in response to a non-hydraulic signal, eg. sorghum (Ebel *et al.* 1994) and sunflower (Sadras *et al.* 1993). In the latter example, leaf expansion rate was much more sensitive than g_S. At first sight, a strategy based on leaf area control appears to be inferior to one based on stomatal control because it is not readily reversible. However, this is not strictly true because leaf expansion may be partially reversible in some species, e.g sunflower (Sadras *et al.* 1993), and with the grapevine, lateral shoots may grow after main shoots have stopped, in response to favourable soil conditions late in the season (Smart and Coombe 1983).

The grapevine may have a combination of strategies because both leaf area and stomatal conductance appear to respond to non-hydraulic signalling. In the case of leaf area, the main effect is on the rate of leaf initiation, and, as will be demonstrated in Chapter 9, the development of lateral leaf area is particularly affected by partial drying; leaf size, a consequence of leaf expansion rate, is less affected. In grapevines, leaf abscission, particularly of mature leaves, is usually a consequence of severe water stress and has not been observed in response to partial drying. Winkel and Rambal (1993) proposed that there are differences between cultivars of *Vitis vinifera* with respect to drought strategies: cultivars of temperate origin, e.g Shiraz, were said to lack efficient stomatal control and tended to reduce leaf area in response to drought. Cultivars of Mediterranean origin, eg. Carignan, were said to have relatively sensitive stomata and a relatively larger leaf area than temperate-origin cultivars under the same conditions. Cabernet Sauvignon is a cultivar of temperate origin and thus could be expected to behave in a similar fashion to Shiraz, ie. shoot growth may be more sensitive than stomata to root signals.

The magnitude of the decrease in average g_S of half-dried vines relative to control over the period of the experiment, ie. 17 to 27%, was similar to that measured with the self-rooted *Vitis vinifera* cultivars in the pot experiments described in Chapters 4 to 6 where there was a single period of half-drying. However, the maximum depression relative to the control was greater than that observed in those pot experiments and in those experiments of Poni *et al.* (1992) and Düring (1992) with *Vitis vinifera*. For example, g_S of both S1 and S2 decreased to ca. 50% of the control level before recovery. It is possible that this was a consequence of the less-controlled conditions of the field experiment. Also, gas exchange measurements were carried out in the afternoon and differences between control and half-dried plants tended to be higher in the afternoon than in the morning (Figures 5.3, 8.5). Nevertheless, it appears to be characteristic of half-drying that the closure of stomata is only partial and this may be beneficial because plant water-use efficiency is increased (Düring 1992).

The partial stomatal closure in response to drying of part of the root system is an illustration of the plant's 'first line of defence' against drought, according to Düring (1992), who cited the model proposed by Mansfield and Davies (1981), in which complete closure in response to severe stress is the 'second line of defence'. It appears that partial closure is more readily reversible than almost complete closure and thus, under conditions of transient drying of the surface roots, there would be minimal impact on carbon gain. So long as the closure was only partial, it would not matter if there was a high degree of sensitivity of stomata to non-hydraulic signals.

There was a positive linear relationship between stomatal conductance and shoot growth rate (as % of control) for both S1 and S2 during the period from the start of drying until the start of recovery. This has also been shown in pot experiments for other cultivars of *Vitis vinifera* in the experiments of Chapter 4, and for apple (Lakso 1985). This suggests that the two physiological processes are influenced by the same root signal, as proposed by Gowing *et al.* (1990), in response to half-drying. Furthermore, the evidence from this and preceding chapters strongly suggests that the signal is non-hydraulic in nature.

Both g_S and SGR of half-dried vines appeared to respond once the soil (0-50cm) on the dry side had decreased to 19% soil water content; however, this can only be regarded as an approximate estimation because it is based on relatively few points. This level was reached eight days after the start of soil drying for both S1 and S2, ie. on D12 and D19 respectively, and is within two days of the half-dried treatments being significantly different from the control for those variables. The relationship between volumetric SWC and soil matric potential was estimated for soil samples taken from 20 and 40cm depths (Appendix V) and, at 19%, this corresponds to -354 and -78kPa respectively. The measured SWC was representative of the soil depth from 0 to 50cm and it is difficult, therefore, to estimate the average soil matric potential of the root-zone. In pot experiments with split-root plants, leaf expansion rate of half-dried plants was not significantly different to controls until the soil in the dry pot had decreased to between -10 and -100 kPa with sorghum (Ebel *et al.* 1994). It is possible that the average soil matric potential of the root-zone for the field grapevine in this study was similar. Zhang and Davies (1989a) found that, as the matric potential of the soil around roots falls towards -200 to -300 kPa, partial dehydration of roots stimulates ABA production. The relationship between soil matric potential and response to partial drying of grapevines is discussed further in Chapters 10 and 11.

Diurnal changes in Ψ_L of grapevines growing in the field have been recently reviewed by Winkel and Rambal (1993) and Naor *et al.* (1993): pre-dawn values of -0.2 MPa decreasing to -1.2 to -1.6 MPa after midday are typical and the results from this experiment are very similar. However, several authors have suggested that shoot growth of field vines will cease if midday Ψ_L decreases to ca. -1.0 to -1.25 MPa (Matthews *et al.* 1987; Peacock *et al.* 1987; Naor *et al.* 1993). It is obvious from the data of Figure 8.3a that a midday Ψ_L of ca. -1.2 MPa was measured at a time (D18) when the SGR of control vines was averaging more than one cm/day. Furthermore, the substantial reduction in SGR of the half-dried treatments relative to the control was not associated with any difference in Ψ_L, either pre-dawn or at any other time of the day.

Both the diurnal variation and actual values of g_S of the control vines were similar to those reported by Downton *et al.* (1987) and Winkel and Rambal (1993) for *Vitis vinifera* field vines at the end of the growing season. The difference between control and half-dried treatments during the course of D18 was very similar to the differences in gas exchange reported from experiments with well-watered and water-stressed field vines (Downton *et al.* 1987; Williams and Grimes 1987; Williams and Matthews 1990; Winkel and Rambal

1993). For example, well-watered and stressed Carignan (*Vitis vinifera)* vines both had g_S values of ca. 200 mmol/m^2/s at 0900h, increasing to ca. 400 mmol/m^2/s by 1400h in the case of well-watered vines whereas stressed vines remained at ca. 200 mmol/m^2/s over the whole day (Winkel and Rambal 1993); however, in their case, there was also a substantial difference in Ψ_L between well-watered and stressed vines (minimum Ψ_L of -0.9 and -1.5 MPa for well-watered and stressed respectively). It is interesting that the increase in g_S of control vines, in response to the more favourable atmospheric conditions after midday (eg. decreased VPD), was not shown to the same degree by the half-dried vines. This is an indication that soil factors may over-ride atmospheric factors in the regulation of stomatal control.

There is strong evidence from the experiments described in this and preceding chapters that changes in shoot function in response to partial drying of the root system are mediated by a non-hydraulic signal produced by the roots in drying soil. This is the first report of the inhibition of shoot growth, independently of changes in leaf water relations, achieved through the use of split-root plants growing in the field. It is also the first report of recovery of shoot function in the field environment, in the absence of rewatering of the 'dry' side of the half-dried plant. The results of this experiment allows acceptance of the hypothesis *that drying of one half of the root system of field vines reduces shoot growth and stomatal conductance, and that shoot function may recover in the absence of rewatering of the dried half of the root system.*

8.5 Conclusions

a. *Drying of one half of the root system of grapevines in the field significantly reduced shoot growth and stomatal conductance relative to control vines watered on both sides.*

b. *Shoot function of half-dried vines 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.*

c. *The rate of node initiation was affected more than internode elongation in response to half-drying.*

d. *There was no significant effect of treatment on* Ψ_L, *confirming that the response to partial drying is most likely mediated by a non-hydraulic signal produced by the roots in drying soil.*

e. *That there was a positive linear relationship between stomatal conductance and shoot growth rate 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. However, the fact that shoot growth appears to be more sensitive to the signal than* g_S *tends to support the first hypothesis.*

f. *Both stomatal conductance and shoot growth of half-dried grapevines in the field appeared to respond once the soil around the drying roots had decreased to specific soil water content or matric potential.*

Chapter Nine - Partial root-zone drying of field vines controls shoot vigour, improves fruit quality and reduces water use.

9.1 Introduction

The results of the experiment described in Chapter 8 confirmed that a single drying period imposed on one-half of the root system of grapevines in the field could significantly decrease shoot growth relative to fully-watered vines, without any associated change in shoot water status. However, as for the pot experiments (Chapters 4,5,6), shoot growth of half-dried field vines recovered without rewatering of the 'dry' side. Therefore, it is likely that significant and long-term reduction in shoot growth using partial drying of the root system will only be possible if the recovery is minimised. This was successfully achieved with split-root vines in pots through the use of a strategy whereby the drying of half of the root system was alternated from one pot to the other pot (Chapter 7). As a result, this same strategy was tested on field vines in the experiments described in this chapter, using the premise that recovery could be minimised by timing the switch so that it occurred at the time of slowing down in the decrease in soil water content of the dried side of the vine.

The problem of excessive vegetative growth (vigour) in viticulture was reviewed in detail in Chapter 2. Control of excess shoot vigour is desirable because it should result in a balanced vine with a more open canopy (with important consequences for yield and fruit composition). The development of techniques to manipulate vegetative growth such that grape quality is maintained or improved, without any adverse effect on yield, has been the aim of much previous research. Some of the experiments with regulated deficit irrigation have shown promise in this regard (Goodwin 1995) and particular strategies have been suggested by Hardie and Martin (1990), McCarthy *et al.* 1996 and others. However, until now, it has not been possible to achieve all of the above aims over a wide range of viticultural environments. In most experiments where shoot growth has been significantly reduced, there has also been a reduction in yield (McCarthy and Staniford 1984; Matthews and Anderson 1988,1989; Goodwin and Jerie 1992; Poni *et al.* 1993; McCarthy 1997).

The imposition of water stress by irrigation management to control vigour in Mediterranean-type climates is largely restricted to the period from flowering to veraison: earlier than flowering it is difficult to induce sufficient soil water deficits to inhibit growth and post-veraison stress generally has little effect on shoot growth (Matthews and Anderson 1989; Poni *et al.* 1994; Naor *et al.* 1993) but may detrimentally affect fruit composition and yield (Williams and Matthews 1990; Naor *et al.* 1993). Nevertheless, there is general agreement that the potential exists for canopy manipulation by strategic irrigation in Mediterranean-type climates because vegetative growth of grapevines is more sensitive to water stress than berry growth (Williams and Matthews 1990), but the optimal timing and level of stress required has yet to be clearly defined.

Reduction in shoot vigour may reduce canopy density and increase bunch exposure. 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 lower concentration of anthocyanins and phenolics (Smart 1992; Botting *et al.* 1996). Experiments in both controlled environments and in the field have shown that increased bunch exposure results in enhanced sugar accumulation and colour development (Smith *et al.* 1988; Dokoozlian and Kliewer 1995a,b). One of the aims of this experiment was to determine if partial root-zone drying (PRD) has any beneficial effects on fruit quality, and if so, is it associated with any improvement in the degree of bunch exposure as a result of a reduction in shoot vigour.

Experiments were conducted at two sites with cv. Cabernet Sauvignon (*Vitis vinifera)* to test the hypothesis *that partial root-zone drying of field vines, with alternation of the drying from one half of the root system to the other, will reduce shoot growth and will impact on fruit quality.*

9.2 Materials and methods

9.2.1 Waite experiment

Experimental design: The same vines were used as for Experiment 13; the 1993/94 season was the first time that they were allowed to bear fruit. Treatments were: a) both sides of the vine irrigated at any one time with two, 2 Lph drippers ('control' C); b) only one side of the vine (either east or west) irrigated at any one time during the 'partial drying' PRD period with a single, 2 Lph dripper ('treated' T). Drippers were positioned on either side of the vine trunk, 40cm from the trunk, and at right angles to the vine-line, ie. the drippers on each vine were 80cm apart for 1993/94 and 1994/95 seasons; they were moved to 50cm from the trunk for the 1995/96 season (Figures 8.1, 9.4). There were four replicates per treatment with three vines per plot (only the middle vine in each plot was used for measurement unless otherwise indicated). Volume of water applied per dripper during each irrigation was measured by using a meter on the inlet valve which supplied all treatments and dividing by the total number of drippers.

1993/94: From 8/11/93 to 23/11/93, all vines received 88L of irrigation, equally applied to both sides of the vine (Appendix VI). PRD started on 24/11/93 (84 days after September 1, = D84) and finished on D181. From 2/3/94 (D182), both sides of C and T vines were irrigated.

1994/95: From 15/9/94 to 25/11/94, all vines received 248L of irrigation, equally applied to both sides of the vine (Appendix VI). PRD started on 26/11/94 (D86) and finished on D165. From 14/2/95 (D166), both sides of C and T vines were irrigated.

1995/96: From 1/9/95 to 15/10/95, all vines received 40L of irrigation, equally applied to both sides of the vine (Appendix VI). PRD started on 16/10/95 (D45) and finished on D178. From 27/2/96 (D179), both sides of C and T vines were irrigated.

Pruning, trellis system, canopy manipulation: In winter 1993, all vines were pruned to ca.10, two-node spurs per vine. In 1994 and 1995, all vines were pruned to 14 and 25, two-node spurs per vine respectively. In 1995, at least one-third of spurs were either horizontal or pointing downwards in preparation for the conversion to the 'Smart-Dyson' (Smart 1994) training system in the 1995/96 season.

1993/94: All vines were thinned to 16 to 20 shoots per vine in late November in order to stimulate the growth of remaining shoots. There was no shoot trimming; some of the longest shoots were wrapped onto a single foliage wire at 190cm.

1994/95: There was no shoot trimming; all shoots were positioned vertically after set using two pairs of moveable foliage wires at 160 and 190cm and some of the longest shoots were wrapped on to the upper wire.

1995/96: The training system was converted to 'Smart-Dyson', ie. approximately half of the shoots on each vine were positioned vertically upwards ('up' shoots) after set using two pairs of moveable foliage wires at 160 and 190cm and the other half combed outwards and retained in a semi-downwards position for the next three weeks with a single foliage wire on each side of the canopy ('down' shoots); they were then positioned vertically downwards by moving the foliage wires to a location on the trellis posts below the fruiting wire. 'Up' shoots were trimmed on December 13 and the trimmings weighed (fresh weight); this removed approximately 33% of main leaves and all of immature leaves of the longest shoots (14.5 and 13.3 leaves per 'up' shoot after trimming for C and T vines respectively). 'Down' shoots were not trimmed at any stage and were allowed to grow horizontally once they had reached the soil surface; at the time of trimming 'up' shoots, there were 21 mature and 6 immature leaves per 'down' shoot for both treatments.

Soil water and meteorological measurements: In 1993/94, volumetric soil water content (expressed as %) was measured in the afternoon, twice each week, by time domain reflectometry using 50cm probes permanently installed 10cm from the dripper on both sides of two vines of each treatment. Capacitance probes (on both sides of one vine per treatment) were installed in winter 1994 and used for measurement of volumetric soil water content (expressed as mm) in the 1994/95 and 1995/96 seasons (Figure 8.1). Meteorological data were recorded daily at the campus weather station, 800m from the experimental site (Appendix II).

Irrigation scheduling: *1993/94*: Irrigation was scheduled with the aim of maintaining soil water content (0-50cm) on the 'wet' side of T vines at 20% or higher (measured by TDR);

there were 24 irrigations from D84 to D182 with an average of 2.9 and 1.45mm per irrigation on each C and T vine respectively (Appendix VI). Irrigation was alternated from one side to the other of T vines on D101, D120, D134, D150 and D166 which resulted in six irrigation periods averaging 16 days (range = 14 to 19 days). The timing of alternation was based primarily on soil water measurement and modified by the occurrence of rainfall events: initially, the strategy was to change sides when soil water on the 'dry' side decreased to 16%. For the last three periods, the 'refill' point was changed to 12%, or every 14 to 16 days, when it became apparent that a refill point of 16% was unrealistic (Figure 9.1).

1994/95: Irrigation was initially scheduled with the aim of maintaining soil water content (15-55cm) on the 'wet' side of T vines at 110mm or higher (measured by capacitance probe); however, this was changed to 100mm from the beginning of the third period (D101) when it became apparent that this was a more appropriate refill point. There were 32 irrigations from D86 to D166 with an average of 2.4 and 1.2mm per irrigation on each C and T vine respectively (Appendix VI). Irrigation was alternated from one side to the other on T vines on D101, D115, D129, D144 and D158, which resulted in six irrigation periods averaging 13 days (range = 8 to 15 days). The timing of alternation was based primarily on the experience of the previous season and secondarily on soil water measurement: the initial strategy was to change sides after 14 days for the first two or three periods and to monitor soil water at the same time. Experience from Exp.13 in the 1992/93 season, and from pot experiments, had shown that shoot growth started to recover at the time when the soil water content started to level off. Therefore, a lower limit of 80mm was chosen for the 'dry' side after the second period because it was observed that the decrease in soil water content began to slow at that level (Figure 9.2) and also, there was a slight recovery in SGR (Figure 9.5b).

1995/96: As a result of experience in the previous season, the irrigation was initially scheduled with the aim of maintaining soil water (15-55cm) on the 'wet' side of T vines at 110mm or higher initially (measured by capacitance probe), then changed to 100 mm later: this change was actually made at the beginning of the seventh period (D121). There were 52 irrigations from D45 to D179 with an average of 2.48 and 1.24 mm per irrigation on each C and T vine respectively (Appendix VI). Irrigation was alternated from one side to the other on T vines on D73, D86, D98, D110, D121, D131, D139, D150, D157 and D167, which resulted in 12 irrigation periods averaging 10.8 days (range = 7 to 14 days). The timing of alternation was initially every 12 to 14 days, based on experience from the previous season, irrespective of the change in soil water on the 'dry' side; this was the case for the first four periods. From the fifth period, timing was based primarily on soil water measurement and the change was made when soil water content on the 'dry' side started to level off at ca. 80mm (Figure 9.3) to minimise the opportunity for recovery of shoot growth, with the proviso that the minimum length of a period would be 7 days.

Shoot growth measurements: *1993/94*: Three actively-growing shoots were selected on each vine on D91 and used for determination of shoot growth rate (SGR) by measuring the increase in shoot length (reference node = ten nodes below shoot tip) every seven days on average and expressing as cm/d (average per vine) since the previous measurement.
1994/95: Ten actively-growing shoots were selected on each vine on D71 and SGR determined as for 1993/94. At the start of measurement, average shoot length was 145cm for both treatments. Ten new shoots were selected on D90 after shoot positioning and measured until D176. The percentage of shoots which had ceased to grow was determined at each measurement .
1995/96: Five actively-growing shoots were selected on each vine on D41 and SGR determined as for 1993/94 (measured 'up' shoots were not trimmed). At the start of measurement, average shoot length was 67cm with 11 nodes for both treatments.

Gas exchange and leaf water potential measurements: *1993/94*: Gas exchange measurements were conducted, twice weekly on average, either mid-morning or early afternoon, on eight leaves per vine (leaves of similar maturity) using a LiCor LI6200 photosynthesis system (first four measurements only) or a Delta T AP4 porometer. Mean stomatal conductance per treatment for each irrigation period and for the whole experimental period were calculated. Leaf water potential was measured on D120 and D133 (both pre-dawn) on two leaves per vine.

1994/95: Gas exchange measurements were conducted every four to seven days on average from D74 to D180, on eight leaves per vine (leaves of similar maturity) using a Delta T porometer (except for the measurement on D133 when a LiCor photosynthesis system was used); measurements were always conducted between 1030 and 1200h, except on D161 when measurements were done between 1030 and 1700h. Mean stomatal conductance per vine was calculated at each date. Mean stomatal conductance per treatment for each 'drying' period, and for the periods immediately before and after the 'drying' periods, were calculated as well as the treatment means for the whole experimental period. Leaf water potential was measured on three occasions between D133 and D143 (pre-dawn and noon) on three leaves per vine.

1995/96: Gas exchange measurements were conducted on D80, D103 and D119, then on seven days between D131 and D154 in association with leaf water potential and xylem sap ABA measurements, on eight leaves per vine (leaves of similar maturity) using a Delta T porometer; measurements were always conducted between 1000 and 1200h. Mean stomatal conductance per vine was calculated at each date. Mean stomatal conductance per treatment was calculated for set to veraison. Leaf water potential was measured on four occasions between D131 and D137 (morning and afternoon) on three to four leaves per vine and on four occasions between D141 and D153 (morning only).

Sap flow measurements: In a related experiment to this study, the sap flow on each side of the root system was measured on a T vine. Sap flow sensors (Greenspan Technology, Warwick, Qld, Aust.) were installed early in the 1994/95 season into the top parts of each root system, ie. each half of the trunk immediately below the split, at about ground level. The trunk halves below the split were approximately hemispherical in section and therefore, the custom-made probes had to be inserted so that the thermistors were located in conductive tissue; this was achieved by trial and error. Sap flow was logged at 30 minute intervals throughout the season but the data presented are for a 10 day period from midday on D126 to midday on D138, coinciding with the start of a PRD period on D129. The volumetric fraction of wood and water were determined gravimetrically through tests on several canes of similar diameter but with different cross-sectional shape.

Leaf area measurements: *1994/95:* Leaf area (main and lateral leaves) was measured on five randomly-chosen shoots per vine on D102 and D202 (very short shoots were not included) and leaf area per vine calculated (= mean leaf area per shoot x shoot number per vine); see Chapter 3 for details of measurements of leaf area components. On D202, the length of these shoots was also measured.

1995/96: Leaf area (main and lateral leaves) was measured on five randomly-chosen shoots per vine on D50 and D110, the latter just before trimming of 'up' shoots. On D180, leaf area was measured on eight (four 'up' and four 'down') randomly-chosen shoots per vine; unlike the first two determinations (described in Chapter 3), ten leaves along the shoot were chosen at random for measurement of mean area per leaf for both main and lateral leaves. Leaf area per vine was calculated (= mean leaf area per shoot x shoot number per vine) for each time. Vine surface area was measured on D180 and used to calculate leaf area to surface area ratio.

Canopy measurements: Just before harvest, leaf layer number and bunch exposure index were determined by point quadrat and ceptometer respectively. Leaf area to surface area ratio (LASA) was calculated using the pre-harvest leaf area measurement.

Fruit composition: *1993/94:* Starting on D159 (23 days before harvest), 50 berries were randomly selected from each vine every four to seven days and used to determine mean berry weight, total soluble solids (°Brix) and pH. For the last two samples on D175 and D182 (immediately prior to harvest), 100-berry samples were collected and also used for determination of titratable acidity, anthocyanins and phenolics.

1994/95: Starting on D167 (19 days before harvest), 50 berries were randomly selected from each vine every six to seven days; for the last two samples on D180 and D186 (immediately prior to harvest), 100-berry samples were collected and also used for determination of titratable acidity, anthocyanins, phenolics and glycosyl-glucose (total and 'red-free').

1995/96: Starting on D160 (19 days before harvest), 60 to 100 berries were randomly selected from each vine every five to seven days; at harvest on D179, 100-berry samples were collected at random from harvested bunches (analysis as for 1993/94).

Yield components: Bird net was applied just after veraison and was left on the vines until harvest. At harvest [March 2 (D182),1994; March 6 (D186), 1995; February 27 (D179), 1996] fruit from all vines in each plot was weighed and bunch number per vine counted. Mean bunch weight per vine and mean berry number per bunch were calculated. In 1995/96, due to severe infection by oidium (*Uncinula necator*) and greater level of infection on C than T vines, yield per vine was corrected as if there was no disease: bunches with severe infection were discarded and 'good' bunches used to determine 'good' bunch weight which multiplied by total bunch number per vine estimated disease-free fruit weight. The percentage of bunches on each vine with severe oidium infection was also estimated.

Pruning weight measurements: At pruning in the winters of 1994, 1995 and 1996, all shoots were cut back to two node spurs and the prunings weighed ('pruning weight' per vine) and mature shoot number counted ('shoot number' per vine). For every main shoot, the main shoot length, node number per main shoot , the length and number of lateral shoots and diameter of main shoot (between nodes 1 and 2) was measured or counted. Mean main shoot weight, mean main shoot weight per unit length, mean and total length of lateral shoots per main shoot, mean internode length per main shoot and main shoot number per m row were calculated. Frequency distribution was determined on a per vine basis and treatment means ± se for each class calculated. In winter 1995, four of the longest shoots were selected from each vine after pruning: shoot length and the length of every internode were measured and the total number of internodes per shoot counted. The mean internode length per treatment was calculated for internodes 0 to 21 and 22 to tip separately because it was estimated that there were 21 internodes per shoot on average formed prior to the start of treatment. In winter 1996, main shoots were separated into 'up' and 'down' categories and measured separately. Some shoots were selected to be photographed and the length of every internode was measured on some of these shoots.

For ‘down’ shoots, the point at which the shoot touched the ground and started to grow horizontally was marked: pruning weight of ‘down’ shoots was divided into two components, proximal and distal to this point. The relationship between these components was used to estimate leaf area of the vertical canopy and thus leaf area to surface area ratio.

Trunk diameter: The diameter of the trunk at 50cm above the ground and the diameter of each half of the trunk immediately below the split, ie. the top part of each root system just above the soil surface, of each vine was measured with dial callipers.

9.2.2 Blewitt Springs experiment

Site preparation: One-year-old *Vitis vinifera* cv. Cabernet Sauvignon (clone LC 14) [grafted to *Vitis champini* cv. Ramsey rootstock] split-root vines (from cuttings) were planted in a north-south row in a vineyard at Blewitt Springs, South Australia in November, 1991. The vines were planted such that each half of the root system from separate pots was separated by a membrane of black polythene (200μm) to 1.5m depth. The membrane was installed several days prior to planting by excavating a 15cm wide trench with a trench-digger and replacing the soil on either side of the membrane by hand; trellis posts were installed at the same time, before the soil was replaced. The row was 3.0m and 4.2m from rows to the west and east respectively and the vines spaced at 2m along the rows. In the first growing season, irrigation was applied frequently by using two, 2 Lph drippers per vine, one dripper on either side of the vine, 30cm from the trunk, ie. 60cm apart. The vines grew vigorously in the first season and in winter 1992, were pruned such that they almost filled the fruiting wire at 1.2m. For the next two seasons, both sides of the vines were well-irrigated. In winter 1994, all vines were pruned to an average of 16 (range 14 to 18), two node spurs per vine.

Experimental design: The vines were used for experimentation in the 1994/95 season (second harvest). Treatments were: a) both sides of the vine irrigated at any one time with two, 2 Lph drippers (C); b) only one side of the vine (either east or west) irrigated at any one time with a single, 2 Lph dripper (T) from 8/12/94 (98 days after September 1, = D100) until 1/3/95 (D181). Originally, there were six replicates in a randomised block design with single vine plots; due to shortage of vines, buffering was incomplete ie. four each of C and T test vines were buffered on both sides by vines receiving the same treatment with two each of C and T test vines buffered on one side only. Fruit weight and pruning weight measures after the experiment had been completed indicated that the incomplete buffering was not completely effective (despite the fact that soil moisture measurements suggested that it was) and therefore, only data from those vines with complete buffering were used and analysed as for a completely randomised design.

Irrigation scheduling: Prior to 8/12/94, all vines were irrigated on both sides but the amount (80L) was only measured from 28/11/94 to 8/12/94 (Appendix VI). From D98 (fruit set) to D181 (24 days after veraison), there were six drying periods when one side of the T vines were not irrigated, achieved by changing sides every 14 days; this length of drying period was chosen as a result of the experience in Exp.14 and, for convenience, the same interval was used for each period. From D181 (when more than 50% of T shoots had ceased to grow) until harvest (D237), both sides of all vines were irrigated. The amount and timing of each irrigation event was determined by the vineyard manager and thus the experimental vines were irrigated at the same time as the other vines in that block. The amount of irrigation applied to the experimental vines each week from D98 to D237 was determined by measuring the output of a single dripper collected in a sealed plastic drum (the output of this dripper was occasionally checked to ensure that it was the same as the drippers on the test vines).

<u>Shoot growth measurements</u>: There was no shoot trimming or shoot positioning. Five actively-growing shoots were selected on each vine on D100 and used for determination of SGR by measuring weekly the increase in shoot length (reference node = six nodes below shoot tip) and expressing as cm/day (average per vine) since the previous measurement. At the start of measurement, average shoot length was 150cm for both treatments. The

percentage of shoots which had ceased to grow was determined at each measurement from D140.

Soil water measurement: Soil water content was measured between 1030 and 1200h on eight occasions, mainly in the middle of a drying period , by time domain reflectometry using 50cm probes permanently installed 10cm from the dripper on both sides of two vines of each treatment. Meteorological data were recorded daily at the Southcorp 'Seaview' vineyard, five km from the experimental site.

Gas exchange and canopy measurements: Gas exchange measurements were conducted weekly on clear days from D104 to D174, and on D190 and D196, on eight leaves per vine (leaves of similar maturity) using a Delta T porometer; measurements were always conducted between 1100 and 1230h. Mean stomatal conductance per vine was calculated for three periods (mean of three occasions for each period) and for the period from set to veraison. Bunch exposure index was measured 30 days after veraison.

Fruit composition and yield components: Starting on D181 (56 days before harvest), 50 berries were randomly selected from each vine every week and used to determine mean berry weight, (°Brix) and pH. For the last two samples before harvest, half was frozen and used for later determination of anthocyanins and phenolics. At harvest on April 24 (D237), fruit from all vines in each plot was weighed and bunch number per vine counted. Mean bunch weight per vine and mean berry number per bunch were calculated. Two hundred berries were randomly selected from harvested bunches; half was used for determination of mean berry weight, total soluble solids (°Brix), titratable acidity and pH immediately and the other half was frozen for later determination of anthocyanins and phenolics.

Pruning weight components: At pruning in winter 1995, all shoots were cut back to two node spurs and the prunings weighed ('pruning weight' per vine) and mature ('main') shoot number counted ('shoot number' per vine). Mean main shoot weight was calculated.

9.3 Results

9.3.1 Waite experiment

Soil water and irrigation: *1993/94*: The second drying period was disrupted by several rainfall events and was terminated before the SWC had decreased to the pre-determined re-fill point; nevertheless, the non-irrigated side was dried down to the re-fill point during the other periods, resulting in an average period length of 16 days. There was little difference between the average SWC of both sides of C vines and the 'wet' side of T vines (Figure 9.1).

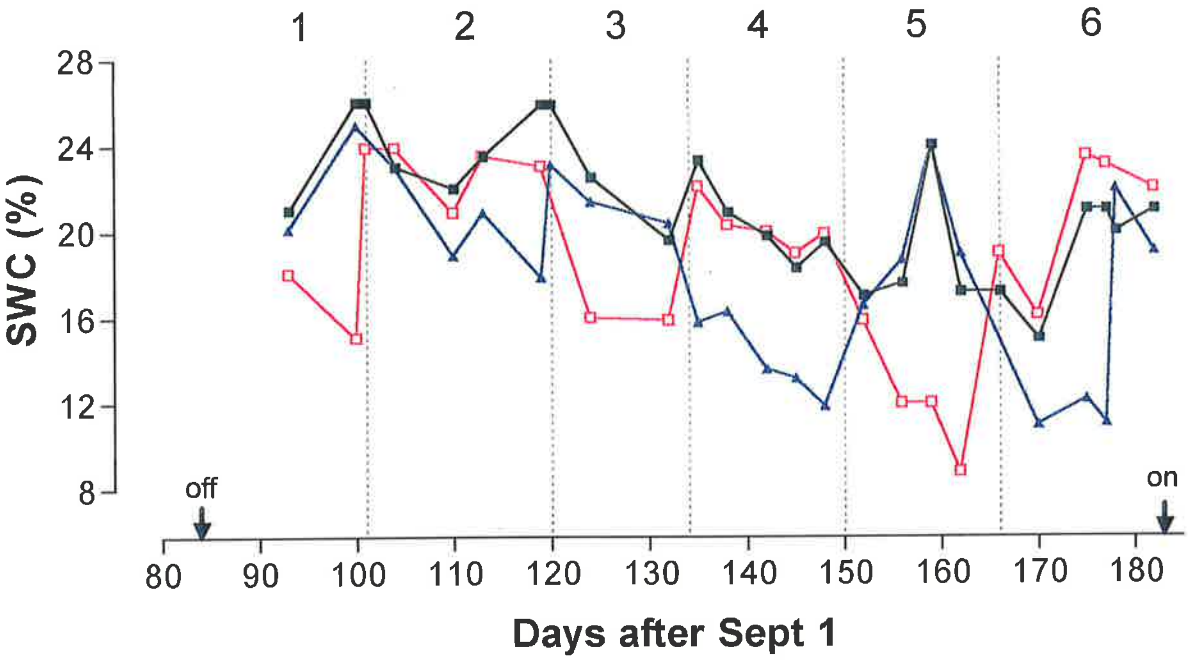


Figure 9.1 Volumetric soil water content (SWC, %) of 'control' (C, mean of both sides, ■, black), west side (▲, blue) and east side (□, red) of 'treated' (T) Cabernet Sauvignon/Ramsey split-root vines, 1993/94, Exp. 14. C: both sides of vine irrigated; T: one side of vine not irrigated at any one time from 83D ('off') until 183D ('on'). Dotted lines indicate days when irrigation of T switched from one side to the other.

1994/95: The SWC of the 'wet' side of T vines was maintained between 100 and 130mm in the 15-55cm layer for most of the time during the PRD periods (Figure 9.2a), while the SWC of the 'dry' side decreased during each period before levelling off at 75 to 80mm (for the last five periods). Between the end of the PRD periods and harvest, both sides of the T vines were excessively dried inadvertently for several brief periods while the C soil water content did not drop below 110mm (Figure 9.2b).

1995/96: The SWC of the 'wet' side of T vines was maintained between 100 and 110mm in the 15-55cm layer until the end of the sixth period, and generally greater than 100 for the remaining periods; during the seventh, ninth and eleventh periods when the west side was irrigated, SWC decreased below 100mm due to a combination of excessive time between irrigations and insufficient amount applied at each irrigation. By comparison, when the east side was irrigated, except for period 12 just before harvest, the same irrigation regime was able to maintain SWC higher than 100mm. For the first four periods, SWC on the 'dry' side was still decreasing when the switch was made at the end of the pre-determined 14 day period; subsequently, the SWC of the 'dry' side usually levelled off at 80 to 85mm before the decision was made to switch sides (Figure 9.3a). The C vines were irrigated at the same time as the 'wet' side of T vines and, at twice the rate per vine during the PRD periods: as a result, SWC was generally maintained between 115 and 135mm in 1994/95 (Figure 9.2a,b) and between 110 and 130mm in 1995/96 (Figure 9.3b). Over the whole season from budburst to harvest, between 35 and 64mm less irrigation was applied to T vines than C resulting in calculated average crop factors of 0.33 and 0.29 respectively (Appendix VI). Average irrigation rate (L/vine/day) during the period from the start of PRD until harvest increased for both C and T vines over the three seasons in response to the increase in vine water-use (due to increased leaf area per vine) and increased irrigation frequency (Table 9.1).

Table 9.1 Average L/vine/day for Exp.14 (Waite, three seasons) and Exp.15 (Blewitt Springs, 1994/95): 'control' (C, irrigated on both sides) and 'treated' (T, partial drying).

Season	Location	Time	C	T
1993/94	Waite	start of PRD to harvest	5.6	2.8
1994/95	Waite	PRD period	7.6	3.8
		start of PRD to harvest	7.6	4.5
1995/96	Waite	start of PRD to harvest	7.9	3.9
1994/95	Blewitt Springs	PRD period	17.2	8.6
		start of PRD to harvest	12.4	7.3

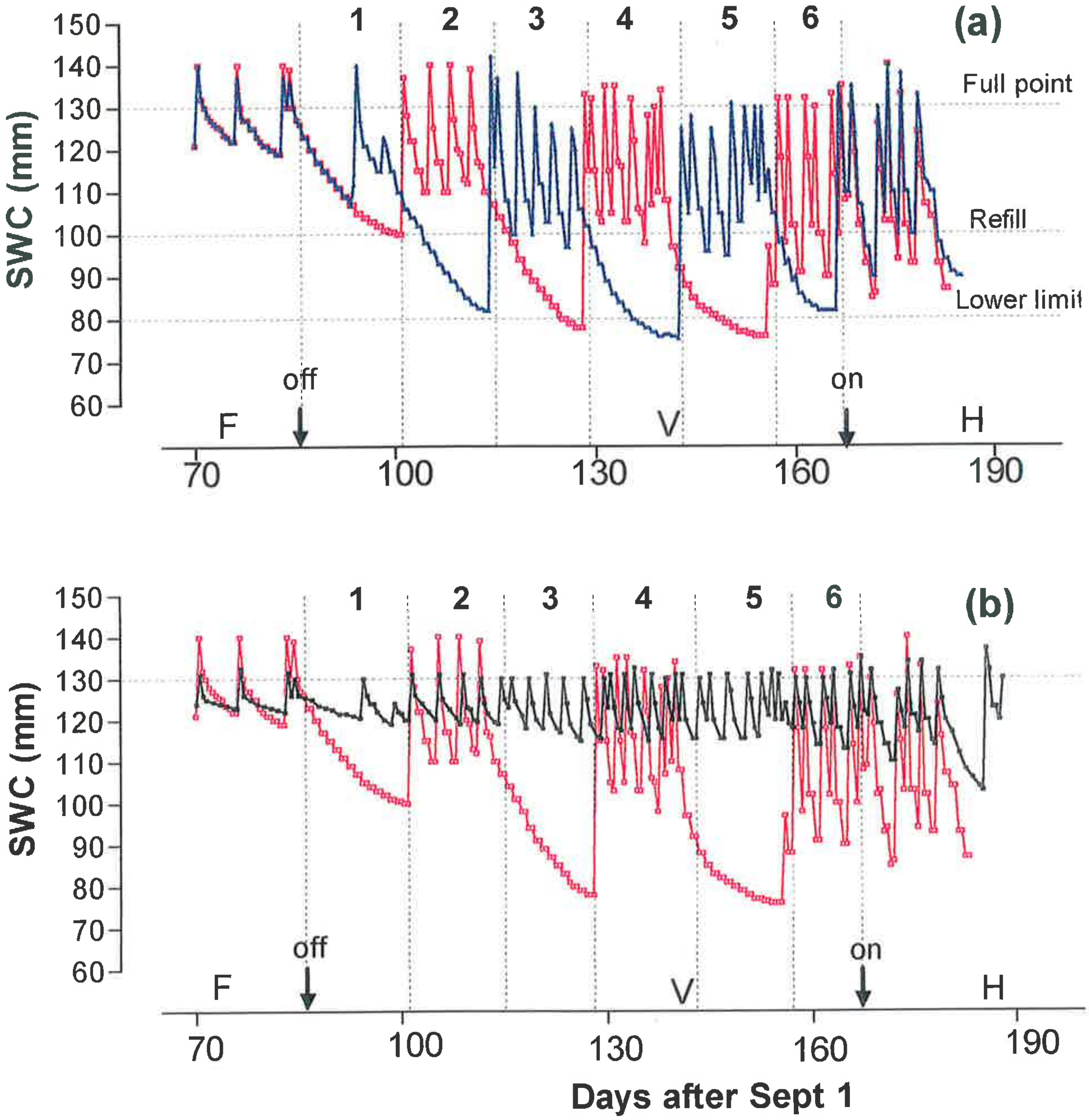


Figure 9.2 Volumetric soil water content (SWC, mm) of 'control' (C, both sides of vine irrigated) and 'treated' [T, one side of vine not irrigated from D86 ('off') until D166 ('on')] Cabernet Sauvignon/Ramsey split-root vines, 1994/95, Exp. 14. F, V, H indicate flowering, veraison and harvest dates respectively; horizontal dotted lines indicate full point, refill and lower limit; numbers in bold represent 'drying' periods.

a) west (□, red) and east (▲, blue) sides of T vines; 15-55cm summed

b) west side of C (■, black) and west side of T vines (□, red); 15-55cm summed

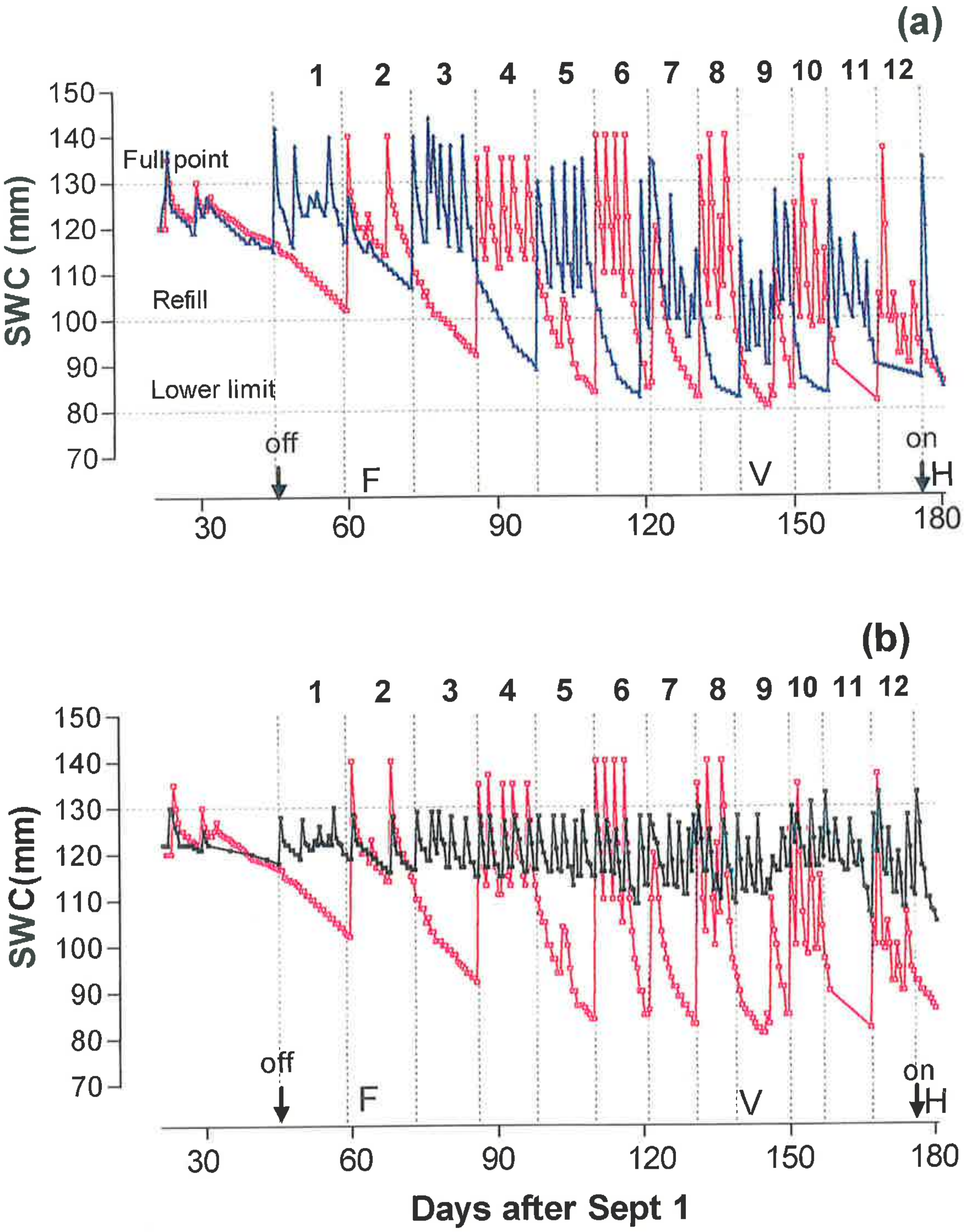


Figure 9.3 Volumetric soil water content (SWC, mm) of 'control' (C, both sides of vine irrigated) and 'treated' [T, one side of vine not irrigated from D45 ('off') until D179 ('on')] Cabernet Sauv./Ramsey split-root vines, 1995/96, Exp. 14. F, V, H indicate flowering, veraison and harvest dates respectively; horizontal dotted lines indicate full point, refill and lower limit; numbers in bold represent 'drying' periods.

a) east (☐) and west (▲) sides of T vines; 15-55cm summed

b) east side of C (■) and east side of T vines (☐); 15-55cm summed

The lateral spread of water in the wetting zone beneath the dripper on each side of the vine was relatively limited. This was confirmed by TDR measurements with 15cm wave guides within 30 minutes of the completion of a three hour irrigation cycle in November 1996: within the boundary of the wet patch on the surface, SWC ranged from 24 to 32% but it was physically impossible to insert the wave guides into the dry soil beyond the boundary of the wet patch.

Figure 9.4 Control vine (both sides of vine irrigated) at the end of an irrigation period; Cabernet Sauvignon/Ramsey split-root vines, Exp. 14. Blue ribbon has been used to delineate the margins of the wet patches associated with each dripper; the west and east sides of the vine are at the bottom and top of the photograph respectively.

On the western side of the vines, the wet patch on the surface was roughly circular with an average radius of 62cm; on the eastern side, because the effect of the slight slope was more pronounced, the wet patch was an irregular oval, with average radius of 48cm in the north-south plane, and 38cm and 15cm west and east of the dripper respectively (Figure 9.4). Although there was some variation between vines with respect to both shape and size of the wet patch, there was no effect of treatment because the output per dripper was the same in each case.

Sap flow: Prior to D129, the east side of the 'treated' vines was irrigated and the west side was dried; the sides were switched on the morning of D129 (Figure 9.2a). Day-time sap flow in the east half of the trunk (below the split) was greater than that in the west half up to the morning of D130 but night-time flow rates were similar (Figure 9.5). Thereafter, night-time flow was higher in the west half than the east half, but it was not until D134 ie. five days after irrigation started on the west side and the east side started to be dried, that day-time flow was significantly greater over the whole day in the west half than the east half. However, for the next three days, there was no difference in day-time flow.

Shoot growth rate: SGR was reduced in all seasons in response to PRD (Table 9.2). In the first two seasons, PRD commenced at fruit set and, after 23 to 32 days of treatment, SGR of T vines was significantly less than that of C (Figure 9.6). In the last season, PRD started ca. 40 days earlier and SGR of T vines was not significantly lower than C until 52 days after the start of PRD; however, this was still earlier than in the other two seasons by 10 days or more. Relative to C vines in 1994/95 and 1995/96, SGR of T vines was successfully maintained at a lower rate from D118 and D97 respectively until after veraison, by which time, the SGR of T vines was less than 50% of C vines. However, in the first season, SGR of T vines was not significantly less than C for much of the time from ca. veraison onwards due to a marked decrease in SGR for both treatments from veraison (Figure 9.6).

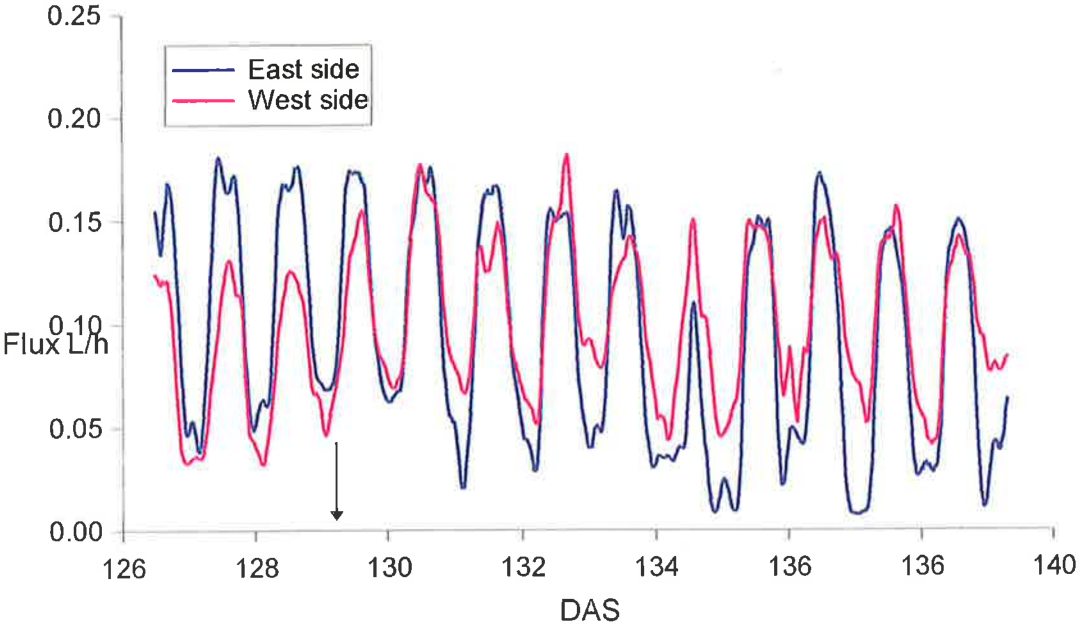


Figure 9.5 Sap flow measured in west (Red) and east (Blue) halves of trunk below split of 'treated' Cabernet Sauvignon/Ramsey split-root vine from 126 to 139 days after September (DAS); 1994/95, Exp.14. One side of vine not irrigated during PRD periods; irrigation switched from east side to west side of vine on morning of D129 (arrow).

Table 9.2 Summary of shoot growth rate (SGR, cm/day) and stomatal conductance (g_S, mmol/m^2/s) from set to veraison[a] for 'control' (C) and 'treated' (T) Cabernet Sauvignon/Ramsey split-root vines, Exp.14: (Waite, three seasons) and Exp.15 (Blewitt Springs, 1994/95).

		Waite:1993/94	**Waite:1994/95**	**Waite:1995/96**	**Blewitt: 1994/95**
SGR	**C**	2.8 ± 0.1	2.4 ± 0.13	2.2 ± 0.23	1.74 ± 0.23
	T	2.3 ± 0.2	1.8 ± 0.24	1.7 ± 0.16	1.21 ± 0.22
	% diff.	-18	-24	-23	-30
	Signif.	<0.05	<0.05	<0.05	<0.05
g_S	**C**	293 ± 12	264 ± 17	275 ± 14	326 ± 7
	T	255 ± 5	185 ± 26	242 ± 11	266 ± 5
	% diff.	-13	-30	-12	-18
	Signif.	<0.05	<0.05	<0.01	<0.01

[a] veraison plus 2 weeks for 1993/94 (both shoot growth and conductance) and 1994/95 (conductance only); veraison plus 1 week for 1995/96 (Waite).

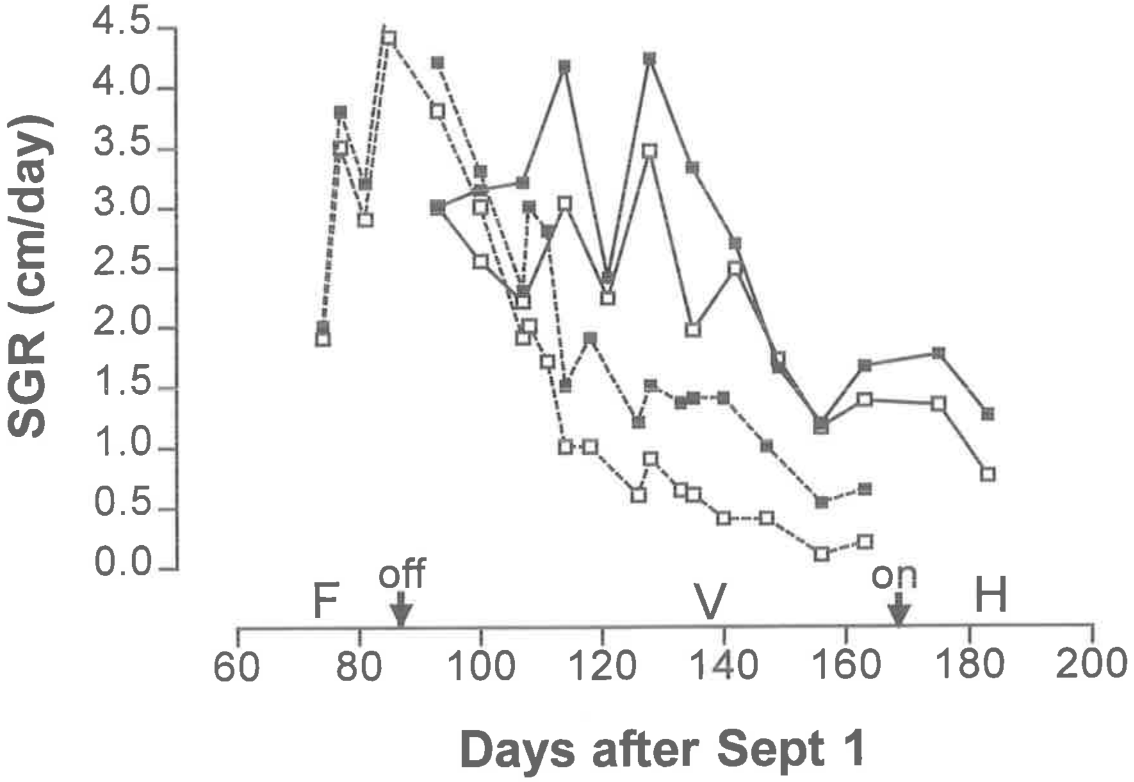


Figure 9.6 Effect of partial drying on shoot growth rate (SGR, mean, cm/day) of Cabernet Sauvignon/Ramsey split-root vines in two seasons, Exp 14. Both sides of vine irrigated (C, ■); one side of vine not irrigated at any one time (T,☐) from D83 ('off') until D183 (1993/94, solid lines) and D86 ('off') to D166 ('on') (1994/95, dotted lines). F = flowering, V = veraison, H = harvest. Means are significantly different ($p<0.05$) from D107 to D128 in 1993/94 and from D118 to D165 in 1994/95.

The reduction of SGR of T vines below 50% of the rate of C vines from veraison in 1994/95 coincided with cessation of growth of more than 50% of shoot tips. By comparison, fewer than 10% of C shoots had stopped growing by veraison and the 50% level was not reached until at least D180, 40 or more days after T vines reached the same level (Figure 9.7). SGR of T vines decreased to ca.10% of C rate by the end of the PRD periods but by this time there were almost no remaining active shoot tips on T vine: by D150, ca. 90% of T shoots had ceased to grow. Similar observations were made in the other seasons. SGR for both T and C vines reached a maximum at set and decreased thereafter (Figure 9.6).

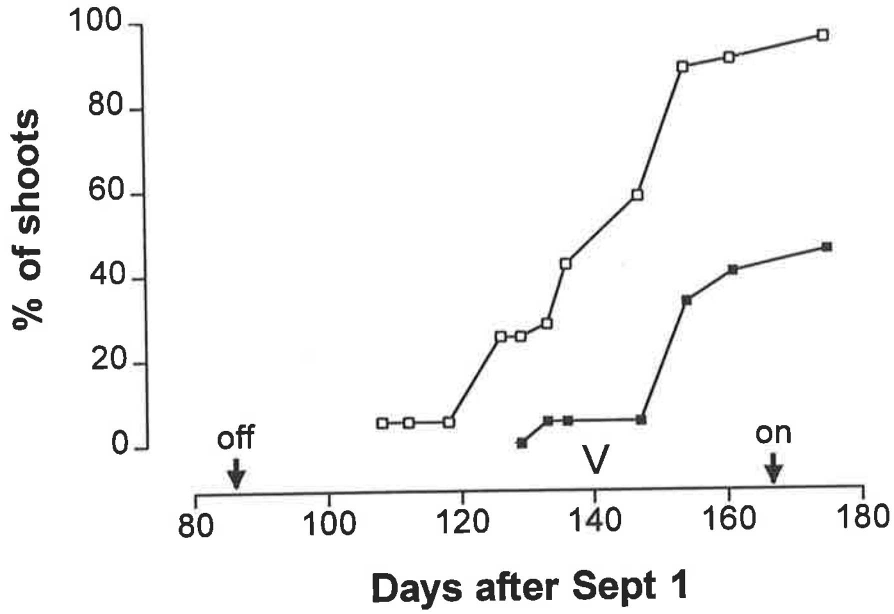


Figure 9.7 Percentage of shoots which had stopped growing on Cabernet Sauvignon/Ramsey split-root vines, 1994/95, Exp.14. Both sides of vine irrigated (C, ■); one side of vine not irrigated during PRD periods (T, □).

In all seasons, the relative decrease in SGR of T vines during the first period of soil drying coincided with the decrease in soil water content on the 'dry' side. In 1993/94, there was a partial recovery during the second period, a decrease again during the third and recovery again during the fourth period (Figure 9.8a). By comparison, in 1994/95, SGR of T vines decreased to ca. 80% of C before a slight recovery at the end of the first period (Figure 9.8b). There was a further decrease of T relative to C during the second and third periods to ca.60% and 50% respectively before a slight recovery prior to the end of each period.

There were no obvious recoveries towards the end of PRD periods in 1995/96, apart from a slight recovery in the first PRD period (Figure 9.8c). Actual SGR decreased for both C and T from 1993/94 to 1995/96 (Table 9.2), in response to increased shoot number per vine. Relative to C, SGR of T vines was 21 ± 3% lower over three seasons.

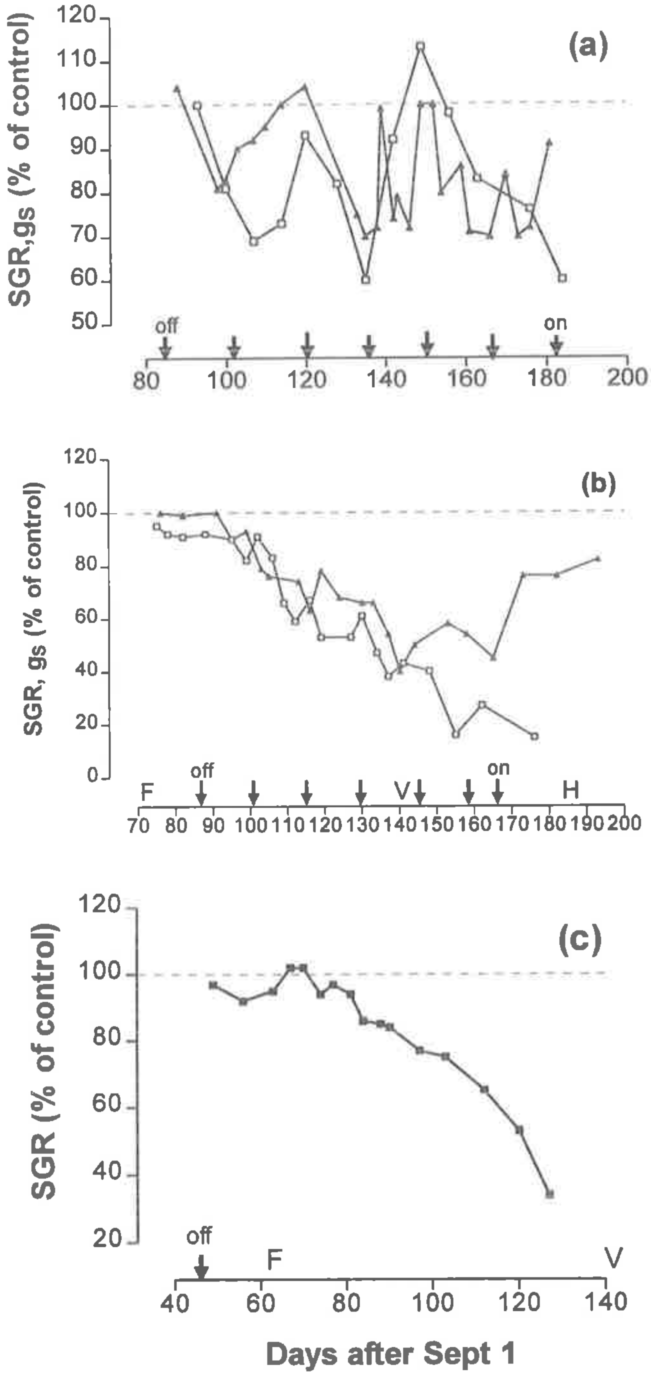


Figure 9.8 Effect of partial drying on shoot growth rate (SGR) and stomatal conductance (g_S) of Cabernet Sauvignon/Ramsey split-root vines in three seasons, Exp 14. SGR (□) and g_S (▲) of 'treated' (T) as % of 'control' (C). Arrows indicate days when irrigation of T vines switched from one side to the other. See captions of Figures 6.1, 6.2 and 6.3 for explanation of treatments.

(a) 1993/94: SGR and g_S.

(b) 1994/95: SGR and g_S. T significantly different ($p<0.05$) to C from D113 to D165 for g_S and from D118 for SGR.

(c) 1995/96: SGR only. T significantly different ($p<0.05$) to C from D97.

Main shoot components: The reduction in SGR from set to veraison (18 to 24%) in response to PRD resulted in significantly shorter shoots (ca. -20%) at maturity (Table 9.3a,b,c). For the second set of shoots in 1995/96, the SGR of the 'up' shoots was 38 and 30% of 'down' shoots for C and T vines respectively. The overall decrease in vegetative growth in response to treatment was reflected by the significantly lower pruning weights in each season (-19 to -29%) which was due to a significant decrease in mean shoot weight (-22 to -35%). In the first two seasons, the lower shoot weight was due to a combination of decreased shoot length (ca. -20%) and decreased shoot weight per unit shoot length (ca. -15%); in 1995/96, this was mainly due to decreased shoot weight per unit shoot length (-13%). Decreased shoot weight per unit shoot length was not the result of decreased shoot diameter but rather due to a reduction in number of lateral shoots per unit shoot length (Table 9.3).

There was greater effect of treatment on the 'down' shoots than the 'up' shoots in the last season (Table 9.3c). 'Down' shoots were twice as long as 'up' shoots on C vines due to the effect of trimming on 'up' shoots.

The frequency distribution of mature shoot length was also affected by treatment: C vines had a higher proportion of long shoots and a smaller proportion of short shoots than T vines (Figure 9.9). The reduction in mature shoot length on T vines in 1994/95 was due to a significantly lower node number per shoot (-25%) with no effect on internode length, either averaged over the whole shoot (Table 9.5) or when individual internodes along the shoot were measured (Figure 9.10). In the latter case, there was also no difference between treatments with respect to the length of internodes formed before or after the application of PRD. Node number per shoot was lower (-15%) on T shoots in 1995/96 but internodes were 11% longer (Table 9.3c).

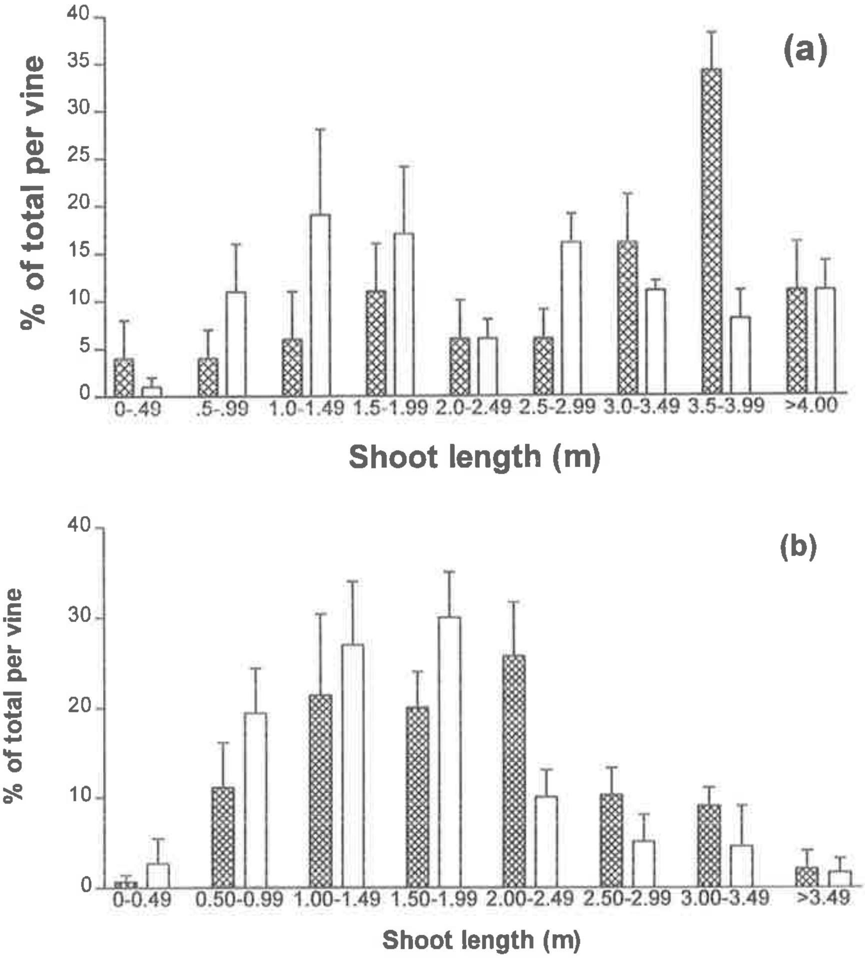


Figure 9.9 Frequency distribution of mature shoot length (mean ± se), measured in winter, of Cabernet Sauvignon/Ramsey split-root vines, Expt 14. Both sides of vine irrigated (C, hatched bars); one side of vine not irrigated during PRD periods (T, clear bars).
a. 1993/94; b. 1994/95

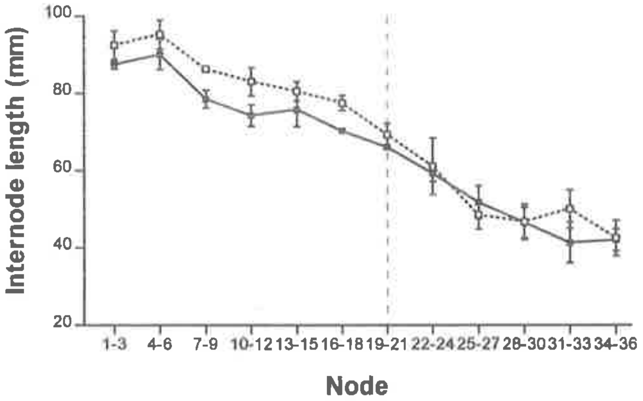


Figure 9.10 Internode length (mean ± se, mm) of Cabernet Sauvignon/Ramsey split-root vines, 1994/95, Expt 14. Both sides of vine irrigated (C, ■); one side of vine not irrigated during PRD periods (T, □). Means of three nodes; nodes 1 to 21 inclusively formed before start of PRD.

Lateral shoots: One of the most significant responses to PRD was the reduction in growth of lateral shoots on T vines (Table 9.3). Both lateral number and total lateral length per shoot were affected with decreases of 28 to 47% and 50 to 66% respectively. Mean lateral length was not affected in the first season but it was reduced by 29 to 35% in subsequent seasons. The effect of treatment on lateral shoot number was greater for 'up' shoots than 'down' in 1995/96, but the reverse was the case for mean lateral length (Table 9.3c). Shoot length and lateral number per shoot were strongly correlated ($p<0.001$): lateral number = 0.0117shoot length +1.38 for C shoots and = 0.011shoot length +0.02 for T shoots (C and T shoots from all vines were pooled).

Table 9.3 Shoot growth components and canopy measurements of Cabernet Sauvignon/Ramsey split-root vines, Exp. 14. Both sides of vine irrigated (C); one side of vine not irrigated during PRD periods (T).

a. 1993/94

Variable	**C**	**T**	**% diff.**	**Signif.**
Pruning wt (kg/vine)	5.2 ± 0.13	3.7 ± 0.31	-29	<0.05
Shoot no/vine	15.5 ± 0.8	17 ± 1.2	+9	ns
Shoot wt (g)	340 ± 20	220 ± 25	-35	<0.05
Shoot length (cm)	296 ± 22	231± 23	-22	<0.05
Shoot wt/unit length (g/cm)	1.15 ± 0.08	0.95 ± 0.1	-17	<0.01
Lateral no/main shoot	4.6 ± 0.5	2.4 ± 0.4	-47	<0.05
Total length of lat. shoots/main sht (cm)	302 ± 16	151 ± 20	-50	<0.01
Mean length lateral sht /main sht (cm)	66 ± 4	63 ± 2	-5	ns
Shoot no/m row	7.8	8.5	+9	ns
Leaf layer number	3.0	1.7	-43	<0.01
Bunch exposure index	146	565	+286	<0.05

b. 1994/95

Variable	**C**	**T**	**% diff.**	**Signif.**
Pruning wt (kg/vine)	4.27 ± 0.17	3.47 ± 0.37	-19	<0.05
Shoot no/vine	31.0 ± 2.6	35.3 ± 0.6	+13	ns
Shoot wt (g)	138 ± 15	98 ± 8	-31	<0.05
Shoot length (cm)	193 ± 6	154 ± 11	-20	<0.05
Shoot wt/unit length (g/cm)	0.7 ± 0.06	0.6 ± 0.02	-13	ns
Lateral no/main shoot	1.78 ± 0.2	0.98 ± 0.2	-45	<0.05
Total length of lateral shoots/main sht	53 ± 14	18 ± 4	-66	<0.05
Mean length lateral sht (cm)	31 ± 6	22 ± 4	-29	ns
Mean node no/sht	29.3 ± 0.5	21.9 ± 1.6	-25	<0.01
Mean internode length (cm)	6.6 ± 0.1	7.0 ± 0.1	+6	ns
Shoot diameter (mm)	10.1 ± 0.3	10.0 ± 0.9	0	ns
Shoot no/m row	15.5 ± 1.5	17.7 ± 0.3	+14	ns
Bunch exposure index	119 ± 37	318 ± 50	+167	<0.05

c. **1995/96**

Variable	**C**	**T**	**% diff.**	**Signif.**
Pruning weight (kg) up	2.26 ± 0.28	1.94 ± 0.23	-14	ns
down	3.0 ± 0.63	2.30 ± 0.22	-24	ns
total / vine	5.27 ± 0.9	4.23 ± 0.39	-20	ns
total / vine (corrected)	6.2 ± 0.9	5.0 ± .5	-19	ns
Shoot number / vine	45 ± 5.4	46 ± 2.0	+2	ns
Shoot wt (g)	118 ± 14	92 ± 6	-22	ns
Shoot length, winter (cm) up	103 ± 7.6	131 ± 3.0	-6	ns
down	206 ± 14.5	191 ± 12.7	-7	ns
ave/vine	154 ± 10.9	144 ± 5.5	-7	ns
Shoot wt / unit length (g/cm)	0.77 ± 0.04	0.64 ± 0.05	-17	ns
Lateral number / shoot up	2.75 ± 0.49	1.8 ± 0.31	-35	ns
down	2.7 ± 0.18	2.1 ± 0.21	-23	ns
ave/vine	2.7 ± 0.3	1.9 ± 0.26	-28	ns
Total length of lat. shts/main sht (cm) up	61 ± 11.5	29 ± 6.2	-52	<0.05
down	72 ± 24	32 ± 7	-55	ns
ave/vine	66 ± 17	31 ± 7	-54	ns
Mean lat shoot length (cm) up	21.8 ± 1.9	15.5 ± 1.3	-29	<0.05
down	25.9 ± 6.9	15.3 ± 2.3	-41	ns
ave/vine	23.9 ± 4.4	15.4 ± 1.7	-35	ns
Node no. / shoot up	14.7 ± 0.83	12.8 ± 0.4	-13	ns
down	31.0 ± 2.2	26.0 ± 2.9	-16	<0.05
ave/vine	22.9 ± 2.4	19.4 ± 1.4	-15	ns
Mean internode length (cm) up	7.0 ± 0.15	7.6 ± 0.25	+9	ns
down	6.6 ± 0.18	7.5 ± 0.35	+13	ns
ave/vine	6.8 ± 0.12	7.6 ± 0.23	+11	ns
Shoot diameter (cm) up	1.15 ± 0.01	1.15 ± 0.06	100	ns
down	1.21 ± 0.02	1.21 ± 0.02	100	ns
ave/vine	1.18 ± .01	1.18 ± 0.03	100	ns
Shoot no / m row	22.5	23.0	+2	ns
Leaf area/surface area	4.0 ± 0.44	3.1 ± 0.21	-22	ns
Leaf layer number (upper bunch zone)	2.3 ± 0.14	1.9 ± 0.27	-17	ns
Bunch exposure index	285 ± 38	396 ± 52	+39	<0.05
Total leaf area / fruit wt (cm^2/g)	24.5 ± 3.0	18.5 ± 1.2	-24	ns
% bunches severely infected with oidium	52 ± 12	34 ± 11	-35	ns

* first shoots only

Leaf area: The relative decrease in vegetative growth of the T vines was reflected by the relatively smaller total leaf area per vine (TLA) at harvest. The difference in TLA (-25% for T) was mainly due to a difference in lateral leaf area [LLA] (-41%) rather than main leaf area [MLA] (-12%) (Figure 9.11, Table 9.4). The ratio of LLA to TLA at harvest was also significantly lower on T vines in both seasons, eg. 36% and 27% of TLA at harvest for C and T respectively in 1995/96 (Figure 9.11b). The reduction in 'leafiness' of T shoots was indicated by the decrease in leaf area/unit shoot length.

The actual differences in TLA measured at harvest were the result of differences in the relative increase in TLA over the season. In 1994/95, TLA of C vines increased by 130% from set to harvest whereas TLA of T vines only increased by 50% (Figure 9.11a); for 1995/96, the increase was 260% and 180% respectively from D50 to harvest (Figure 9.12). The changes in leaf area over the whole season were closely monitored in 1995/96 (Figure 9.11b): by D110 (before trimming of 'up' shoots), TLA of C vines was 23% greater than T, due to a similar (%) effect on MLA and LLA. At that time, LLA was 27% of TLA for both treatments. From D110 to harvest, the biggest effect of treatment was on the LLA

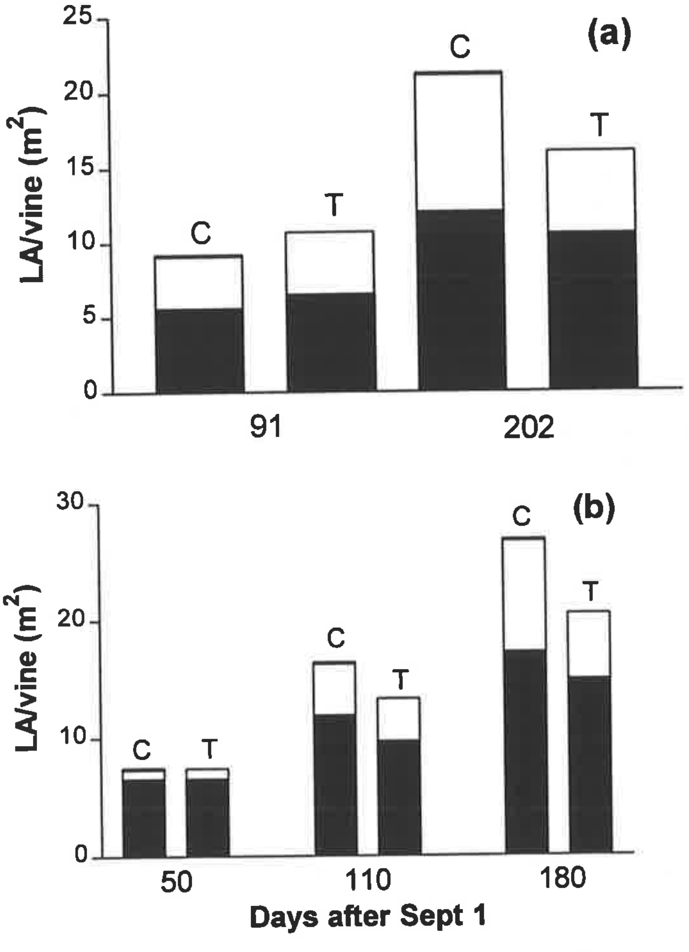


Figure 9.11 Total, main (solid) and lateral (clear) leaf area per vine (LA, m^2) of Cabernet Sauvignon/Ramsey split-root vines, Exp.14. Both sides of vine irrigated ('C'); one side of vine not irrigated during PRD periods. ('T').

a. 1994/95: D91(1/12/94) and D202 (22/3/95); total and lateral leaf area of T vines significantly ($p<0.05$) less than C on D202.
b. 195/96: D50, D110) and D180); total and lateral leaf area of T vines significantly ($p<0.05$) less than C on D110 and D180.

component: LLA of C vines increased by 113% (relative to D110) whereas LLA of T vines increased by just 56%. The actual increase in MLA was similar for both treatments (ca.5 m^2 per vine) as was the percentage increase (ca.50% relative to D110). The effect of treatment on leaf number per shoot (both main and lateral) was greater than the effect on leaf size. For example, lateral leaf area per shoot at harvest in 1994/95 was significantly less on T vines (-45%) due to a combination of fewer lateral leaves per shoot (-34%) and smaller mean area per leaf (-21%) [Table 9.4].

Table 9.4 Leaf area components of 'control' (C, both sides of vine irrigated) and 'treated' (T, one side of vine not irrigated during PRD periods); Cabernet Sauvignon/Ramsey split-root vines, 1994/95, Exp.14.

Variable	**C**	**T**	**% diff.**	**Signif.**
Leaf area per shoot				
1/12/94				
Main leaves				
Mature leaf no	13.4 ± 0.2	14.4 ± 0.4	+7	ns
Mean mature leaf area (cm^2)	123 ± 6.4	120 ± 2.0	-	ns
Mature leaf area (cm^2)	1675 ± 47	1742 ± 55	+3	ns
Total main leaf area (cm^2)	1809	1862	+3	ns
Total lateral leaf area (cm^2)	1160 ± 115	1190 ± 153	+3	ns
Total leaf area: main + lat (cm^2)	2969 ± 140	3052 ± 176	+3	ns
Lateral/total area	0.37 ± 0.02	0.37 ± 0.02		ns
22/3/95				
Main leaves				
Mature leaf no	33.6 ± 1.7	26.5 ± 2.8	-21	ns
Mean mature leaf area (cm^2)	113 ± 2.6	112 ± 4.6	-2	ns
Mature leaf area (cm^2)	3845 ± 222	2969 ± 255	-23	<0.05
Total main leaf area (cm^2)	3845 ± 222	2969 ± 255	-23	<0.05
Lateral leaves				
Lateral leaf no	63.4 ± 3.8	42.1 ± 8.8	-34	<0.05
Mean lateral leaf area (cm^2)	46.3 ± 2.8	36.5 ± 2.7	-21	<0.05
Total lateral leaf area (cm^2)	2940 ± 97	1539 ± 401	-48	<0.05
Total leaf area: main + lat (cm^2)	6785 ± 270	4508 ± 596	-34	<0.05
Lateral/total area	0.40 ± 0.01	0.29 ± 0.03	-27	<0.05
Shoot length (cm)	267 ± 8	211 ± 15	-21	<0.05
Leaf area/shoot length (cm^2/cm)	25.5 ± 1.7	20.7 ± 1.2	-19	<0.05
Nodes/sht	44 ± 0.5	34 ± 4	-22	<0.05
Lateral leaf no/total leaf no	0.65 ± .02	0.60 ± 0.07	-8	ns
Leaf area per vine				
Main leaf area/vine (m^2)	12.0 ± 1.6	10.5 ± 1.0	-12	ns
Lateral leaf area/vine (m^2)	9.2 ± 0.9	5.5 ± 1.7	-41	<0.05
Total leaf area/vine (m^2)	21.2 ± 2.5	15.9 ± 2.5	-25	<0.05
Total leaf area/fruit wt (cm^2/g)	43.6 ± 3.9	33.2 ± 5.9	-24	ns

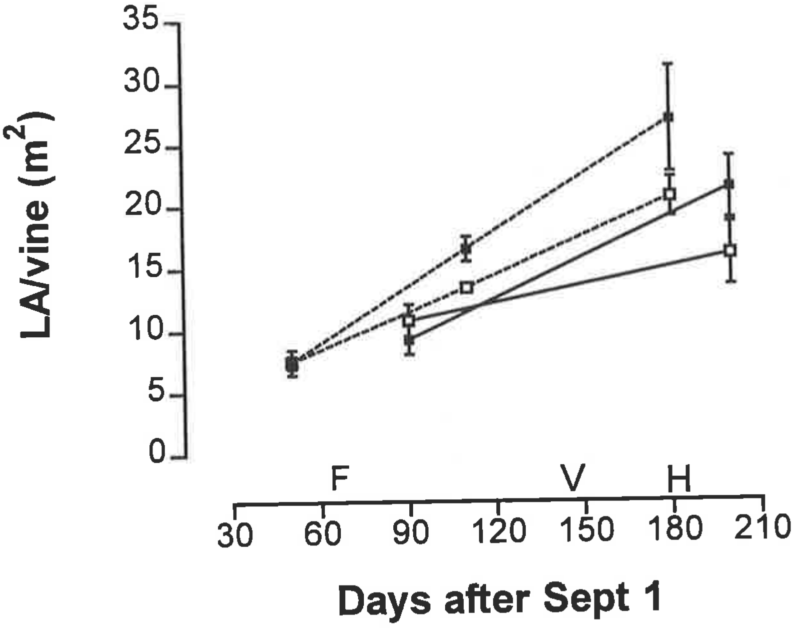


Figure 9.12 Leaf area per vine (LA, mean ± se, m^2) of Cabernet Sauvignon/Ramsey split-root vines, Exp.14; 1994/95 (solid lines), 1995/96 (dotted lines). Both sides of vine irrigated (C, ■); one side of vine not irrigated during PRD periods (T, □). F, V, H indicate flowering, veraison and harvest dates respectively for 1995/96 season.

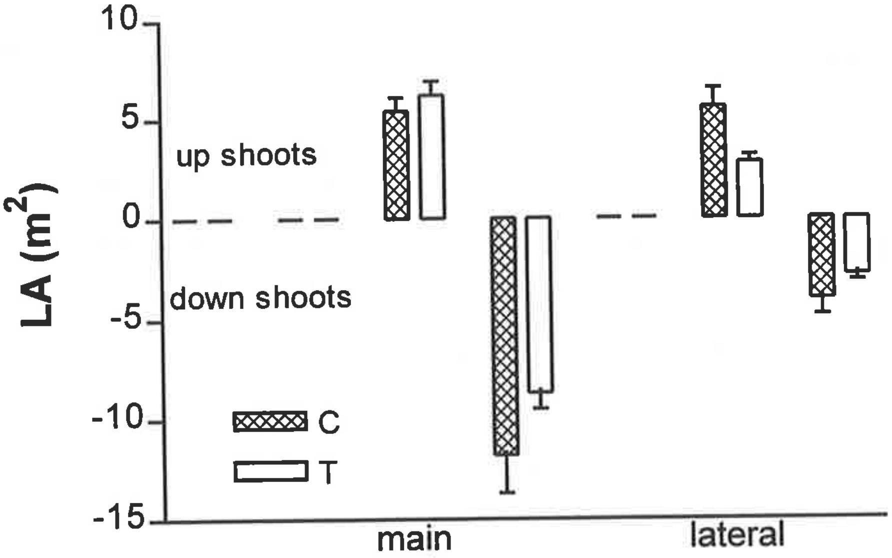


Figure 9.13 Leaf area (LA, mean ± se, m^2) of main and lateral leaves of 'up' and 'down' shoots of 'control' (C, hatched bars) and 'treated' (T, clear bars) Cabernet Sauvignon/Ramsey split-root vines, 1995/96, Exp.14. C: both sides of vine irrigated; T: one side of vine not irrigated during PRD periods.

The relative effect of treatment on 'up' and 'down' shoots was examined in 1995/96. TLA on 'up' shoots was reduced on T vines due to a 26% decrease in LLA (Figure 9.13). TLA on 'down' shoots was also less due to a combination of reduced MLA (-26%) and LLA (-30%) but, because LLA only comprised ca.25% of the 'down' total for both treatments, the effect on MLA in real terms was more important. By comparison, lateral leaves were a more significant component of TLA of the 'up' shoots (50 and 30% for C and T respectively). Figure 9.14 shows differences in lateral shoot development on trimmed 'up' shoots from C and T vines: (a) is representative of 'average' shoots of both treatments with respect to mean lateral number and total lateral length per main shoot (Table 9.3c) whereas (b) is representative of high 'vigour' shoots. Both show the greater response of C shoots to trimming, particularly at distal nodes; shoots from T vines with long laterals were likely to have them at basal nodes (Figure 9.14b).

Trunk diameter: Diameter of the trunk and of the top part of the root systems below the split (either side) were not significantly affected by treatment.

Gas exchange: Stomatal conductance was reduced in all seasons in response to PRD. For the period from set to veraison, average g_S of T vines was 12% to 30% lower than C (Table 9.2) and, in general, was significantly lower from the first PRD period. In 1993/94, apart from lower values during the third and sixth periods (coincidental with high temperatures and VPD during the third period), average g_S for each period remained relatively constant for C vines and that of T followed a similar pattern (Figure 9.15); nevertheless, average g_S of T vines was always less than that of C. In 1994/95, average g_S of C remained relatively constant from set until just before veraison, then decreased around veraison; by comparison, g_S of T decreased from set to veraison (Figure 9.15). Both treatments remained relatively constant from veraison to harvest.

As for SGR, the relative decrease in g_S of T vines during the first period of soil drying coincided with the decrease in SWC on the 'dry' side but the response was greater in 1993/94 than the other two seasons. In 1993/94, g_S recovered during the second period,

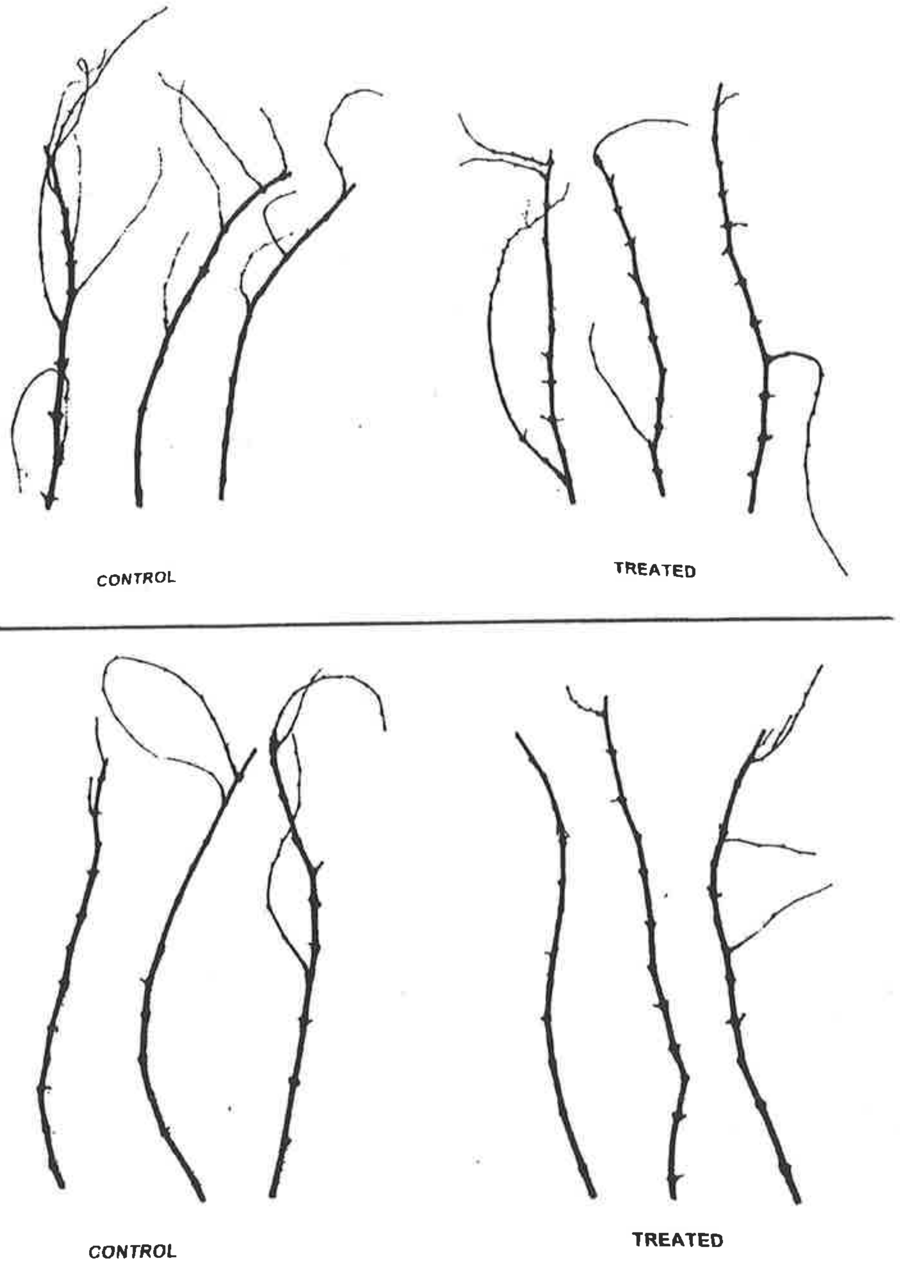


Figure 9.14 Shoots (air-dried, distal 12 nodes, tendrils and bunch stems removed) with similar diameter of 'control' (C) and 'treated' (T) Cabernet Sauvignon/Ramsey split-root vines, 1995/96, Exp.14. C: both sides of vine irrigated; T: one side of vine not irrigated during PRD periods. Upper = 'high vigour' shoots: mean lateral number and total lateral length per main shoot of 5.0 and 150cm, and 2.7 and 70cm for C and T respectively. Lower = average shoots: mean lateral number and total lateral length per main shoot of 2.3 and 64cm, and 1.7 and 34cm for C and T respectively. Scale is 1:12.5 and 1:11.0 for 'upper' and 'lower' respectively.

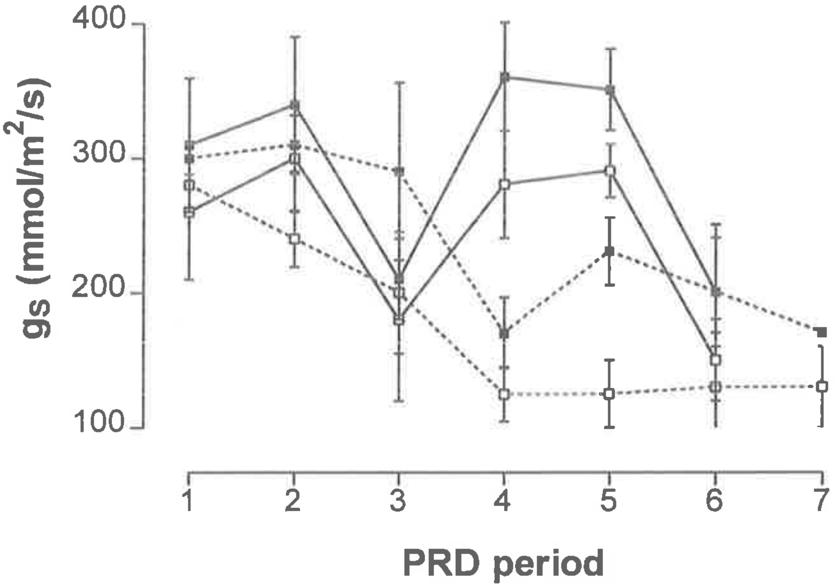


Figure 9.15 Effect of partial drying on stomatal conductance (g_S, mean of each PRD period ± se, mmol/m^2/s) of Cabernet Sauvignon/Ramsey split-root vines in two seasons (1993/94, solid lines; 1994/95, dotted lines), Exp.14a,b. Both sides of vine irrigated (C, ■); one side of vine not irrigated at any one time (T, □) during PRD periods 1 to 6.

decreased again during the third and recovered again during the fourth (Figure 9.8a). The maximum decrease of T relative to C at any one time was 30%, compared to 40% for SGR. In 1994/95, g_S of T vines relative to C decreased only slightly during the first drying period (Figure 9.8b) but by the end of the second, had decreased to ca. 65% and reached the lowest level of ca. 40% of C at veraison. There was an apparent partial recovery of T relative to C from veraison; however, this was not so much due to a change in actual g_S of T but rather a result of a reduction in g_S of C vines (Figures 9.7b, 9.15).

Diurnal changes in g_S were measured five days before the end of the last drying period in 1994/95: T was 36% lower than C by mid-morning and that relative difference was maintained until late afternoon. Actual g_S of C vines remained relatively constant but g_S of T was lower in 1994/95 than the other two seasons (Table 9.1). Relative to C, g_S of T vines was much lower in 1994/95 (-30%) than in the other two seasons (-12 to -13%).

Leaf water potential: There was no significant difference between treatments, at any time of the day, in any season (Table 9.5).

Table 9.5 Summary of leaf water potential (mean ± se, MPa) of Cabernet Sauvignon/Ramsey split-root vines, Exp. 14. Both sides of vine irrigated (C); one side of vine not irrigated (T).

Season	**Dates**	**Time of measurement**	**C**	**T**	**Signif.**
1993/94	30/12, 12/1	Pre-dawn	-0.51 ± 0.01 (n=2)	-0.55 ± 0.02 (n=2)	ns
1994/95	12/1 to 22/1	Pre-dawn	-0.50 ± 0.02 (n=3)	-0.57 ± 0.017 (n=3)	ns
	12/1 to 22/1	Midday	-1.30 ± 0.05 (n=3)	-1.30 ± 0.06 (n=3)	ns
1995/96	10/1 to 2/2	Mid-morning	-0.85 ± 0.07 (n=8)	-0.91 ± 0.06 (n=8)	ns
	10/1 to 2/2	Mid-afternoon	-1.22 ± 0.08 (n=4)	-1.20 ± 0.03 (n=4)	ns

Canopy measures: In all seasons, as a result of decreased vegetative growth, T vines had a more open canopy than C. This was indicated by lower LLN (1993/94,1995/96), greater bunch exposure and lower LA/SA (1995/96) of T vines relative to C (Tables 9.3, 9.12). A further indication of the more open canopy of T vines was the reduction in percentage of bunches severely infected by oidium in 1995/96 (Table 9.3c).

Yield components: There was no significant effect on yield per vine in response to treatment in any season (Figure 9.16). Also, there was no effect on any component of yield including berry weight (Table 9.6). Actual fruit weight was higher on T vines in 1995/96 if there was no correction for oidium infection (Table 9.6c). Fruit weight for both treatments increased from ca. 4 t/ha in 1993/94 (first crop) to ca. 14 t/ha in 1995/96 (third crop) and fruit weight/pruning weight ratio (FW/PW) increased over the same period as the vines became more balanced (Figure 9.16). FW/PW ratio was 23 to 28% higher for T vines in all seasons due to the reduction in pruning weight per vine.

The irrigation efficiency (fruit weight/volume irrigation) increased significantly from the second to the third season for both treatments due to the increase in fruit weight (Figure 9.16). The irrigation efficiency of T vines was up to twice that of C because the latter received twice as much irrigation during the PRD period (Table 9.1). Leaf area/fruit weight ratio on T vines was 24% less in both 1994/95 and 1995/96 due to lower TLA per vine (Tables 9.3c, 9.4).

Table 9.6 Yield components of Cabernet Sauvignon/Ramsey split-root vines, Expt 14. Both sides of vine irrigated (C); one side of vine not irrigated during PRD periods (T).

a. 1993/94

Variable	C	T	% diff.	Signif.
Fruit wt (kg/vine)	3.4 ± 0.5	3.1 ± 0.8	-9	ns
Bunch no/vine	33 ± 1.8	35 ± 4	+6	ns
Bunch wt (g)	103 ± 17	89 ± 12	-14	ns
Berry wt (g)	0.94 ± 0.03	0.95 ± 0.05	0	ns
Berry no/bunch	110 ± 17	94 ± 14	-15	ns
Fruit wt / pruning wt	0.65 ± 0.16	0.83 ± 0.13	+28	ns
Fruit wt/volume irrigation[a] (g/L)	5.5	9.0	+63	

b. 1994/95

Variable	C	T	% diff.	Signif.
Fruit wt (kg/vine)	4.88 ± 0.38	4.95 ± 0.42	+2	ns
Bunch no/vine	72.3 ± 8	75.3 ± 7	+3	ns
Bunch wt (g)	65.9 ± 4.3	65.7 ± 3.5	0	ns
Berry wt (g)	0.71 ± 0.03	0.73 ± 0.04	+3	ns
Berry no/bunch	93.2 ± 7.9	90.5 ± 7.4	-3	ns
Bunch no/sht	2.36 ± 0.3	2.15 ± 0.2	-9	ns
Fruit wt/pruning wt	1.15 ± 0.1	1.47 ± 0.17	+28	ns
Fruit wt/volume irrigation[a] (g/L)	4.9	7.2	+47	

c. 1995/96

Variable	C	T	% diff.	Signif.
Fruit wt[b] (kg/vine)	10.8 ± 0.74	11.1 ± 0.83	+3	ns
Bunch wt (g)	103 ± 9.8	112 ± 9.8	+9	ns
Bunch no/vine	107 ± 10.1	100 ± 3.8	-7	ns
Berry wt (g)	0.72 ± 0.02	0.78 ± 0.01	+8	ns
Berry no /bunch	143 ± 11.9	144 ± 14.5	+1	ns
Bunches / shoot	2.47 ± 0.33	2.20 ± 0.24	-11	ns
Fruit wt / pruning (wt)	2.16 ± 0.22	2.66 ± 0.23	+23	ns
Nodes / vine (winter 1995)	49 ± 1.8	51± 0.9	+4	ns
Shoot no / vine	45 ± 5.4	46 ± 2.0	+2	ns
Shoots / node	0.92 ± 0.04	0.90 ± 0.04	+2	ns
Fruit wt / volume irrigation[a] (g/L)	10.1	20.2	+100	-

[a] Budburst to harvest

[b] Corrected fruit wt to account for oidium infection; actual = 7.3 ± 1.0 and 9.2 ± 0.7 for C and T respectively

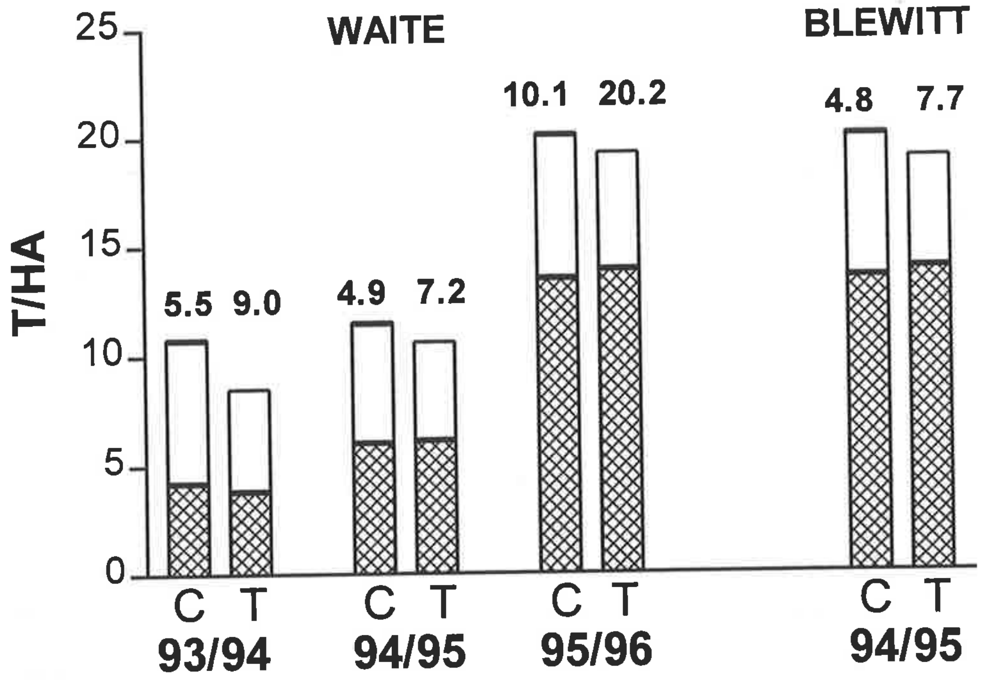


Figure 9.16 Summary of fruit weight (hatched) and pruning weight (clear) expressed as t/ha: C (irrigated on both sides) and T (partial drying) from Exp.14 (Waite: 1993/94, 1994/95, 1995/96) and 15 (Blewitt Springs: 94/95). Numbers in bold above bars are fruit weight/volume irrigation over the whole season (t/ML); see Appendix VI for irrigation data.

Fruit composition: There was no effect of treatment on the timing of veraison, nor on phenology in general (Table 9.7) in any season. However, T vines ripened earlier than C, reaching ca. 23°Brix at least one week earlier in the first two seasons (Table 9.8). For the last two to three weeks of ripening before harvest, °Brix of T vines was always higher than C but not always significantly so (Figure 9.17). In the last season, T vines ripened at a similar rate to C with respect to sugar accumulation.

Table 9.7 Phenological stages and timing of partial drying (PRD) experiments at Waite and Blewitt Springs (date and days after September 1).

Stage	Waite:1993/94		Waite:1994/95		Waite:1995/96		Blewitt:1994/95	
	Date	**Day**	**Date**	**Day**	**Date**	**Day**	**Date**	**Day**
Budburst	2/9	1	4/9	3	1/9	0	-	-
Flowering	12/11	72	12/11	72	3/11	63	-	-
Set	23/11	83	24/11	84	15/11	75	6/12	96
Veraison	14/1	135	20/1	141	22/1	143	5/2	157
Harvest	2/3	182	28/2, 6/3	180, 186	27/2	179	26/4	237
PRD start	24/11	84	26/11	86	16/10	45	8/12	98
PRD finish	2/3	182	15/2	167	27/2	179	1/3	181

In each season, compositional parameters were compared at the same concentration of total soluble solids (°Brix ± 0.1) irrespective of harvest date. Titratable acidity (expressed as tartaric acid) and pH of fruit from T vines were significantly higher and lower than fruit from C vines respectively in the first two seasons, but not the last. No other parameter was significantly different in 1993/94. In 1994/95, anthocyanins, phenolics and glycosyl-glucose (G-G) of T vines were all significantly higher, both on a per-berry and per-berry mass basis: for example, 45% higher for anthocyanins (mg/g berry mass). Similarly, in 1995/96, there was no significant difference except for anthocyanins which were significantly higher on T vines, both on a per-berry (+20%) and per-berry mass basis (+13%). The difference in anthocyanins between treatments was measurable up to three weeks prior to harvest (Figure 9.18a,b) and was higher on T vines at the same °Brix, ie. independent of the rate of sugar accumulation (Figure 9.18c).

Table 9.8 Fruit composition of Cabernet Sauvignon/Ramsey split-root vines, Exp.14. Both sides of vine irrigated (C); one side of vine not irrigated during PRD periods (T).

a. 1993/94

Variable	**C**	**T**	**% diff.**	**Signif.**
Similar °Brix				
Date	**2/3/94**	**23/2/94**		
TSS (°Brix)	22.8 ± 0.15	22.9 ± 0.23	0	ns
Titratable acidity (g/L)	10.0 ± 0.1	11.5 ± 0.14	+15	<0.01
pH	3.07	3.15		<0.05
Berry wt (g)	0.94 ± 0.03	0.85 ± 0.03	-10	ns
Total anthocyanins (mg /berry)	1.28 ± 0.08	1.23 ± 0.05	-4	ns
Total anthocyanins (mg /g berry mass)	1.36 ± 0.2	1.46 ± 0.09	+7	ns
Total phenolics (ab. units /berry)	2.39 ± 0.08	2.24 ± 0.07	-6	ns
Total phenolics (ab. units /g berry mass)	2.54 ± 0.13	2.67 ± 0.11	+5	ns
At harvest (2/3/94)				
TSS (°Brix)	22.8 ± 0.15	23.2 ± 0.2	+2	ns
Titratable acidity (g/L)	10.0 ± 0.1	9.8 ± 0.23	-2	ns
pH	3.07	3.24		<0.05
Berry wt (g)	0.94 ± 0.03	0.95 ± 0.06	+1	ns
Total anthocyanins (mg /berry)	1.28 ± 0.08	1.30 ± 0.1	+2	ns
Total anthocyanins (mg /g berry mass)	1.36 ± 0.2	1.36 ± 0.03	0	ns
Total phenolics (ab. units /berry)	2.39 ± 0.08	2.57 ± 0.15	+8	ns
Total phenolics (ab. units /g berry mass)	2.54 ± 0.13	2.70 ± 0.1	+6	ns

b. 1994/5

Variable	**C**	**T**	**% diff.**	**Signif.**
Similar °Brix				
Date	**6/3/95**	**28/2/95**		
TSS (°Brix)	22.8 ± 0.5	22.9 ± 0.7	0	ns
Titratable acidity (g/L)	5.8 ± 0.2	8.4 ± 0.6	+15	<0.01
pH	3.44 ± 0.04	3.24 ± 0.03		<0.01
Berry wt (g_s)	0.76 ± 0.04	0.74 ± 0.02	-2	ns
Total anthocyanins (mg /berry)	0.89 ± 0.05	1.28 ± 0.05	+43	<0.01
Total anthocyanins (mg /g berry mass)	1.19 ± 0.12	1.72 ± 0.1	+45	<0.05
Total phenolics (ab. units /berry)	133 ± 5.8	167 ± 5.3	+26	<0.01
Total phenolics (ab. units /g berry mass)	176 ± 15	225 ± 12	+28	<0.05
Total G-G (µmol /berry)	1.98 ± 0.18	2.78 ± 0.14	+40	<0.01
Total G-G (µmol /g_s berry mass)	2.64 ± 0.37	3.75 ± 0.26	+42	<0.05
RF G-G (µmol /berry)	0.28 ± 0.14	0.34 ± 0.11	+19	ns
RF G-G (µmol /g berry mass)	0.39 ± 0.14	0.46 ± 0.16	+18	ns
At harvest (6/3/95)				
TSS (°Brix)	22.8 ± 0.5	24.5 ± 0.5		<0.05
Titratable acidity (g/L)	5.8 ± 0.2	5.1 ± 0.2		<0.01
pH	3.44 ± 0.04	3.46 ± 0.02		ns

c. 1995/96

Variable	C	T	**% diff.**	**Signif.**
TSS (° Brix)	22.6 ± 0.1.	22.7 ± 0.25	0	ns
Titratable acidity (g/L)	9.4 ± 0.5	8.6 ± 0.4	-9	ns
pH	3.30 ± 0.01	3.25 ± 0.02	-2	ns
Berry wt (g)	0.71 ± 0.02	0.76 ± 0.01	+7	ns
Anthocyanins (mg /berry)	0.84 ± 0.09	1.01 ± 0.03	+20	<0.05
Anthocyanins (mg /g berry mass)	1.17 ± 0.1	1.33 ± 0.04	+13	<0.05
Phenolics (ab. units /berry)	88 ± 4.5	95 ± 1.5	+8	ns
Phenolics (ab. units /g berry mass)	124 ± 3.0	124 ± 2.1	0	ns

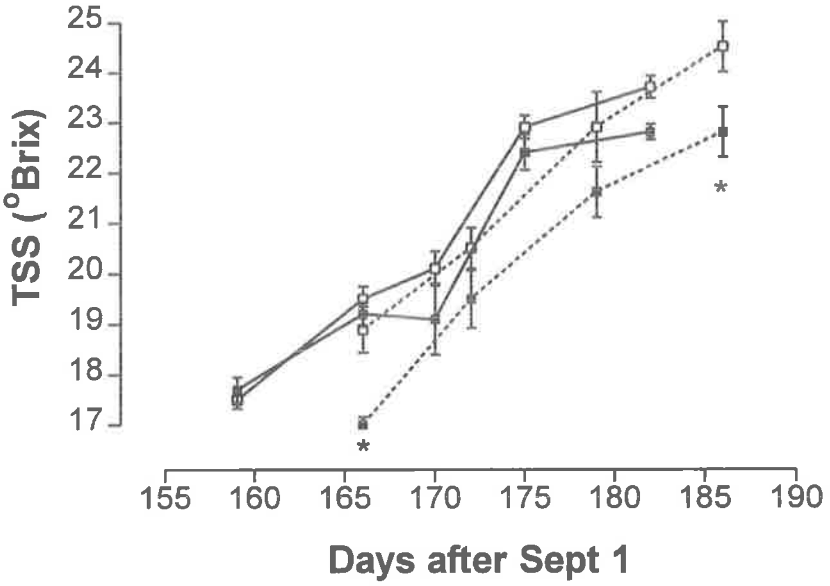


Figure 9.17 Effect of partial drying on total soluble solids (TSS, mean ± se, °Brix) of 'control' (C, ■) and 'treated' (T, □) Cabernet Sauvignon/Ramsey split-root vines in two seasons (1993/94, solid lines; 1994/95, dotted lines), Exp 14a,b. See caption of Figure 9.2 for explanation of treatments. * and ** indicate means are significantly different at p<0.05 and 0.01 respectively.

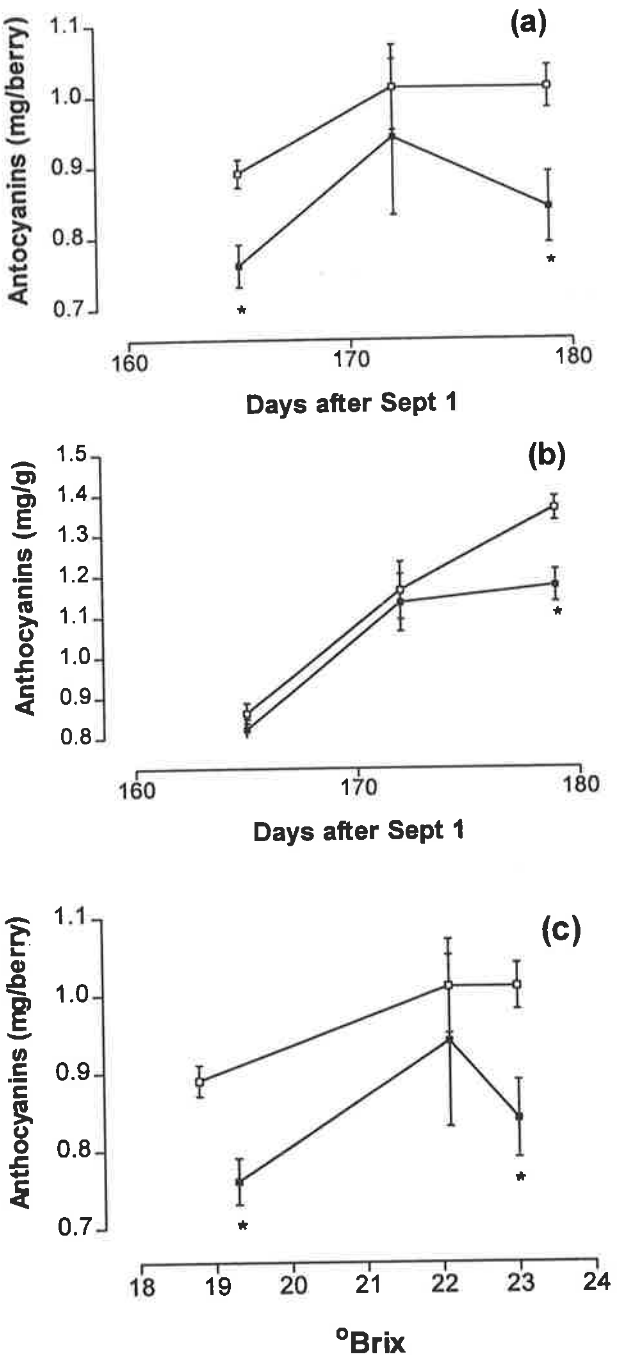


Figure 9.18 Anthocyanins (mean ± se) of Cabernet Sauvignon/Ramsey split-root vines, 1995/96, Exp. 14. Both sides of vine irrigated (C, ■); one side of vine not irrigated from D45 until D179 [= 27/2/96] (T, □). * indicates means are significantly different ($p<0.05$).

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 **

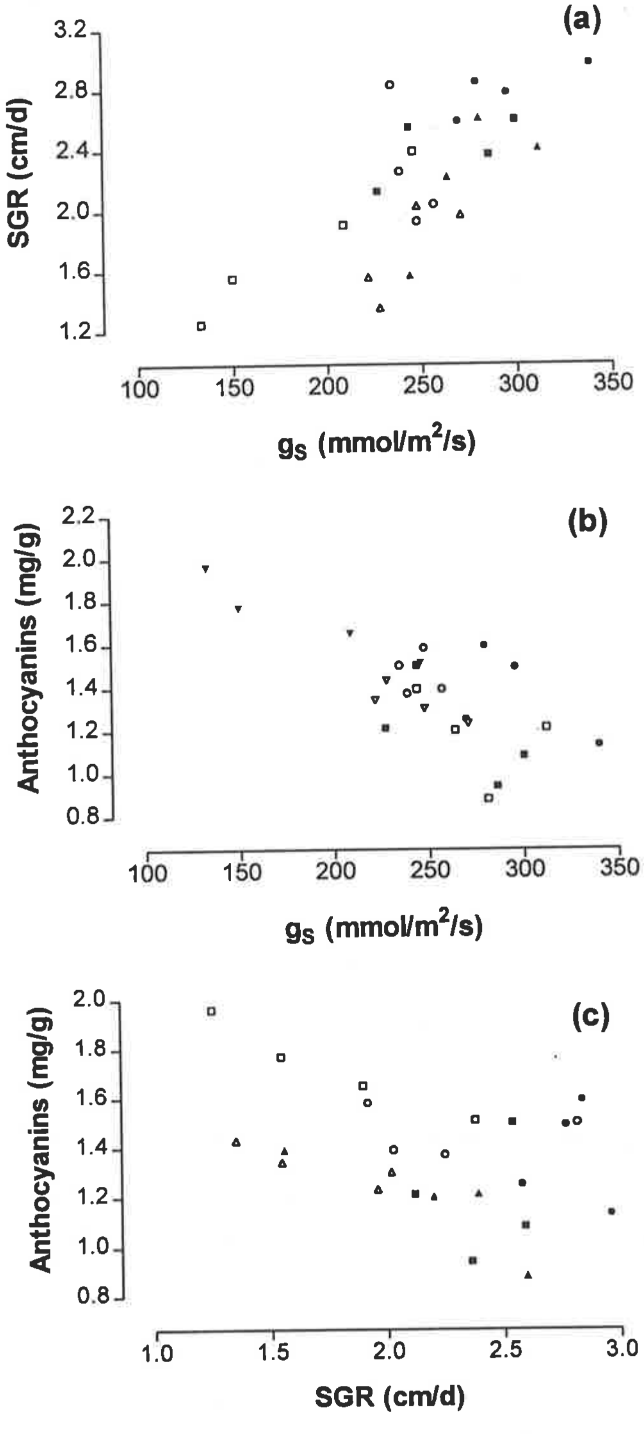


Figure 9.19 Relationships between stomatal conductance (g_S, mmol/m^2/s), shoot growth rate (SGR, cm/day) and anthocyanins (mg/g berry mass) of C (irrigated on both sides, closed symbols) and T (partial drying, open symbols) Cabernet Sauvignon (Exp.14) over three seasons: 1993/94 (circles), 1994/95 (squares),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

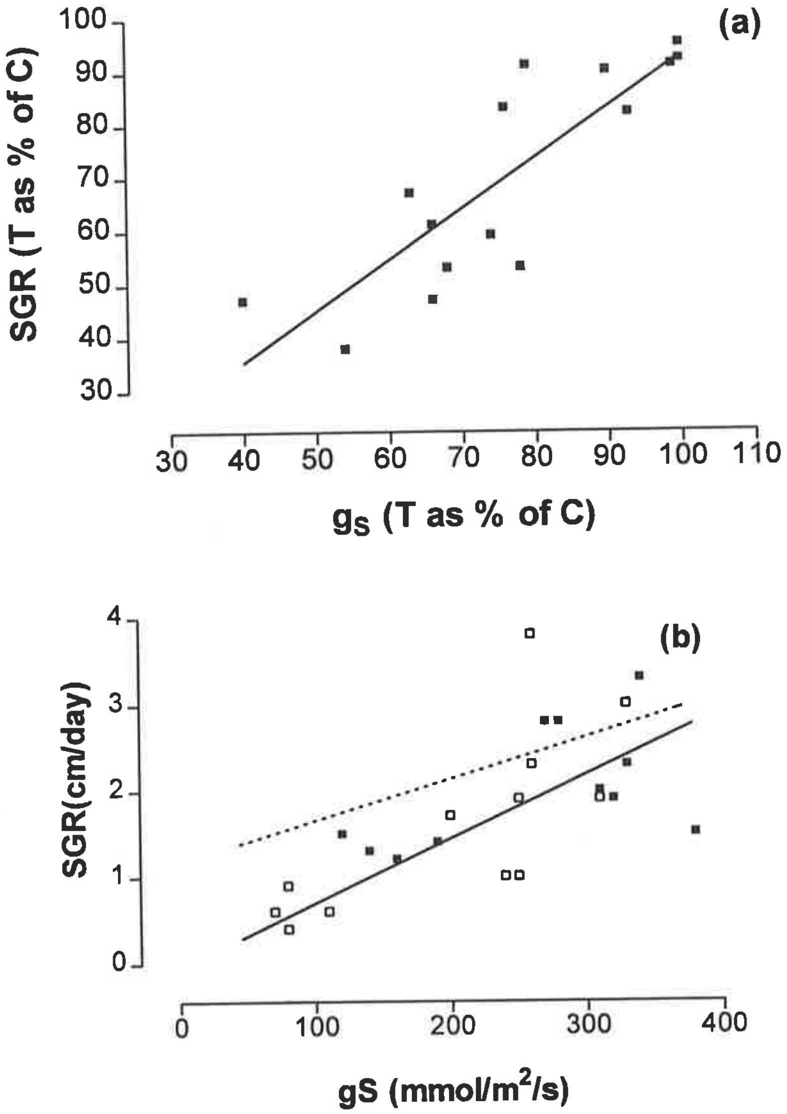


Figure 9.20 Relationship between stomatal conductance (g_S) and shoot growth rate (SGR) from D75 to D141; Cabernet Sauvignon/Ramsey split-root vines, 194/95, Expt 14. C: both sides of vine irrigated; T: one side of vine not irrigated during PRD periods; g_S and SGR values within three days of each other.

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).

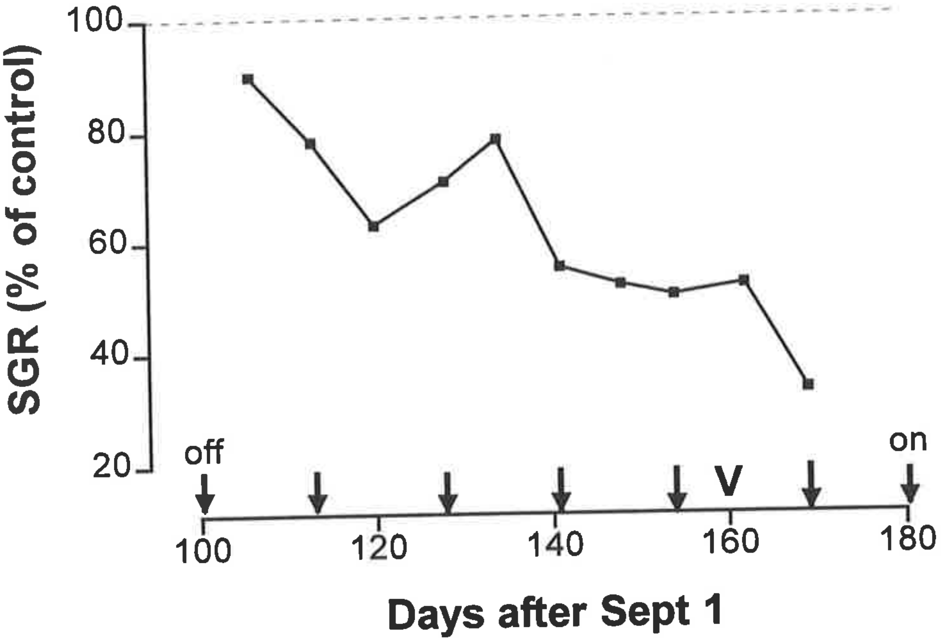


Figure 9.21 Effect of partial drying on shoot growth rate (SGR, cm/d) of 'treated' (T) as % of 'control' (C): Cabernet Sauvignon/Ramsey split-root vines, Exp.15. C: both sides of vine irrigated; T:one side of vine not irrigated from D98 ('off') until D181 ('on'). Arrows indicate days when irrigation 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 '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.

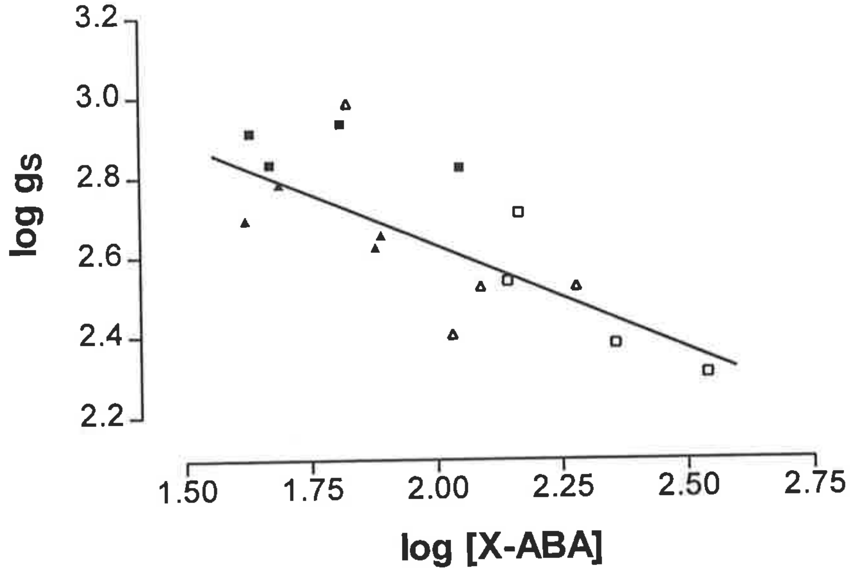


Figure 10.1 Relationship between stomatal conductance (g_S, mmol/m^2/s) and xylem sap ABA concentration ([X-ABA], nmol/mL) of four shoot types of half-dried Shiraz on D10 (code for symbols: upper shoots = triangles, lower = squares; wet shoots = solid, dry = open); Exp. 8. One pot not irrigated from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

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.

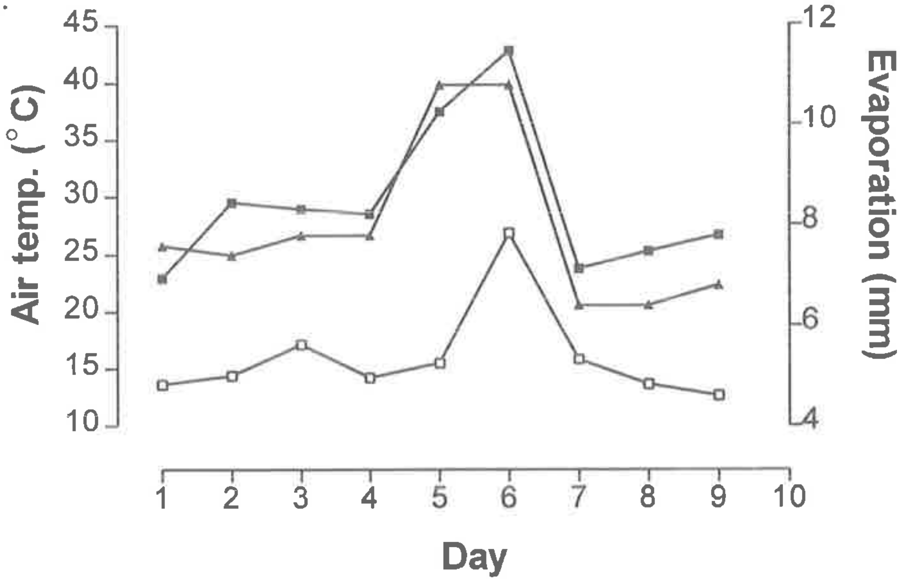


Figure 10.2 Maximum (■) and minimum (□) air temperature (°C) and class A pan evaporation (mm/d, ▲) during PRD period 8; Exp.16, Cabernet Sauvignon/Ramsey, Waite, 1995/96.

'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' 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).

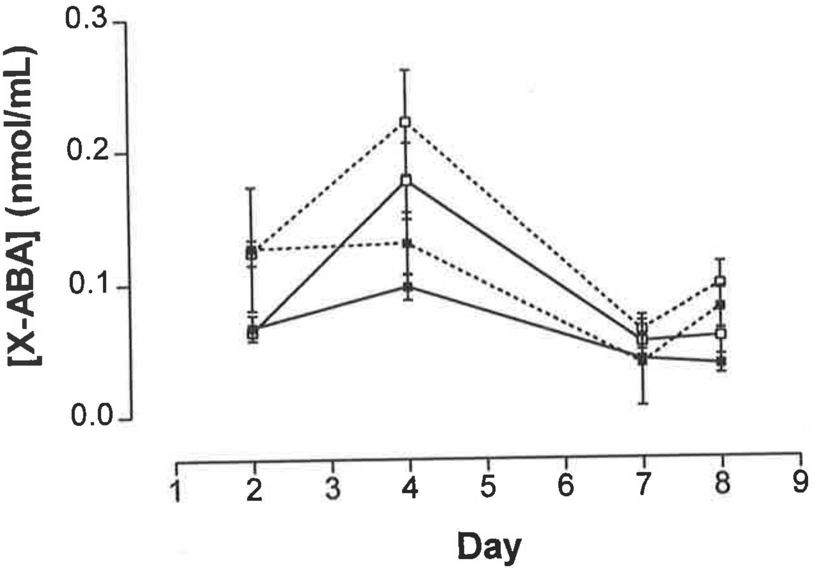


Figure 10.3 Effect of partial drying on xylem sap ABA concentration ([X-ABA], mean ± se, nmol/mL) during PRD period 8; Exp.16, Cabernet Sauvignon/Ramsey, Waite, 1995/96. PRD period started on Day 2. Both sides of vine irrigated (■); partial drying (□). Sap collected in morning (solid line) and afternoon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

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).

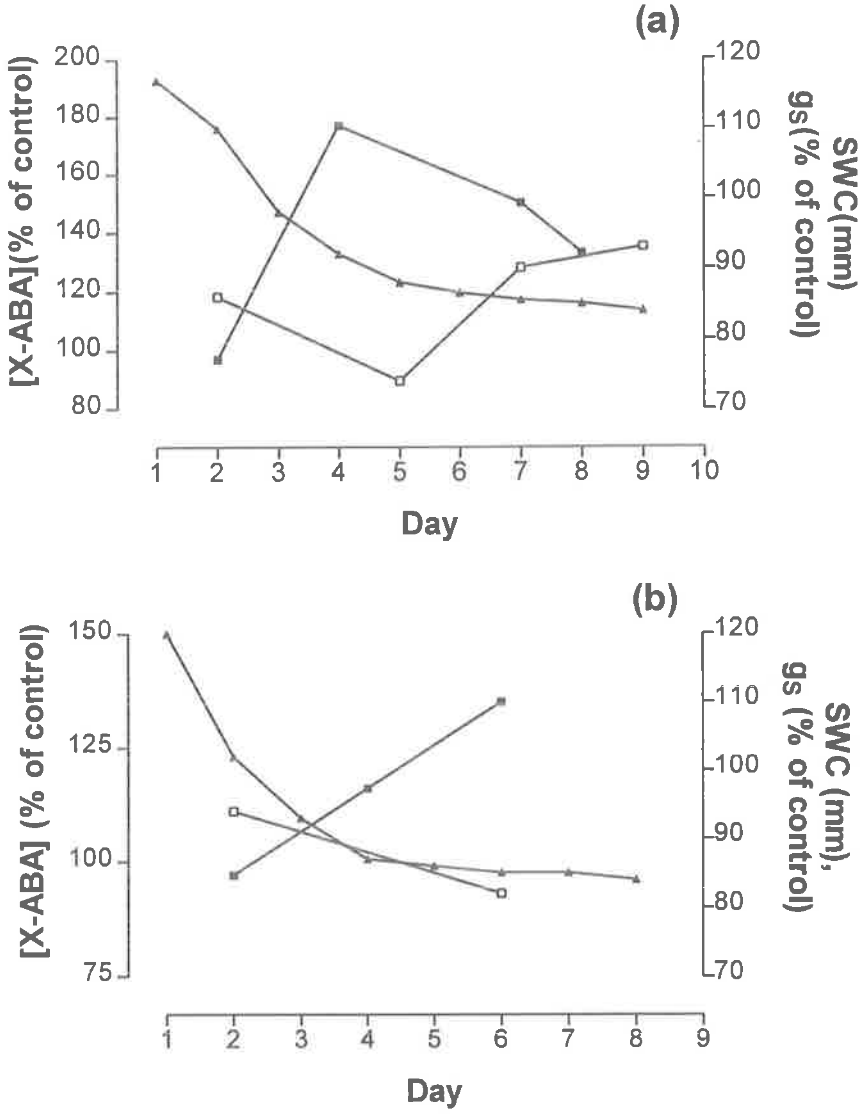


Figure 10.4 Effect of partial drying on leaf xylem sap concentration ([X-ABA], mean of am and pm samples, ■) and stomatal conductance (g_S, □): 'treated' (partial drying) as % of 'control' (both sides of vine irrigated); Exp.16, Cabernet Sauvignon/Ramsey, Waite, 1995/96. Volumetric soil water content (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.

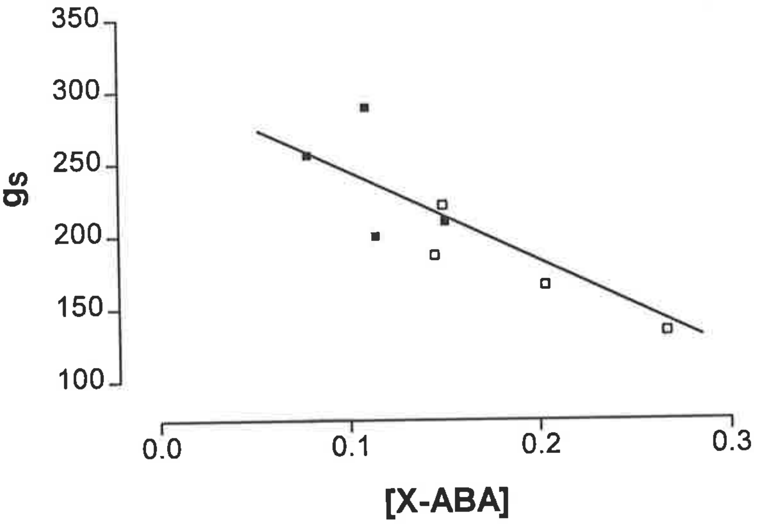


Figure 10.5 Relationship between stomatal conductance (g_S, mean per vine, am, Day 5, mmol/m^2/s) and leaf xylem sap concentration ([X-ABA], mean per vine of am and pm on Day 4, nmol/mL) of 'control' (■) and 'treated' (partial drying, □) during PRD period 8; Exp.16, Cabernet Sauvignon/Ramsey, Waite,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

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 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

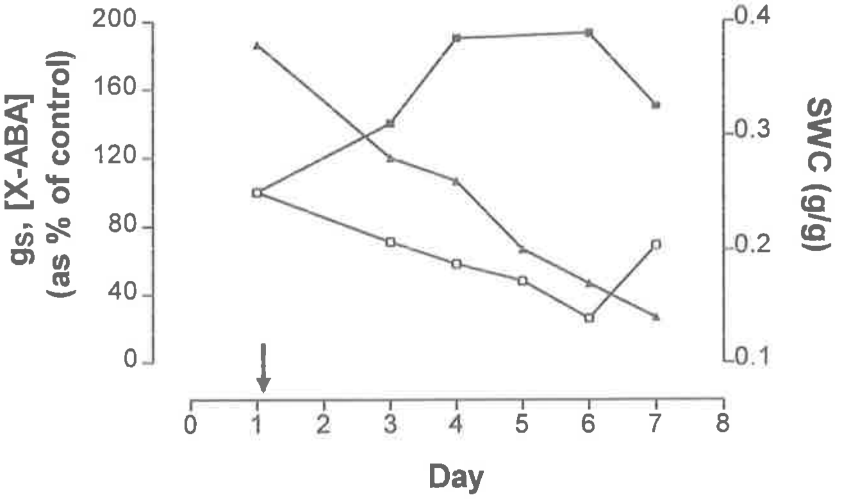


Figure 10.6. Effect of half-drying on xylem sap concentration ([X-ABA],) and stomatal conductance (g_S, ≤) of split-root sycamore: 'treated' (one pot irrigated) as % of 'control' (both pots irrigated). Gravimetric soil water content (SWC, g/g, ▲) of 'dry' pot; drying started on Day 1(arrow). This figure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

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.

<u>The relationship between soil water status and ABA</u>: 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 the current expansion of vineyards. Furthermore, current irrigation practice can be damaging to the environment through depletion of ground water reserves, rising water tables, and salinity and nutrient leaching. There is potential for this new technique to be refined and developed for a wide range of horticultural crops, thus leading to major reductions in water use.

11.10 Cytokinins

The experiments of Chapter 10 together with the discussion in this chapter have tended to emphasise the potential role of ABA as a root signal. However, the fact that lateral shoot growth was one of the major components of shoot growth affected in response to PRD suggests that the potential role of cytokinins should also be investigated.

Cytokinins are known to have an important role in the promotion of budburst and lateral branching (Phillips 1975). Intact plants may respond to applied cytokinin by formation of new buds and burst of lateral buds may be induced by cytokinin application ((Phillips 1975, Li and Bangerth 1992). Various studies have shown a correlation between endogenous cytokinin levels and bud development. For example, the concentration of cytokinins in xylem increases just before and during budburst of apple (Tromp and Ovaa 1990) and coincides with outgrowth of axillary buds in rose plants (Dieleman *et al.* 1997). The level of cytokinins in buds increased during the period of active bud development of *Picea abies* (Chen *et al.* 1996) and the induction of new lateral bud primordia in *Pinus radiata* (Taylor *et al.* 1984). The level of cytokinins appears to play an important role in the establishment of differences in bud size and thereby architecture of the tree crown of *Picea abies* (Bollmark *et al.* 1995).

It is widely accepted that cytokinins can be produced in root tips and transported via the xylem to shoots (Skene 1975). Root-derived cytokinins transported to buds appear to be involved in induction of budburst (Letham 1994). Lateral shoot production of peach seedlings was inhibited by removal of 50% of the root system but this effect could be reversed by exogenous application of the cytokinin 6-benzylaminopurine (Richards and Rowe 1977).

Optimum levels of soil moisture and macronutrients promote cytokinin biosynthesis in plants (Mullins et al. 1992; Jackson 1993). Reduced xylem transport of cytokinins to shoots has been detected in droughted plants of some species (Livne and Vaadia 1972) but there was no correlation between soil drying and concentration of cytokinins in xylem sap of almond trees (Fusseder et al. 1992). Davies et al. (1986) speculated that reduced leaf extension of maize in a split-root experiment may be the result of a reduced supply of cytokinins from roots in response to soil drying. However, according to Davies et al. (1994), there have not been any reports of studies in which the flux of cytokinins has been quantified and compared to changes in growth induced by drought.

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. Nevertheless, the possibility of an interaction between ABA and cytokinins cannot be dismissed. Cytokinins are known to modify stomatal response to ABA: exogenous application of cytokinin can override ABA-mediated stomatal closure in maize (Blackman and Davies 1985). Therefore, there is a possibility of an interacting effect of ABA and cytokinins on growth, as suggested by Davies et al. (1994). Future research could test the hypothesis that the reduction in lateral shoot growth in response to PRD is the result of a decreased supply of cytokinins caused by root drying.

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'.

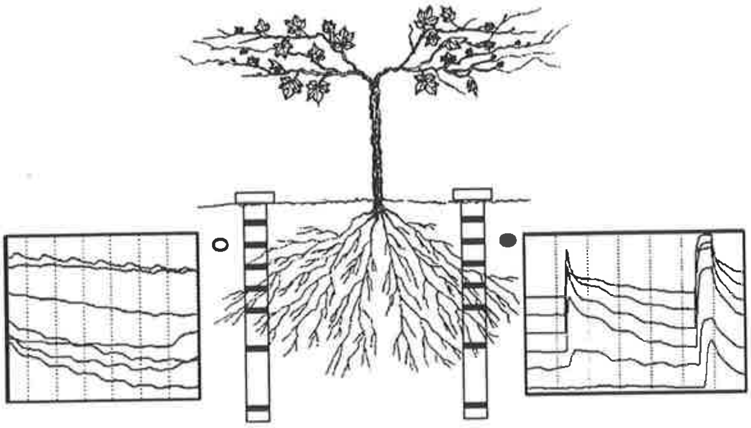


Figure 11.1 Diagrammatic representation of a grapevine irrigated using the PRD technique. Sub-surface drip lines supply water to one side or the other of the vine. In this figure, water is supplied through two irrigation cycles to the right hand line. The soil water content at various depths is shown 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 supplics 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
III. Characteristics of soil at Waite Campus site
IV Calculation of anthocyanins, total phenolics, total G-G and 'red-free' G-G of berry homogenate
V. Soil water retention curve
VI. Irrigation, rainfall, evaporation and crop factors

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.

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

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

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

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

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

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

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

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

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-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

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[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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