Achieving water use efficiency and improved drought tolerance with rootstocks



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Achieving water use efficiency and improved drought tolerance with rootstocks
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Abstract

The recent drought across the Murray-Darling basin demonstrated some of the difficulties that Australia's wine industry will face during future droughts and as a result of climate change. To overcome these threats the industry will need to improve water use efficiency compared with current practice. Consumer preferences prevent any large scale change to more water use efficient varieties, but replanting with rootstock grafted vines provides an opportunity to re-position for the future. This project aims to determine the impact rootstocks have on the relationships between vigour, yield and water use, together with an assessment of drought and salinity tolerance and mechanisms behind them. Using a field trial in a warm climate viticulture region, Sunraysia, and complementary glasshouse experiments the project has demonstrated that low to moderate vigour rootstocks have an improved yield / canopy area ratio at a given canopy size and an improved Crop Water Use Index (yield / water transpired). This offers the proposition of tailoring irrigation requirements more specifically to individual rootstocks and altering vine planting density to improve vineyard yield and water use efficiency. A series of glasshouse experiments have examined various rootstocks, grafted with Shiraz, for drought tolerance, salinity tolerance and potential mechanisms for conferred vigour. The results suggest that rootstocks can vary in their production of root:shoot signals (abscisic acid) in response to the same soil moisture deficit and that this results in a different leaf level response. Data also point strongly towards vine hydraulics being important in driving rootstock conferred vigour.

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Executive summary

The ‘millennium’ drought demonstrated the impact that reduced irrigation availability can have on the Australian wine industry. When the next such drought will occur is difficult to know, but we can be sure that such a drought will re-occur. Climate change is likely to exacerbate the situation, with predictions of reduced winter rainfall suggesting the water availability in the future will be reduced and the predictions of warmer air temperatures suggesting that the vine will have a greater demand for water. It is clear that Australian viticulture will have to continue to improve water use efficiency compared with current practice.

Improving the scion genetics in this respect requires changing long-established consumer preferences, which is a long-term prospect. However, by utilising more water use efficient rootstocks the industry has an opportunity to improve water use efficiency in the vineyard as part of the normal turnover of plantings. Indeed, through their effect on vine vigour rootstocks have the potential to have a large effect on vine water use, but there is a difference between water use and water use efficiency. This project has attempted to explore the relationship between rootstock conferred vigour, yield and water use, together with their role in drought and salt tolerance.

The field based component of the project utilised seven rootstocks, embedded in a larger, fully replicated, trial at the CSIRO field station in Sunraysia. This trial was over 20 years old and included both high and low vigour rootstocks, all grafted with Shiraz scions. The second component of the project was a series of glasshouse studies that examined the links between rootstock conferred vigour, drought tolerance and salt tolerance.

The field work demonstrated a two-fold range in canopy size between the rootstocks and a four-fold range in pruning weight, but only a 1.7 fold difference between the highest and lowest yields. Actual vine water use (transpiration) was measured using sap flow sensors and was strongly correlated with rootstock conferred vigour, with a greater than three-fold range. Differences in yield were smaller than differences in vigour or transpiration, so water use efficiency, defined as crop produced per unit of water transpired (crop water use index: CWUI), was greater in vines grafted to the lower vigour rootstocks.

There was a strong relationship between canopy size and fruit production over four seasons of measurement, so in general smaller vines produced less fruit. However, the relationship was non-linear, so as rootstock conferred vigour got greater, the increase in yield got smaller. Furthermore, there was also some variation in this relationship, for instance Dog Ridge produced less fruit per unit of canopy than Ramsey, while Merbein 5512 produced more fruit per unit of canopy than 140 Ruggeri.

Whole vine water use has two plant based drivers, canopy size and stomatal conductance. Both were influenced by rootstock choice. Consequently, while vigour, yield and transpiration appear to all be inter-related, rootstock genotype can ‘stretch’ these relationships, allowing rootstocks of moderate vigour to have improved water use efficiency with little or no yield penalty.

Understanding how rootstocks alter scion vigour and vine water use will assist breeders to produce rootstocks that optimise the vigour-yield-transpiration relationship. The glasshouse studies, undertaken with various rootstocks grafted to Shiraz scions, found strong relationships between vine size (vigour) and root length specific hydraulic conductance. A large component of root hydraulic conductance is typically due to proteins called aquaporins. These can have a rapid

turnover and are, at least partially, regulated by the shoot. In the glasshouse, differences in hydraulic conductance were not explained by expression of the genes encoding for aquaporins. In contrast, aquaporin gene expression was highest in rootstocks with the lowest conferred vigour. The simplest interpretation is that, in the glasshouse at least, rootstock conferred vigour was driven by physical differences in the vine hydraulics. Further work is required to confirm or refute this hypothesis.

The glasshouse studies also found differences in rootstock tolerance of drought and salinity stress, including in combination. The impact of stress on plant biomass was generally greater in the rootstocks with greater conferred vigour, e.g. Ramsey and Dog Ridge both had a larger proportional difference in biomass between drought stressed and non-drought stressed vines than Schwarzmann or Merbein 5512. There were also differences in shoot physiological responses dependent on rootstock. However, the trends in all of the measured variables were similar across rootstocks. For example, root hydraulic conductance was lower under water deficit and root fraction (the proportion of whole plant biomass in the root system) was higher under both drought and salinity stress and highest when the two were combined.

In summary, this project has demonstrated the inherent negative relationship between rootstock vigour and water use efficiency, but that water use efficiency, drought tolerance and salt tolerance are separate traits and thus that there is a potential for rootstock breeding to generate a rootstock that possesses all of these attributes.

Chapter 1

Project Background

Through much of the first ten years of the 21st century the Murray-Darling basin was in the grip of possibly the most serious drought since records were kept. Practically all winegrape production regions in Australia were affected by the drought in some way, with the warmer climate areas hit particularly hard by substantial reductions in irrigation allocations. The Murray Valley regions are known as the 'engine room' of Australia's wine export success, producing 60% of all winegrapes crushed, and contributing 85% of total wine exports. To survive the drought, growers had to assess their entire plantings to decide which patch was to receive more or less water, with some deciding to exit the industry completely. Recurrence of drought in Australia is "when" not "if" and the 2000's have demonstrated that improving water use efficiency and 'drought-proofing' against future drought episodes is essential for the long term sustainability of the wine industry.

The consequences of climate change can only exacerbate the effects of the next drought. Predictions of precipitation over the next 30-40 years are difficult and vary, but even without a general decline in rainfall, warmer air temperatures will result in greater evaporation rates, reduced run-off into the river system and, therefore, less water available to irrigated horticulture. Furthermore, increased evaporation rates increase the water requirements of grapevines. Consequently, the combination of a future drought with the expected effects of climate change is one that cannot be ignored by the industry and cannot be overcome without improvements in water use efficiency compared with current viticultural practice.

Whilst there are few positives associated with a large-scale drought, one potential opportunity is that of re-development to improved planting material. A consequence of the drought during the 2000's was that some varieties were taken out of production by some growers. Re-planting with improved material, such as rootstock grafted vines, provides that opportunity, together with a re-positioning for the future.

Due to long established and robust consumer preferences, Australia has a reliance on a small number of dominant wine grape varieties and this is unlikely to change in the near future. We can, however, go some way to achieving improved water use efficiency and increased drought tolerance through the use of rootstocks, while at the same time continuing to use the dominant varieties as scions.

The rootstock, more so than management practices, appears to be the primary determinant of vine water use efficiency if measured by crop water use index (CWUI: crop produced per unit of water transpired). Our prior GWRDC funded work has shown that regulated deficit irrigation (RDI) and sustained deficit irrigation do not necessarily improve CWUI because reductions in water use are offset often by reductions in yield.

What is needed is rootstocks with improved water use efficiency and if possible drought tolerance. However, these two traits may have different physiological drivers and it is not known if they can be combined in a single rootstock genotype. Ideally, such a genotype should also have a capacity for chloride and sodium exclusion to provide protection against salinity. We previously reported that a CSIRO bred, low to moderate vigour, rootstock (Merbein 5489) more than doubled water use efficiency, as measured by crop water use index, when compared with 1103 Paulsen, although this was a single experiment with immature vines. If confirmed this would be a significant step

forward in understanding grapevine water use efficiency, but it does not tell the full story in terms of drought tolerance. Water use efficiency and drought tolerance are not necessarily the same trait and the term 'drought tolerance' can refer to actual tolerance of soil water deficit or drought avoidance. We do not know, for example, whether water use efficient rootstocks also confer drought tolerance.

This project focuses on these two desired rootstock traits and aims to establish the possibility of combining them. Furthermore, it is also provides a further opportunity to assess the relationship between salt tolerance and plant vigour and whether salt exclusion can also be combined with the aforementioned traits.

Project objectives

Utilising a unique field collection of 60 mature rootstocks grafted with Shiraz, that are established in a replicated trial design and growing in the representative warm climate Sunraysia region, combined with a series of intensive glasshouse based studies of rootstock physiology, our aims were:

- Determine relationships between harvest index, crop water use index and maximum yield (based on direct measurement and indirect measurements of vine transpiration using sapflow and stable isotope techniques respectively).
- Assess rootstocks for drought tolerance, in terms of yield (field trial), growth (glasshouse trial) and comparative physiology, including water use under a well irrigated treatment and a significantly reduced irrigation treatment, the latter equating to a drought scenario.
- Determine potential inter-relationships between rootstock crop water use index, drought tolerance and 'salt tolerance index' based on a combination of plant vigour and chloride and sodium exclusion rankings.

Chapter 2

Linking rootstock effects on vigour, yield and water use in the field

Background

The field component of the project assessed the links between rootstock conferred vigour, fruit yield and whole vine water use. This required a fully replicated rootstock trial with mature fruit bearing vines, managed under commercial like conditions. Such a trial was available at the CSIRO farm in Sunraysia (Figure 1), comprised of 60 rootstocks, having been originally established over 20 years previously as part of a GWRDC funded project (CSH 94/3: Field assessment of selected rootstock hybrids for quality wine production). This trial included a range of industry 'standard' rootstocks as well as a number of lines from the CSIRO breeding program. The current study made use of the three rootstocks most commonly planted in recent years (Whiting 2012), 1103 Paulsen, 140 Ruggeri and Ramsey, as well as the three recently released, CSIRO bred, low to moderate vigour rootstocks, Merbein 5489, Merbein 5512 and Merbein 6262. The study has also utilised Dog Ridge, a rootstock known for its high conferred vigour.



Figure 1. CSIRO rootstock trial in Sunraysia.

Materials and Methods

Experimental site: history, irrigation and environmental monitoring

The rootstock trial consisted of a single block and each rootstock replicate was a two vine panel, with six replicates assigned to random positions. All rootstocks were grafted with Shiraz scions (clone PT23) and the vines were planted in 1989. The block was irrigated uniformly using an overhead sprinkler system from establishment until the end of the 2008/9 season. The sprinklers

were replaced with a drip irrigation system for the 2009/10 growing season, the first season of this study. All rootstocks were nominally irrigated with the same volume of water at all times during the study, with no attempt to match irrigation to potential differences in rootstock water requirements. It was not possible to establish irrigation treatments in the trial due to the limited number of vines and replicates available. No fertiliser was applied during the course of the experimental period as neither fruit nor prunings were removed from the site (See Chapter 3 for estimates of vine nitrogen status). The vines were mechanically 'hedged' following leaf fall in each season.

Environmental conditions were monitored at 10 minute intervals throughout the year by an Automatic Weather Station (MEA, Magill, SA) and included, air temperature, relative humidity, FAO56 reference evapotranspiration (ET_0), wind speed and direction, solar radiation and rainfall. Soil moisture was monitored at one hour intervals at 30 and 60 cm depth using Delta-T Theta probes (also MEA), radio-linked back to the weather station, with two replicates for each of the seven rootstocks studied being instrumented.

Scion vigour

Vine growth and vigour were assessed by a series of leaf area index (LAI) measurements each season, using a LiCor LAI2000 Plant Canopy Analyser (John Morris Scientific, Keswick, SA), and ceased once canopy growth was complete. Measurements were made using a 90° view cap facing along the row from the trunk of the first vine in each two vine replicate. As a result of the limited number of vines per replicate, the LAI measurements had to be restricted to a maximum of approximately 2.7 m of row length, the distance from the trunk of the first vine in the rep to the mid-point between the second vine and its subsequent neighbour, to ensure that only canopy from the replicate was included and not adjacent vines. However, due to this, the LAI estimates excluded part of the inter-row space, where canes were thinnest and had the least cover and thus were overestimated to some extent. Consequently, the LAI measure is referred to as 'apparent LAI'. This did not impact the conclusions in regards to the differences between rootstocks in their conferred vigour.

Pruning weights were measured on the same vine that was used for the harvest estimate in any given season, for each replicate after leaf fall. Pruning was matched to that of the machine hedging used on the block and a nominal 'vine' was assigned as being between the mid-point from the selected vine and its neighbour on one side to the mid-point on the other side. In 2012/13 leaf fall was incomplete when the measurements were made (late May) due to the impending end of the project and any remaining leaves had to be removed.

Finally, trunk diameters were measured at the start and end of each season for the vines with sapflow loggers installed.

Whole vine transpiration

Whole vine water use was monitored at 15 minute intervals from September to June in the 2010/11 season and onwards using SFM sap flow logging systems (ICT International, Armidale, NSW). These systems were installed shortly before budburst in four replicates for each of the seven rootstocks. Sap flow was logged throughout the growing season and the systems were removed shortly after leaf fall each year.

Harvest yield

Once maturation monitoring indicated that the fruit had a total soluble solids (TSS) content of

approximately 24° Brix, a harvest estimate was made. For each replicate a single vine was selected and all the fruit removed by hand, with the nominal vine being defined as for pruning weights (above). The fruit was then weighed to provide a fruit weight per vine.

Table 1 - Rainfall, irrigation and crop evapotranspiration at Sunraysia field site.

Season	Rainfall (mm)	Irrigation (mm)	Et_c (mm)
2009/10	254*	c. 350†	778*
2010/11	886	265	650
2011/12	258	335	675
2012/13	89	458	757

**Data obtained from Bureau of Meteorology station at Mildura Airport.*

†Estimated.

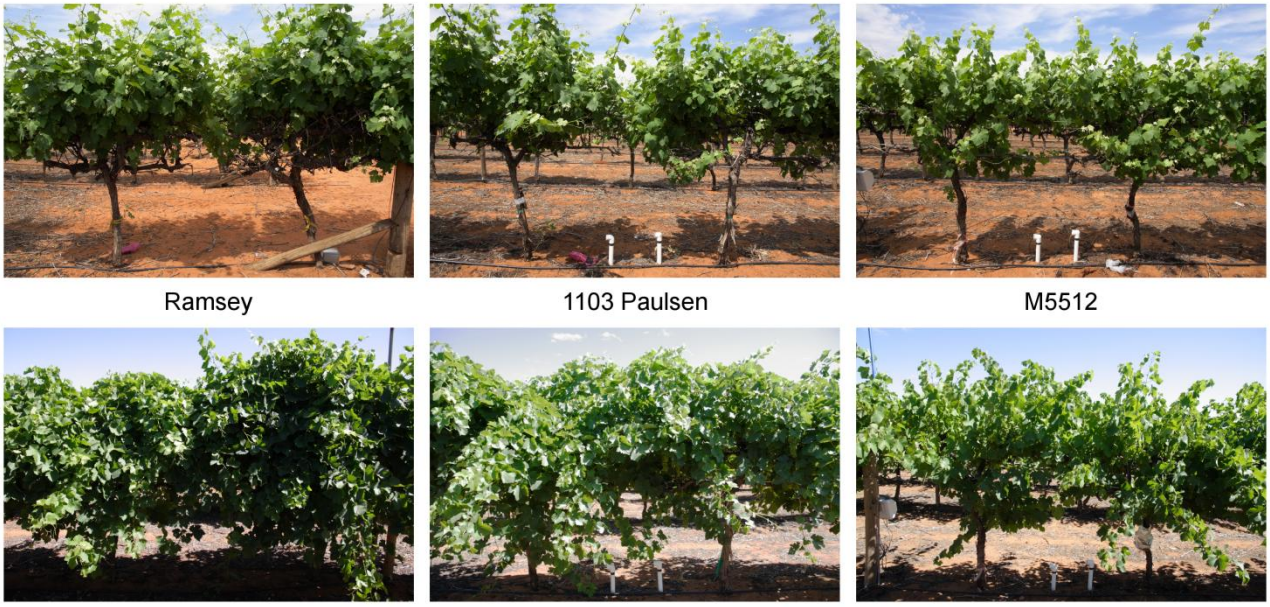


Figure 2. Photographs Shiraz canopies on Ramsey (left), 1103 Paulsen (centre) and M5512 (right) rootstocks at flowering (upper row) and canopy closure (lower row) during the 2011/12 season.

Results and discussion

The existing field trial at the CSIRO vineyard in Sunraysia was a suitable site due to its location in a major warm-climate winegrape production area, its age and the wide range of vigour imparted on the scion by the rootstocks present. Figure 2 provides a visual example of that range.

Rootstock conferred scion vigour

Canopy size was determined as apparent LAI. However, the need to restrict the scope of those LAI measurements does mean caution is needed when comparing these apparent LAI results to published data where multiple vines and rows allowed LAI to be determined with a greater absolute accuracy. By averaging the peak LAI value (full canopy) for each individual replicate over the four seasons of the experiment it was possible to separate the inherent effect of the rootstock

on scion vigour from the inter-seasonal differences. Doing this, the rootstocks split into three clear vigour groups, with Ramsey and Dog Ridge having the largest canopies, 1103 Paulsen and 140 Ruggeri being intermediate and the three Merbein series rootstocks having the smallest canopies (Figure 3). LAI of the high vigour group was 96% larger than the Merbein series group with the highest vigour rootstock, Dog Ridge, having an LAI more than double (240%) the smallest, M6262.

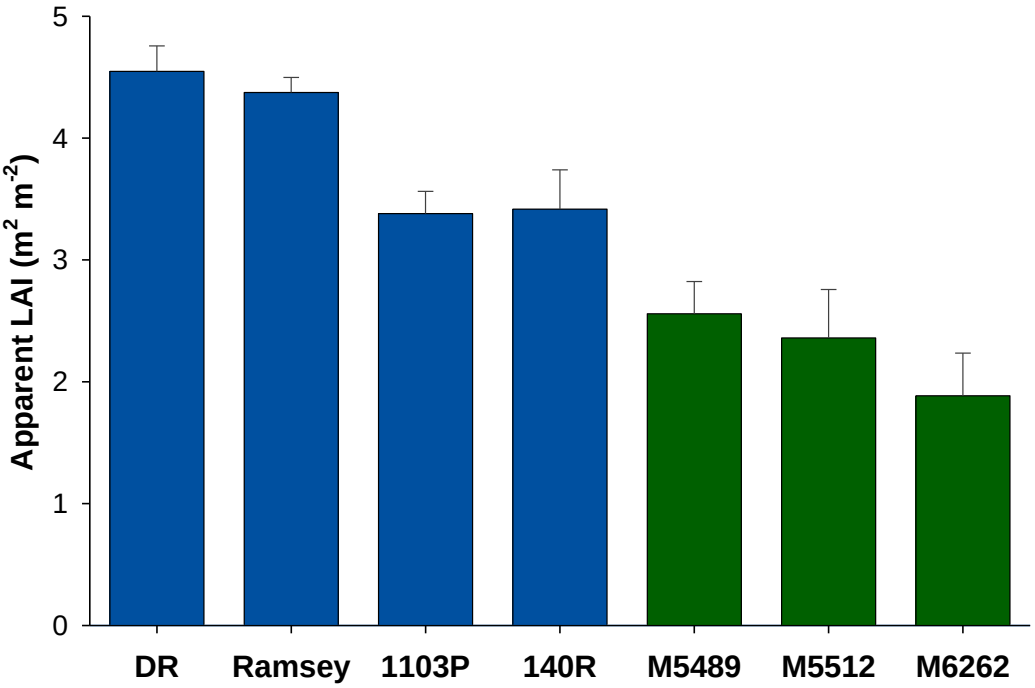


Figure 3. Apparent leaf area index (LAI) of Shiraz scions on seven rootstocks, four 'standards' (blue) and three CSIRO Merbein series (green). Data are mean of six replicates +SE, where each replicate is a mean of four LAI measurements, collected once per season at peak canopy size.

Canopy size represents the area available for carbon fixation via photosynthesis to the vine, with the caveat that the sun exposed proportion of that area reduces with increasing canopy size due to self shading. However, estimating canopy size is labour intensive and even using instrumentation to measure LAI does not perfectly predict the whole vine leaf area. Growers do not typically have access to such instrumentation and thus the fresh weight of prunings is more commonly used to gauge vine vigour and inter-seasonal differences in growth.

The impact of rootstock on pruning weight was similar to that on LAI, with the same three vigour groups present (Figure 4). The difference between those groups was much larger though, with Dog Ridge and Ramsey having approximately five times the pruning weight of the three Merbein rootstocks, compared with double the LAI. This was due to an exponential relationship between pruning weight and LAI, irrespective of whether individual vines, seasons or rootstocks were examined. Consequently, whilst both measures can be used to quantify rootstock conferred vigour, care must be taken in interpreting pruning weight results as a large increase in pruning weight will not represent an equivalent increase in canopy size.

Rootstock effects on fruit yield at harvest

Averaged over the four seasons the difference in yield between the two highest vigour rootstocks, Dog Ridge and Ramsey, and the following group of 1103 Paulsen and 140 Ruggeri was small (Figure 8). Yield of the three Merbein stocks, was reduced to a greater extent compared with the other

rootstocks, but the difference was much less than the difference in either LAI or pruning weight.

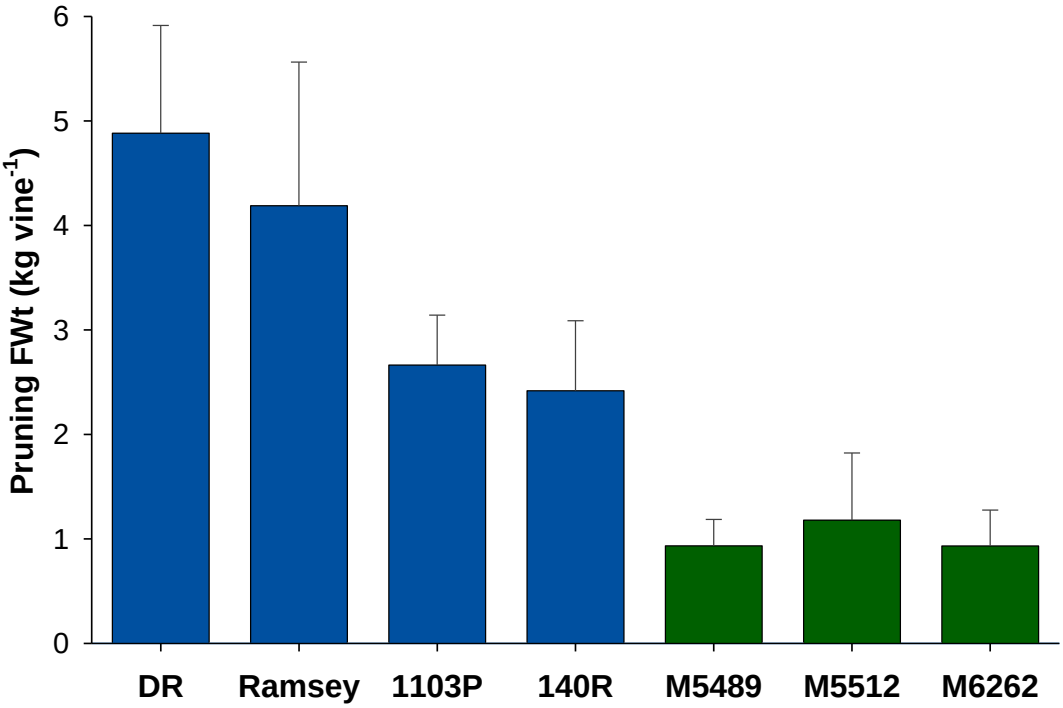


Figure 4. Fresh weight of pruning from Shiraz scions on seven rootstocks, four 'standards' (blue) and three CSIRO Merbein series (green). Data are mean of six replicates +SE, where each replicate is a mean of four measurements, collected once per season after leaf fall.

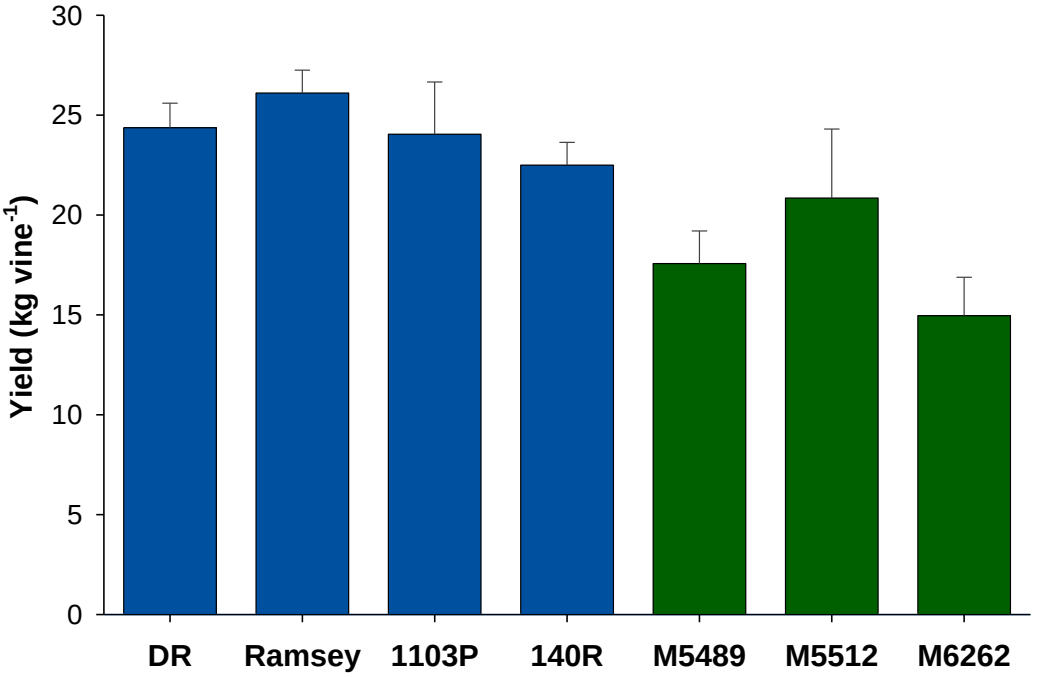


Figure 5. Fresh weight of fruit at harvest from Shiraz scions on seven rootstocks, four 'standards' (blue) and three CSIRO Merbein series (green). Data are mean of six replicates +SE, where each replicate is a mean of four measurements, collected once per season.

Jones *et al.* (2009) examined yield and pruning weight over six seasons (1993-1998) from all 60 rootstocks in the trial site used by this project, finding a ten-fold range in pruning weights and a six-fold range in yield. As with the data presented here, Dog Ridge had the highest pruning weight and Ramsey the highest yield, demonstrating the long-term stability of rootstock conferred vigour in mature vines.

The ability to predict whether a rootstock selection is likely to maintain a trait during its commercial usefulness is clearly valuable and the maintenance of both vigour and yield responses suggests that factors driven by those traits, such as water use efficiency are also likely to be maintained over the lifespan of the planting.

Inter-seasonal impacts on rootstock conferred vigour and yield differences

Examining the long-term means of vine traits, as above, was an effective way to examine the inherent effect of the rootstock irrespective of seasonal differences due to the scale of rootstock driven effects. However, as Table 1 demonstrates, there were large differences in weather conditions between the four seasons and these resulted in inter-seasonal differences in the measured parameters. The potential was that vines on different rootstocks would respond differently to those variations in climate.

Yield, pruning weight and LAI all exhibited large inter-seasonal differences, presumably driven by differences in weather conditions between the years. For LAI, the range as a result of seasonality was approximately 50% in all seven of the rootstocks (Figure 6), compared with the rootstock driven differences of approximately 100% (see above). Consequently, the absolute differences in LAI between seasons were higher for the high vigour rootstocks and the lower vigour rootstocks had almost half the canopy size irrespective of growing conditions within a given season.

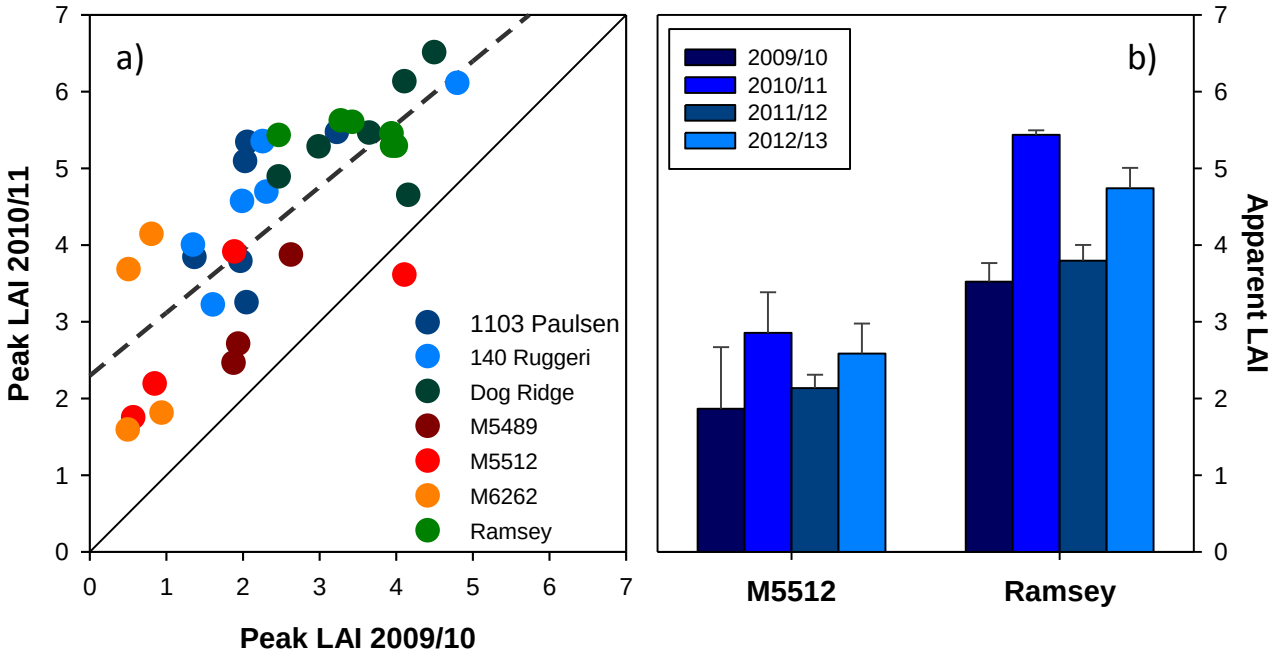


Figure 6. Comparison of peak seasonal apparent LAI of Shiraz scions on seven rootstocks between the 2009/10 and 2010/11 seasons (a) and on two of those rootstocks over four seasons of growth (b, means + SE), where n = 4-6 per rootstock.

In the 2009/10 season, however, Merbein 6262 had a much lower LAI than any of the other rootstocks and the LAI for 1103 Paulsen and 140 Ruggeri was as low as Merbein 5489. The 2009/10 season was the start of the current work and was also the season that the rootstock trial was converted from overhead sprinkler irrigation, watering the entire vineyard floor, to drip irrigation. It is likely that vine performance was impacted by this change due to the different requirements of the two systems in terms of root mass and position (Soar and Loveys 2007). LAI of all vines was higher in the following season, 2010/11, driven by the record high rainfall received in Sunraysia that year. Comparing the two seasons, the rootstocks all fell on a similar relationship, with no rootstock deviating greatly from this, even when examining individual replicates (Figure 6a). In absolute terms this meant that, although relative differences in canopy size between seasons were similar for the remainder of the project, the low vigour rootstocks had a greater difference in canopy size between 2009/10 and 2010/11 than the high vigour rootstocks. The limitation in absolute canopy size that occurred in all seasons, irrespective of weather, suggests that additional management regimes could be implemented when utilising the low vigour rootstocks, such as high-density planting.

The seasonal impact on canopy size was also evident in pruning weights, as might be expected due to the two measures typically being related. As with apparent LAI, the relative effect of seasonality on pruning weights was similar across all rootstocks (Figure 7), but the differences were much larger, approximately three-fold (but less than the four-fold rootstock effect – see above). Also the inter-seasonal differences in pruning weights didn't always match the inter-seasonal differences in LAI, in particular the differences between the 2010/11 and 2011/12 seasons seen in LAI were not observed in pruning weights (Figure 6b cf. Figure 7b). This suggested that the relationship between pruning weight and canopy size was not simple and emphasises the care needed in interpreting pruning weight results relative to leaf cover. The relationship between the two variables was curved, not linear, with all rootstocks falling on a single curve within a season, but that curve varying between seasons (Figure 8). The very high vigour 2010/11 season that followed the change in irrigation method in 2009/10 had the greatest deviation from the other seasons of the project, but the final year (2012/13) also differed from the other three.

The inter-seasonal effects on yield were not as simple as those on vigour. The year to year relationships varied (Figure 9a, c & d) and there were differences between rootstocks (Figure 9a-d). For example, yield of Dog Ridge in 2010 was more than double that of 2011, with the difference in Ramsey vines almost as high (Figure 9b). Those differences were the opposite of the seasonal effect on canopy size. Similarly, the low yield of Merbein 5512 and Merbein 5489 in 2010 compared with subsequent years suggested that mechanisms other than vine vigour were responsible for the yield variation.

Although not directly assessed, there was a high incidence of downy mildew and Botrytis in 2010/11, due to the extremely high rainfall that occurred in Sunraysia (Table 1), as in many areas. This was much more severe in the higher vigour rootstocks, leading to a greater reduction in yield from those rootstocks than the low vigour vines. The differences in Merbein 5512 and Merbein 5489, however, were likely to be due to depressed yields in 2010 as a result of the irrigation moving from overhead sprinkler to drip, which were not seen in Ramsey and Dog Ridge, combined with the less severe effects of disease in 2011.

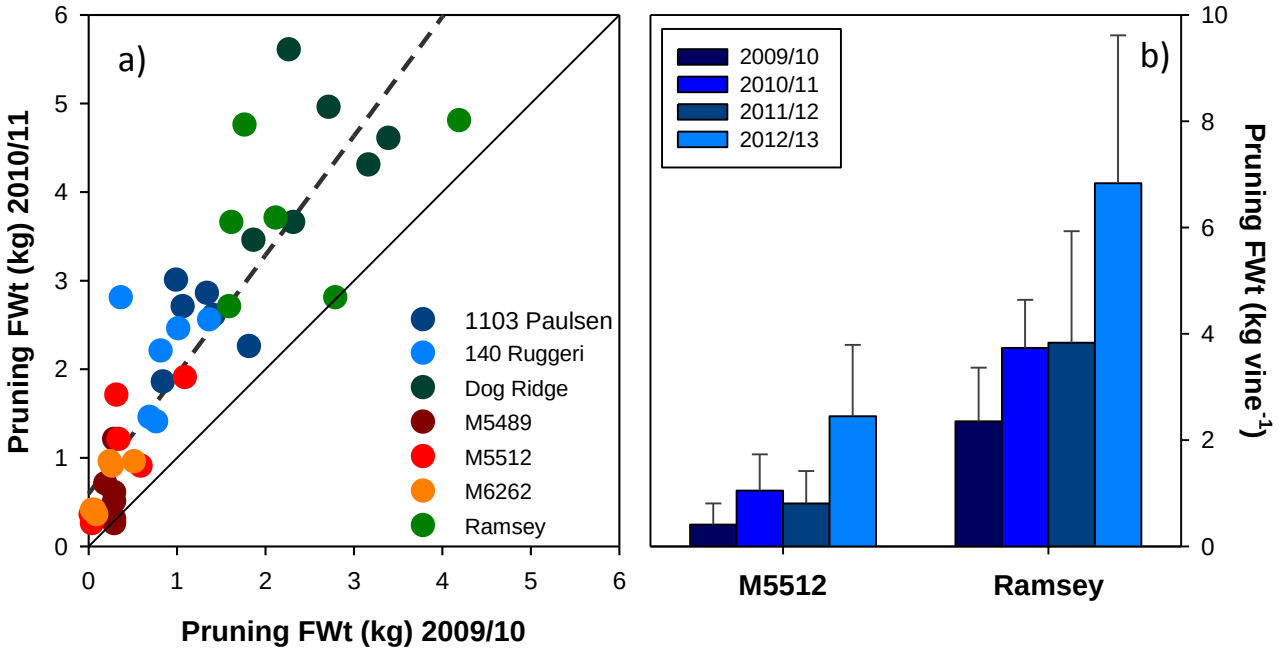


Figure 7. Comparison of pruning weights from Shiraz scions on seven rootstocks between the 2009/10 and 2010/11 seasons (a) and on two of those rootstocks over four seasons of growth (b, means + SE), where n = 4-6 per rootstock.

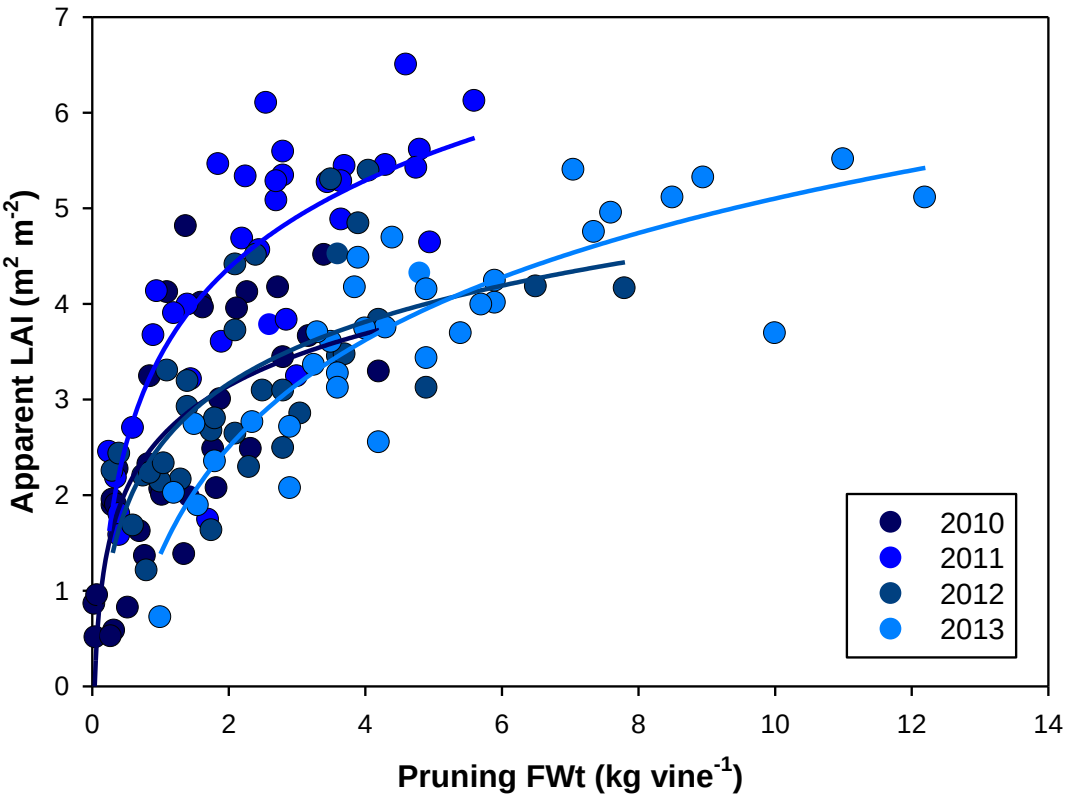


Figure 8. Relationship between pruning weight and apparent LAI in Shiraz scions on seven rootstocks over four seasons.

When comparing the yield of individual replicates between seasons, unlike the vigour measures, there was no relationship between seasons. In other words a vine that had a high yield in one season was no more likely to have a high yield in the subsequent season than one that had low yield in the first season. The only clear inter-seasonal effect was between 2010 and 2011, where the low yield of the low vigour rootstocks in 2009 coupled with the low yield of the high vigour rootstocks in 2011 for the reasons stated above, led to a complete separation of the two (Figure 9a). There were no relationships apparent between other seasons, even 2012 and 2013, when there were no major external factors (weather/disease/irrigation) to influence yield (Figure 9d). Such a result could be indicative of biennial bearing, but another season without a major external factor would be needed to provide data that could support it.

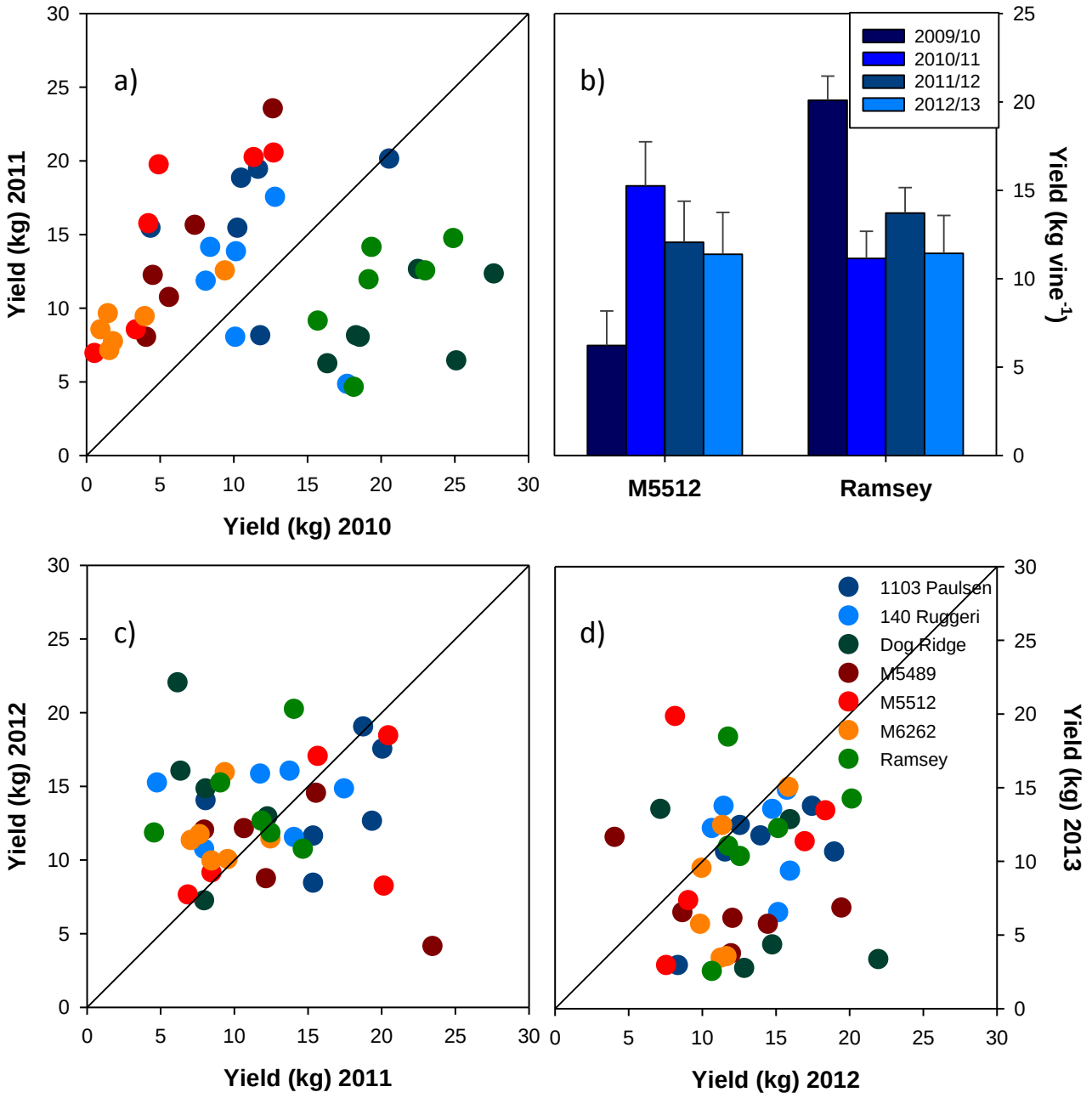


Figure 9. Comparison of yield from Shiraz scions on seven rootstocks between 2010 & 2011 (a), 2011 & 2012 (c), 2012 & 2013 (d) and on two of those rootstocks over four seasons of growth (b, means + SE), where n = 4-6 per rootstock.

Average bunch weight and number were estimated at harvest in all four seasons, so it was possible to examine those yield components for a driver of the rootstock and inter-seasonal variation observed. Using the multiple regression function provided by SPSS (IBM, St. Leonards, NSW), a simple model incorporating bunch weight, bunch number and rootstock was run for each season's yield result (Table 2). In 2010 the major factor, accounting for 80% of the observed variation in yield, was rootstock type. This was due to the low yield of the low-vigour rootstocks and high yield of the high-vigour rootstocks and demonstrated that neither bunch number nor bunch weight explained those rootstock differences. As noted above, this was likely to be a result of the change in irrigation method. In 2011 bunch number explained over 40% of the yield, with rootstock type a similar proportion. The direct impact of rootstock (separate to any effect of rootstock on yield components) in this season was likely to have been via the differential disease impact, which occurred after bunch number was set. The bunch number result suggested that the yield within the low vigour rootstocks was being driven by inflorescence number rather than fruit filling.

Table 2. Proportion of variance in yield explained by bunch number, bunch weight and rootstock type.

Vintage	Bunch No.	Bunch FWt	Rootstock
2010	0.07	0.13	0.80
2011	0.40	0.19	0.41
2012	0.83	0.17	0.00
2013	0.88	0.12	0.00

In the following two seasons, where there was no further change in irrigation method and the weather conditions were less extreme, yield was almost entirely explained by bunch number (>80% of the variance), with no further impact of rootstock. Consequently, in these two seasons the influence of rootstock on yield was almost entirely via the difference in bunch number, rather than a difference in fruit filling. This is clearly illustrated in Figure 10, which plots bunch number against yield for each season.

The importance of bunch number as the main driver of yield across both the seasons and rootstocks is consistent with previous studies with Shiraz, which indicate that Shiraz is sensitive to environmental cues impacting fruitfulness, i.e. 3-fold differences in bunch number across seasons (Clingeleffer 2010). In this study node number and shoot numbers were not recorded, hence it is not possible determine if the differences in bunch number across the rootstocks can be attributed to differences in those factors. It is possible that the high vigour rootstocks may have had lower shoot fruitfulness due to the effects of shading during the fruit bud initiation period as reported for Sultana on Ramsey rootstock, compared to Sultana (Sommer *et al*. 2001) although Stevens *et al.* 2010 found no effect of rootstock on Shiraz shoot fruitfulness across a range of rootstocks.

Yield and vigour relationships

Many studies have examined the relationship between canopy area and crop yield, producing recommendations of ideal ratios for a particular cultivar or region (e.g. Kliewer and Dokoozlian 2005). However, these studies are often concerned with thinning the canopy, more rarely characterising the innate relationship between rootstock vigour and yield. Di Filipo and Villa (2011) recently examined these characteristics of six rootstocks, including 140 Ruggeri, classifying them into four categories, depending on the combination of yield and canopy size.

In our study, the relationship between yield and canopy size was non-significant when the data from individual vines were used in all except the 2009/10 season (data not shown), with an R^2 value of 0.41 for that season. This was also true for the relationship between pruning weight and yield. However, separating the rootstock conferred effect from seasonal or vine to vine variation, by examining the four season means of all three parameters, demonstrated highly significant correlations between both canopy area (estimated from LAI) and pruning weight with yield (Figure 11). In both cases the relationships were non-linear, with an 'exponential to maximum' curve being the best fit. As a result of the non-linear relationship between canopy size and pruning weight the shapes of these curves were different and pruning weight had limited predictive power at its extremes. This suggests that, where available, measures of canopy size are likely to give more easily interpretable estimate of effects on yield than pruning weight.

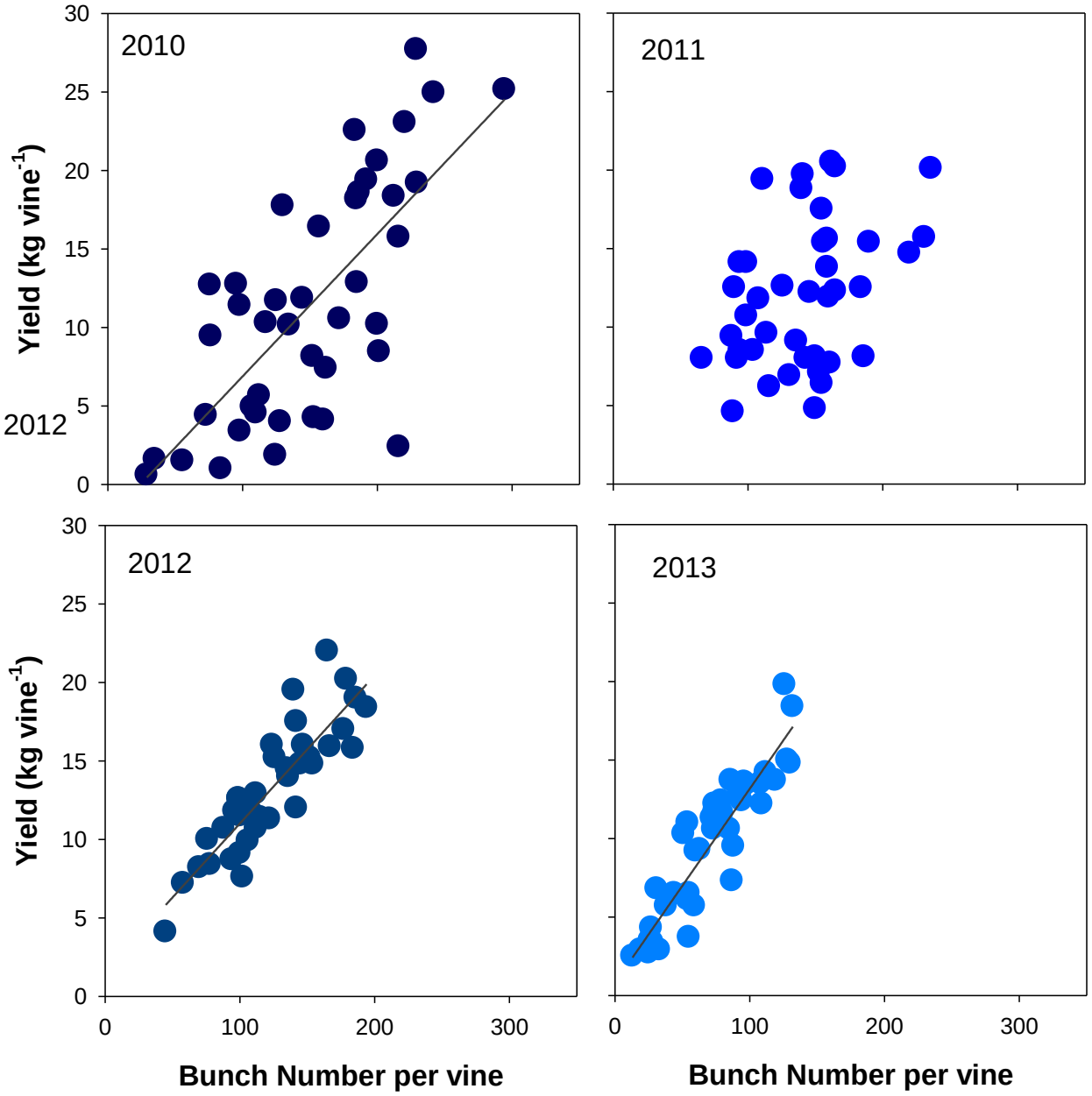


Figure 10. The relationship between bunch number and yield for individual Shiraz scions grafted to seven different rootstocks over the four seasons of the project, n=42 for each vintage.

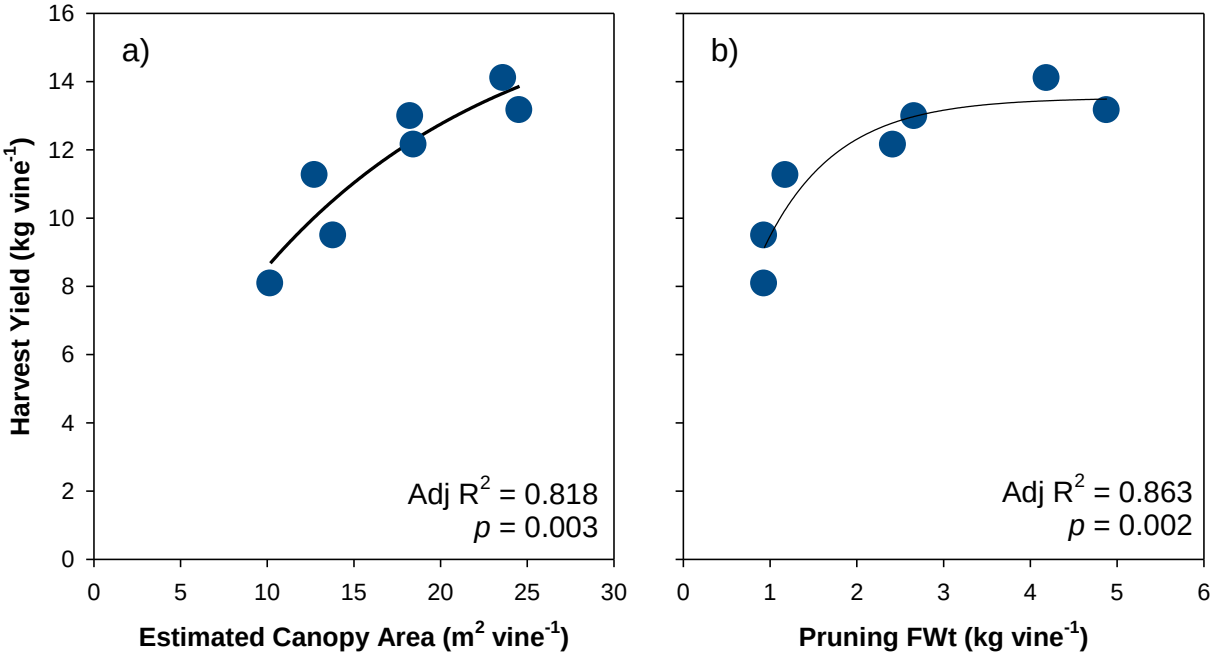


Figure 11. The relationship between the effect of rootstock on canopy size (a) or pruning weight (b) and fruit yield. Data are means of six replicates, with each replicate datum being a mean of four seasons. The curve fits are of the form y=a(1-exp(-b*x).*

Apart from being the line of best fit, the curve fit chosen was justified by a negative correlation between yield per unit canopy area against canopy area itself (data not shown), which suggested that yield must have been related to canopy size, but with a factor below one, i.e. the exponential rise to maximum response. The lack of any correlation when data from individual vines were used, but the high correlation achieved using the multi-season, multi-vine, rootstock means suggests that, for an individual vine in a given season, variation in yield:canopy ratio from factors other than the rootstock are larger than that caused by the rootstock itself. However, Figure 11a demonstrates that rootstock effects on vigour do indeed impact on the vines 'efficiency' in terms of yield per unit canopy area over a longer timescale. Furthermore, there was no significant effect on yield of the seasonal impact on vigour (i.e. re-plotting Figure 11 using mean data for each season instead of each rootstock - data not shown), suggesting that rootstock effects may have been greater than the seasonal effects in this respect. Jones *et al.* (2009) found a high degree of correlation between rootstock and yield, and rootstock and pruning weight over six years, using the same trial site. That work consists of data collected 15 years previously and in conjunction with the data presented here indicates that the effect of rootstock choice on these variables is not only large, but also consistent over a long period.

Rootstock effects on whole vine transpiration and crop water use index (CWUI)

All the vines in the study received the same irrigation in any one season, so water use efficiency, in terms of yield per unit water applied, was a result of yield itself (Figure 5). However, no irrigation system has 100% efficiency and the rootstocks were likely to have differences in rooting volume and root density, as rootstocks vary in both their absolute root biomass and the root fraction of total biomass (Tandonnet *et al.* 2010) and can have different rooting patterns (Smart *et al.* 2006). Furthermore, canopy size is a major factor in whole plant water use (Williams and Ayars 2005). Crop water use index (CWUI), the yield per unit water transpired, is a measure of water use efficiency based on the plant itself, not the irrigation system. Calculating CWUI requires that whole

plant water use be measured. Whilst this can be achieved accurately using weighing lysimeters, the most practical method in a large scale field context is to use sap flow sensors. The system used in this study allowed four vines per rootstock to be monitored and produced data with very little noise that clearly allowed the impacts of weather changes on transpiration to be discerned. For example, Figure 12a reproduces the sap flow recorded over 11 days in January 2011, with the effects of partially cloudy (e.g. 9/1/11) and rain (e.g. 13/1/11) days to be observed. The data can be integrated on a daily basis over a season, allowing the same weather impacts on sap flux to be observed together with the effects of seasonal changes in growth (Figure 12b). Integrating daily sap flux over a season estimates the water used by the vine to produce a crop, allowing CWUI to be calculated.

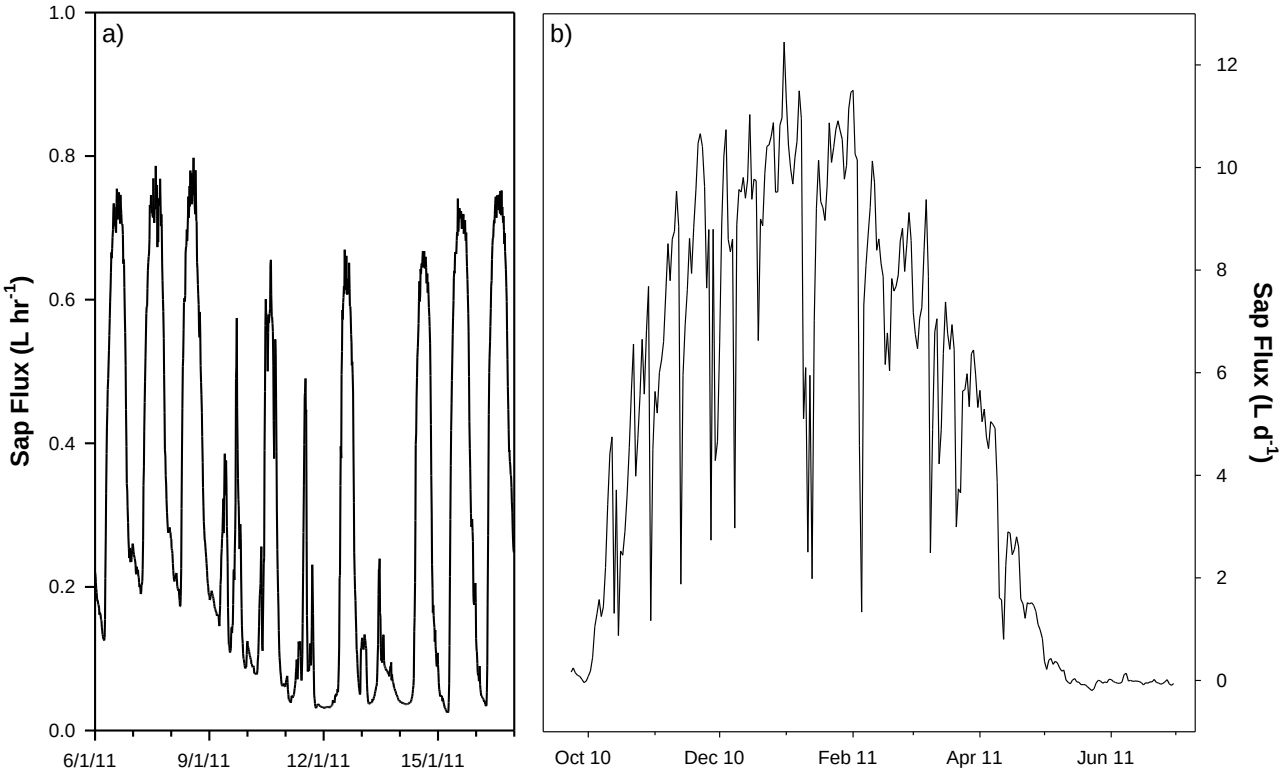


Figure 12. Sap flow measurements from a single Shiraz vine on Ramsey rootstock, a) 11 days of data recorded at 15 minute intervals, b) total daily flux recorded over the entire 2010/11 growing season.

Sap flow data was not collected in the 2009/10 season, but was available for the following three seasons. The results indicated that over an entire growing season the vines transpired between a low of 0.68 ML ha^{-1} for Merbein 6262 and a high of 4.13 ML ha^{-1} for 1103 Paulsen, both extremes occurring in 2012/13. As the whole trial was evenly irrigated there was limited value in examining irrigation water use efficiencies. For example, during 2010/11 record rainfall was received, with 886 mm being recorded between 1/9/10 and 30/6/11 at the Koorlong site. The rainfall was equivalent to almost 9 ML ha^{-1} of irrigation, whereas actual irrigation during the season was 2.7 ML ha^{-1} (Table 1). Although much of the received rainfall would have run off, particularly as the trial plot was located on a slight slope, calculating an irrigation use efficiency would be meaningless. However, over the project as a whole the highest recorded seasonal transpiration value, that for 1103 Paulsen in 2012/13, was equivalent to almost 90% irrigation efficiency. There was a large

range down from that value, which depended on rootstock and season.

Expressed per vine, the seasonal transpiration (Figure 13) was roughly in line with estimates of vigour (Figure 3), the exception being Dog Ridge. This is difficult to explain, as Dog Ridge had the largest canopy. Furthermore, the daily sap flow responded to atmospheric conditions (ET_o, data not shown) in the same way as the other vines. There are a number of factors involved in scaling from two point measurements of sap flux to whole vine transpiration (Hatton *et al.* 1990) and it is possible that estimates of one of these are poor for that very high vigour vine. Alternatively, differences in leaf level physiology, e.g. stomatal conductance, may be involved (see Chapter 3).

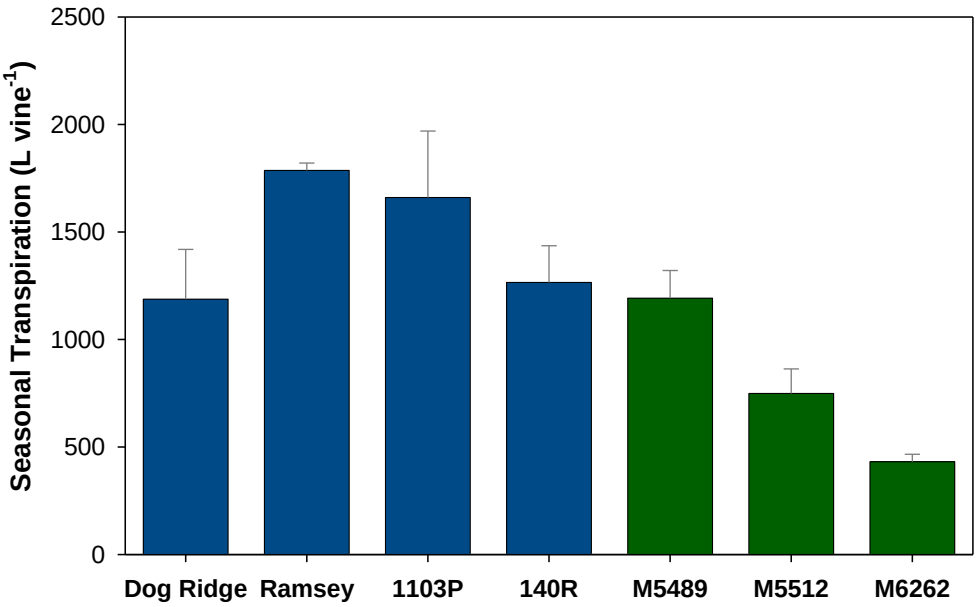


Figure 13. Total seasonal sapflow (from 1st September to 31st May) of Shiraz scions on seven rootstocks, four 'standards' (blue) and three CSIRO Merbein series (green). Data are means of three seasons +SE, where each value for each season is a mean of 2-4 vines (typically 4).

Calculated using the sap flow for the whole season, the CWUI varied from 6.8 to 22.6 t ML^{-1} between rootstocks, when averaged across the three seasons (Figure 14). With the single season maximum and minimum being 26.8 and 4.6 t ML^{-1} for Merbein 6262 in 2011/12 and 1103 Paulsen in 2012/13, respectively. Although the exact values varied, the four standard rootstocks and Merbein 5489 had a similar CWUI, whereas Merbein 5512 and Merbein 6262 had much higher values.

The results demonstrate the trade off between water use and yield when considering water use efficiency. For instance, Merbein 6262 had the highest CWUI, but the lowest yield, whereas Ramsey had the highest yield, but the lowest CWUI. However, the water use/yield relationship was not a simple negative correlation; Merbein 5512 had a CWUI almost double that of the standard rootstocks, probably due to Merbein 5512 having a much smaller canopy than the standard rootstocks (but see Chapter 3), yet had a yield not significantly different from 140 Ruggeri.

Whilst the results in a single season may vary, when considering the water use efficiency of a planting that may be in the ground for 30 years or more, it is a longer-term multi-season analysis that is required. That analysis demonstrates that rootstocks can drive water use efficiency in the

field and that this can be done without significant loss of productivity.

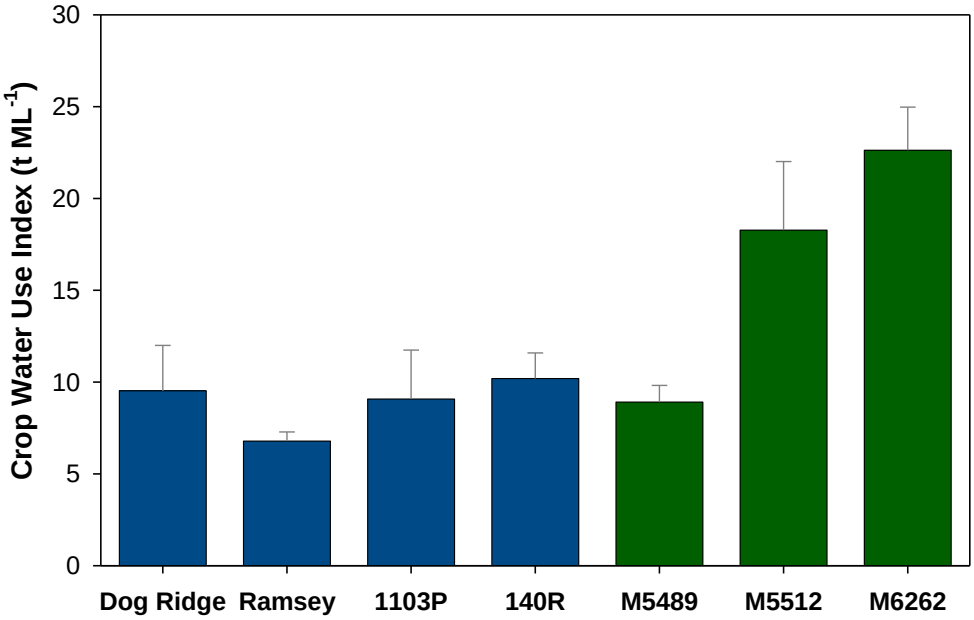


Figure 14. CWUI of Shiraz scions on seven rootstocks, four 'standards' (blue) and three CSIRO Merbein series (green). Data are mean of three seasons +SE, where each value for each season was calculated from a mean of 6 vines for yield and 2-4 vines for water transpired (typically 4).

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Chapter 3

Rootstock effects on scion physiology

Background

Whole vine water use is the product of the evaporative demand of the air around the canopy, stomatal conductance (g_s) and canopy area. The previous chapter demonstrated the potential of rootstock choice to influence the canopy size of the scion and that this, in turn, affected whole vine water use. But rootstocks also have the potential to alter g_s, either through differences in the ability of the hydraulic system to meet shoot demand for water or through differences in plant hormone production and/or transport. This chapter examines the leaf level physiology of the Shiraz scions grafted to seven rootstocks that were studied in Chapter 2 in order to determine whether the rootstock is affecting g_s of the scion. In addition, the mechanism whereby rootstocks affect scion vigour in *Vitis* has not yet been elucidated and the results reported here were also utilised to form the basis of further work to investigate those mechanisms.

Materials and methods

Leaf gas exchange

Gas exchange measurements were made with a LiCor 6400 Portable Photosynthesis System (John Morris Scientific, Adelaide, SA, Australia), using young, fully expanded leaves from the northern side of the trellis, typically the eighth leaf from a cane tip. Measurements consisted of light saturated assimilation (A_{sat}) measured using a reference air CO_2 concentration of 400 ppm, providing a cuvette CO_2 concentration approximately the same as the ambient air. Cuvette air temperature was maintained at the expected typical ambient air temperature for the measurement period and humidity was not controlled.

Transpiration (E), stomatal conductance (g_s) and leaf temperature were also recorded with each A_{sat} measurement. Leaf water use efficiency was calculated for 'instantaneous water use efficiency' (WUE_i) as A_{sat} / E and for 'intrinsic water use efficiency' (WUE_t) as A_{sat} / g_s.

No gas exchange measurements were made during the 2009/10 season. A set of measurements was made over 2-3 days at flowering and veraison in 2010/11, canopy closure and veraison in 2011/12 and veraison in 2012/13. Each set of measurements utilised a single, sun exposed leaf for each replicate of each rootstocks, i.e. n=6 for each rootstock mean.

Porometry

Stomatal conductance (g_s) was measured on the abaxial side of leaves chosen as for gas exchange measurements (above), using a Delta-T AP4 Porometer (MEA, Magill, SA, Australia). Measurements were made in the late morning, normally between 9:30 and 11:30 AM on sunlit outer canopy leaves that were expected to be in direct sunlight for most of the day. Calibration of the porometer was always performed immediately before a set of measurements in the ambient conditions, and a recalibration was performed half way through the measurements if the duration of measurement was expected to exceed 45 minutes.

Measurements were made at veraison in 2009/10, canopy closure in 2010/11, flowering and canopy closure in 2011/12 and flowering, canopy closure and veraison in 2012/13. A measurement set consisted of a single leaf per replicate as for gas exchange.

Elemental analysis
A single sunlit leaf, as used for the various leaf physiology measurements (above), was sampled from each replicate once per season for leaf nitrogen and $\delta^{13}C$ analysis. In 2009/10 the leaf was taken at harvest, in the other seasons the sampling occurred at veraison. Leaves were oven dried at 60°C, ground to a fine powder and analysed by the CSIRO Plant Industry Analytical Chemistry Group using Isotope-Ratio Mass Spectrometry.

Leaf water potential
Leaf water potential was measured using the balance pressure method with a Soil Moisture Equipment Corp. Model 3000 Plant Water Status Console (ICT International, Armidale, NSW, Australia) fitted with a digital peak hold meter (MEA, Magill, SA, Australia). Midday leaf water potential measurements were made within an hour of local solar noon, using sunlit (Ψ_l) leaves as above. Pre-dawn measurements (Ψ_{pd}) were timed to be finished by civil twilight (30 minutes before dawn).

No leaf water potential measurements were made in 2009/10, both measurement types were made at canopy closure in 2010/11, veraison 2011/12, flowering 2012/13 and veraison 2012/13, whilst only Ψ_l was measured at canopy closure 2011/12. A measurement set typically consisted of a single leaf per replicate as for the other leaf physiology measurements, above.

Results and discussion

Physiological measurements, gas exchange, porometry, water potential and stable isotope analysis were used to address the possibility that rootstocks could affect transpiration (E) at the leaf level independently of effects on vine vigour and to examine possible mechanisms whereby rootstocks may drive vine vigour.

Did rootstock affect vine water use at the leaf level?
Direct measurement of leaf E was made using a gas exchange system on one to two occasions in each season except 2009/10 (Figure 15). Absolute rates were dependent on atmospheric conditions, with the highest rates recorded at veraison in 2012/13, when leaf temperatures were approximately 38°C and the vapour pressure deficit (VPD) in the leaf chamber was over 4 kPa. The lowest rates were recorded at canopy closure in 2011/12, when leaf temperatures were 26°C and the VPD was under 2 kPa. Irrespective of absolute rate, vines on Ramsey always had the highest E of any vine grafted on the standard rootstocks and in four out of five sets of measurements the highest rates of all. The exception was at veraison in 2010/11, possibly to greater disease incidence in leaves from vines on standard rootstocks compared to those on the lower vigour Merbein series rootstocks.

Vine vigour *per se*, via canopy density, could have an effect on E of inner canopy leaves due to differences in humidity around those leaves. However, all measurements of E were done on outer canopy leaves, which were exposed to the ambient atmospheric conditions. Thus, the results suggested that water use at the leaf level was indeed altered by rootstock choice. Intriguingly, despite having higher vigour, vines on Dog Ridge had lower rates of E than those on Ramsey in all the measurements, matching the sapflow results. In the absence of disease, the lower vigour Merbein rootstocks had slightly lower rates of E than the standard rootstocks.

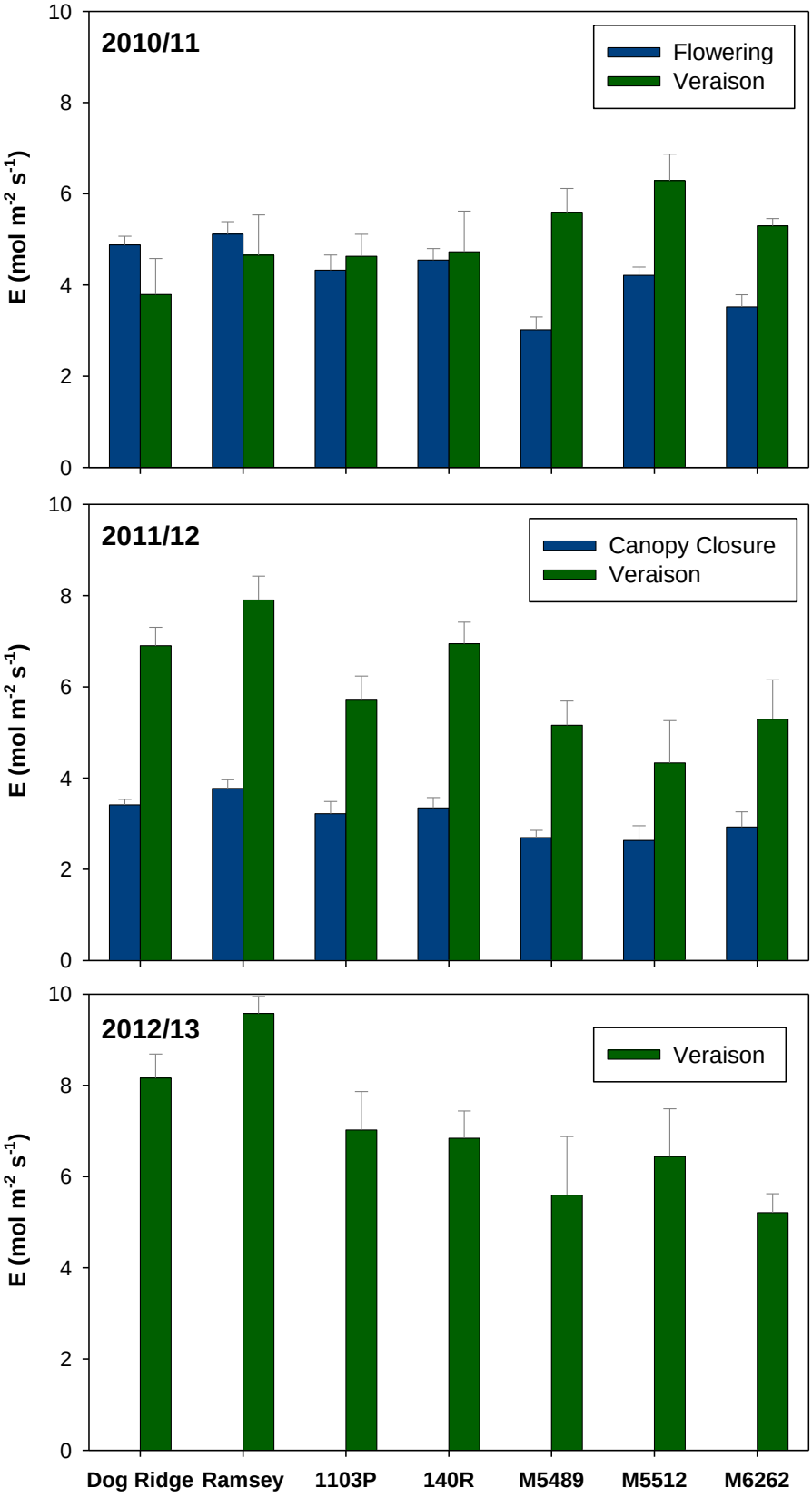


Figure 15. Transpiration of Shiraz scions on seven rootstocks at key phonological periods in the 2010/11 to 2012/13 seasons. Data are means +SE, n=6.

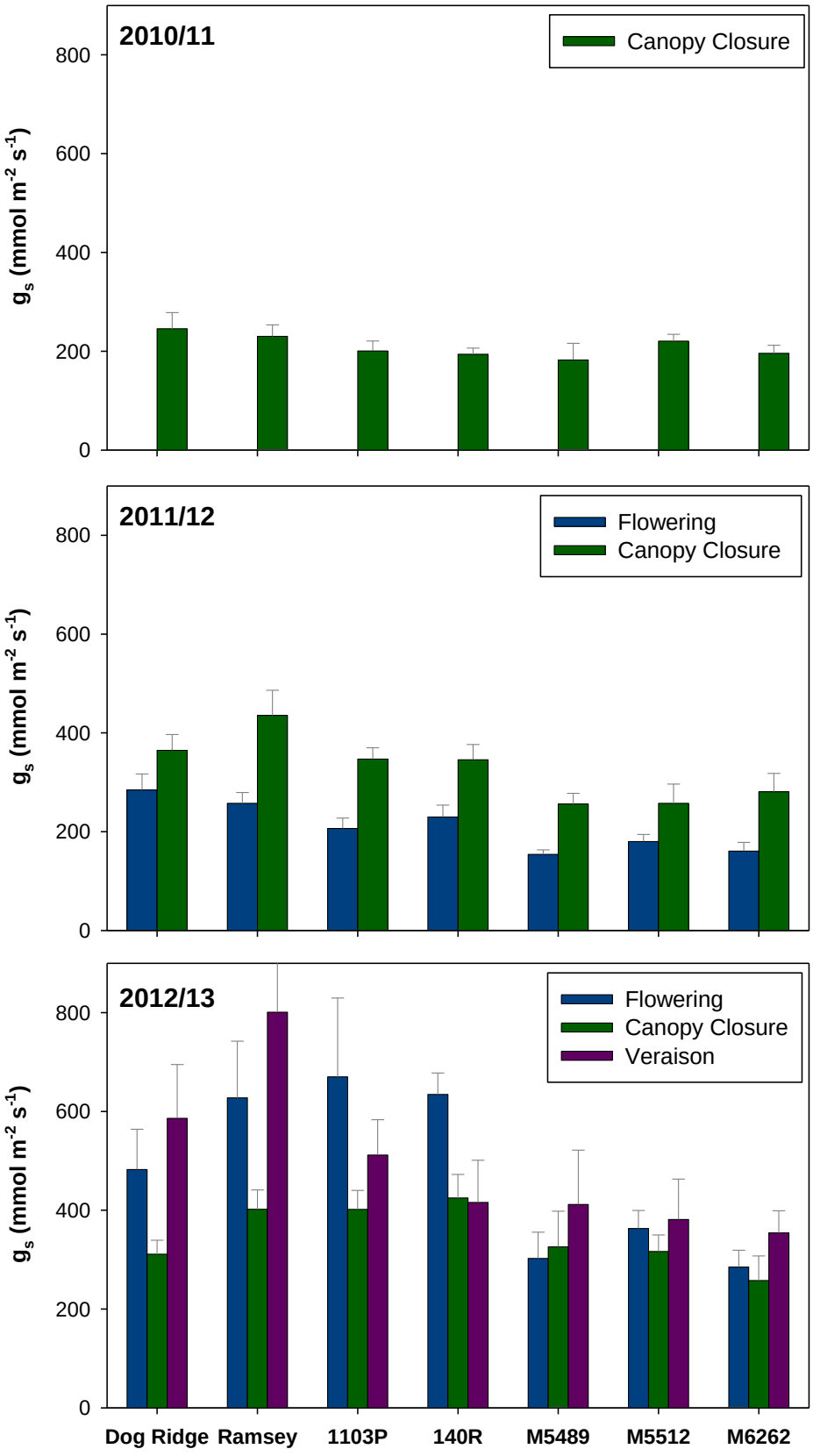


Figure 16. Stomatal conductance of Shiraz scions on seven rootstocks at key phenological periods in the 2010/11 to 2012/13 seasons. Data are means +SE, n=6.

As all the vines were in the same environment and all leaf physiology measurements were made on outer canopy leaves, the observed differences in E were necessarily due to differences in g_s. During the 2010/11 season g_s was only measured on a single occasion due to the weather conditions that season. There were no large differences between the rootstocks and all the results were lower than is typically seen in Shiraz vines in this region, probably also due to the cool, damp, weather conditions (Figure 16a). In the subsequent seasons, the standard rootstocks typically had higher g_s than the lower vigour rootstocks and, within those standards, Ramsey had the highest g_s of all (Figure 16b & c).

There are a number of factors that control g_s and not all are well understood (e.g. Jones, 1998). In addition, there are typical diurnal cycles in g_s and measurements are normally made in late morning when g_s is expected to be highest. The data from 2012/13 (Figure 16c) suggest that there may be a greater range of response to environmental conditions in the standard rootstocks than the lower vigour Merbein rootstocks. This may have been due to differences in root-shoot signalling, or due to differences in hydraulic conductivity, with the lower vigour rootstocks unable to support the high transpiration rates that occurred in the higher vigour rootstocks during veraison 2012/13. Consequently, it may be that typical measurements on a fine day prior to midday are not representative of the long-term effect of rootstock on g_s.

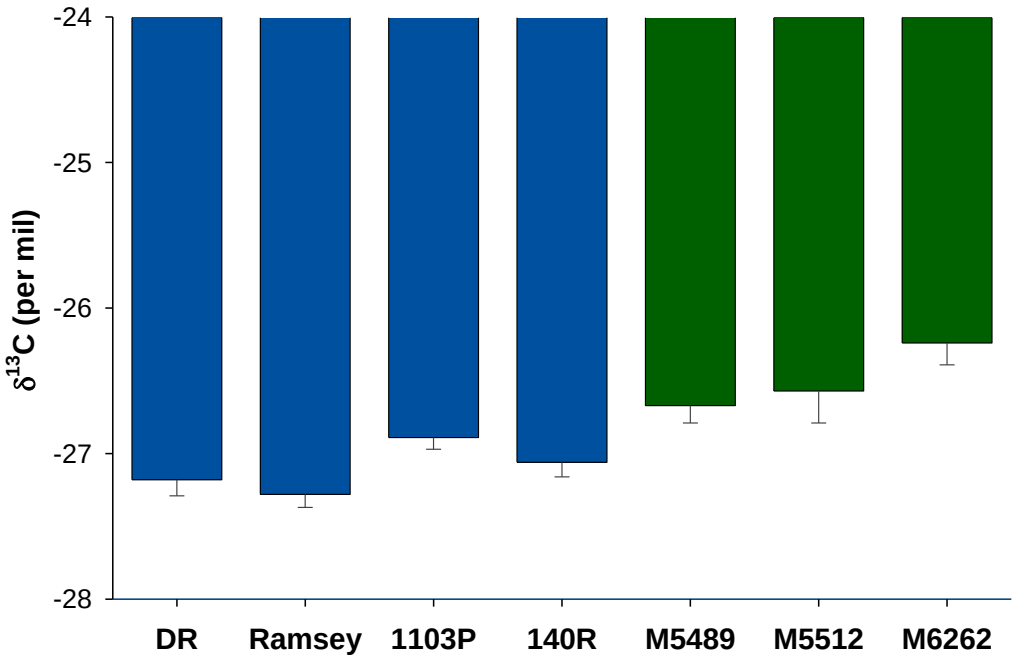


Figure 17. ^{13}C discrimination in leaves from Shiraz scions on seven rootstocks, four 'standards' (blue) and three CSIRO Merbein series (green). Data are mean of six replicates +SE, where each replicate is a mean of four measurements, collected once per season.

The $\delta^{13}C$ of carbon in the leaf is directly related to the transpiration efficiency (molecules of CO_2 fixed per molecule of H_2O transpired) at the time when it was assimilated by the plant (Farquhar & Richards, 1984). Thus the $\delta^{13}C$ signature of the leaf represents an integral of the transpiration efficiency during the time it has been actively photosynthesising. Transpiration efficiency is, in turn, a product of photosynthetic capacity and g_s. Where photosynthetic capacity is unchanged, leaf $\delta^{13}C$, therefore, is representative of g_s integrated over time. A reduction in g_s will result in a higher $\delta^{13}C$ value for a given photosynthetic capacity, whereas an increase in g_s will result in a

lower $\delta^{13}C$.

Leaves were collected once per season, at harvest in 2009/10 and at veraison in the three subsequent seasons and analysed for $\delta^{13}C$. The average over the four seasons was lowest for Ramsey, which also had the highest g_s, and highest in the low vigour rootstocks (Figure 17). This suggests that the transpiration efficiency of the lower vigour rootstocks was greater than the standard rootstocks. As photosynthetic capacity was *not* greater in the lower vigour rootstocks (see below) it also suggests that, over the lifetime of the canopy, g_s was lower in the lower vigour rootstocks.

Overall, the leaf level data collected indicated that rootstock choice had an effect on whole vine water use by altering stomatal conductance as well as canopy size. This was supported by short-term g_s measurements from two different methods (g_s estimates from gas exchange not shown) as well as the stable isotope analysis.

Was there evidence for leaf physiology driving rootstock conferred vigour?
It was clear from Chapter 2 that rootstock conferred vigour was a major driver of vine water use and water use efficiency. There are a range of potential mechanisms whereby differences in vine vigour could be caused by alterations in scion physiology as a result of the rootstock to which it was grafted, including:

- reduced water transport to the scion resulting in reduced carbon assimilation; due either to resistance in the graft union itself, or reduced hydraulic conductance in the rootstock
- an effect on the partitioning of assimilate, either between root and shoot or between components of the shoot
- alteration of the production and/or transport of plant hormones
- changes in the nutritional supply to the scion.

Leaf level measurements had the potential to provide an insight into whether some of these may or may not be occurring using the rootstock trial within this project.

Differences in g_s, as seen in this work, typically result in differences in assimilation due to a reduction in CO_2 entry into the leaf. Lower values of g_s result in more water use efficient photosynthesis, but lower absolute rates. Measurements of A_{sat} were similar to published values of A_{sat} for red wine grapes in the Sunraysia region (e.g. Edwards & Clingeleffer, 2011) and were generally higher in the higher vigour rootstocks (Figure 18). The exception to this was in 2010/11 when the higher incidence of disease (see above) in the standard rootstocks resulted in lower A_{sat}. Differences were not large, but the 2011/12 data suggested that, in the absence of disease, they were consistent through the season (Figure 18b).

The observed effect of rootstock on scion photosynthetic rate was due, in part at least, to the effects on g_s. However, differences in nitrogen availability, perhaps due to differences in water uptake, could also be responsible for lower rates of A_{sat} in the lower vigour vines. Leaf nitrogen concentration is well correlated with photosynthetic capacity (Wright *et al.* 2004) due the high proportion of leaf nitrogen that is incorporated into the photosynthetic system (Harrison *et al.* 2011). Nitrogen concentration was determined on the same leaves that were used for $\delta^{13}C$ determination, which ensured that any conclusions on the likely photosynthetic capacity of those leaves, from leaf N, could be directly linked with estimates of transpiration efficiency, from leaf $\delta^{13}C$.

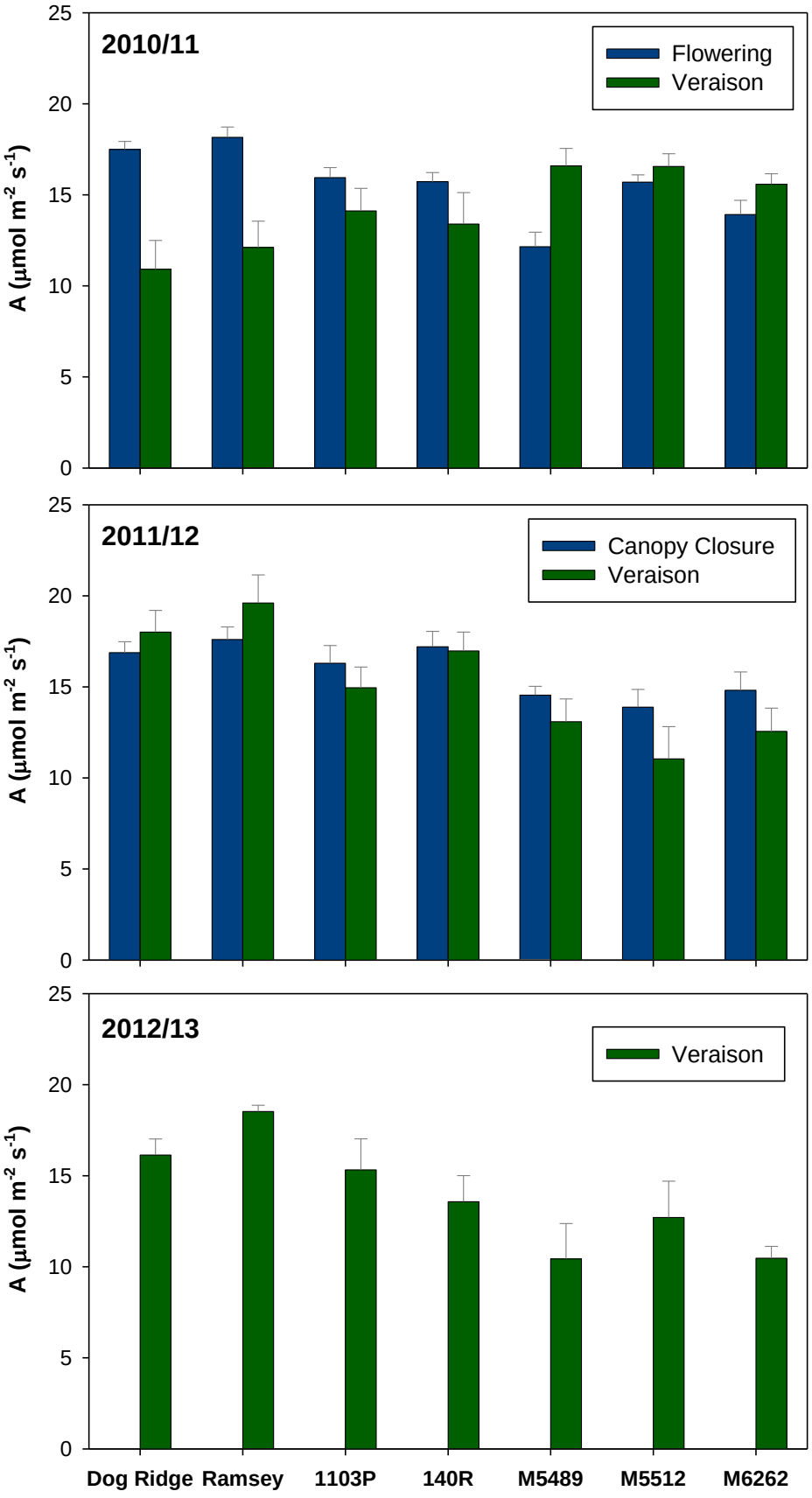


Figure 18. Assimilation under saturating light (A_{sat}) of Shiraz scions on seven rootstocks at key

phenological periods in the 2010/11 to 2012/13 seasons. Data are means +SE, n=6.

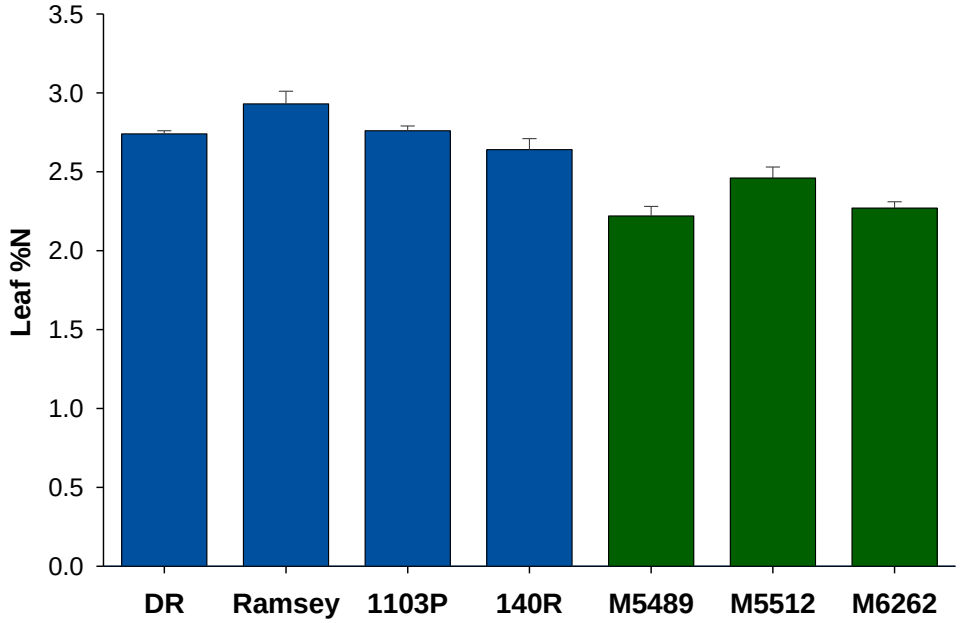


Figure 19. Leaf nitrogen concentration in leaves from Shiraz scions on seven rootstocks, four 'standards' (blue) and three CSIRO Merbein series (green). Data are means of six replicates +SE, where each replicate is a mean of four measurements, collected once per season.

Although no nitrogen fertiliser was applied to the trial during the life of this project the leaf N concentrations were again typical of red winegrape vines in the region (Figure 19). Neither prunings nor fruit were removed from the block (other than those used for the sampling described in this report), so the only N lost from the system would have been through N mineralisation in the soil. Despite this, there were small differences in scion leaf N content between the rootstocks, with Merbein 5489 being the lowest at 2.2% and Ramsey the highest at 2.9%. With the exception of Merbein 5489, the ranking of the rootstocks for scion leaf N was the same as for water transpired, although the relative differences are much smaller, (Figure 13). One factor in N uptake from the soil is bulk flow of water to the roots and if the lower vigour rootstocks had smaller root systems this may have been the case. If so, the differences in leaf N may have been avoided if nitrogen fertiliser had been applied via irrigation system, thereby ensuring that all of the available nitrogen was within the rooting zone.

Estimations of maximum carboxylation rate (a measure of photosynthetic capacity) made from A_{sat} (data not shown) suggested that at veraison, but not earlier in the season, carboxylation rate was lower in the lower vigour vines. That estimate takes account of differences in g_s and reflects either a change in Rubisco activation or in the amount of Rubisco. As Rubisco can account for 50% of the protein in the leaf, lower Rubisco content in the leaf is correlated with lower leaf N content. However, the photosynthetic measurements were made on an area basis, whereas leaf N concentration was measured on a dry weight basis, so the two measurements cannot be combined. Despite this, the lower N concentrations strongly suggest that the lower maximum carboxylation rates were due to lower Rubisco concentrations.

To summarise, rootstock conferred vigour was correlated with leaf N concentration and assimilation rate, resulting in the lower vigour vines fixing less carbon per unit leaf area. Modelling

whole vine assimilation was beyond the scope of this project, but, combining the lower assimilation rates with the reduced canopy size of low vigour vines, it is clear that whole vine carbon assimilation would be less than in the higher vigour vines. However, growth is not a linear process and these results do not provide any direct evidence for such differences being the origin of the differences in vigour. Furthermore, due to self-shading in the larger canopies, the difference in whole vine carbon uptake between high and low vigour vines would have been much less than might be suggested by multiplying A_{sat} and canopy size, particularly as differences in A_{sat} were less during the period where the canopy was actively growing.

Cell, leaf and stem expansion are all reliant on water and can be impacted by low water potentials. Consequently, any difference between rootstocks in their ability to maintain turgor pressure in the scion during the period of active shoot growth could affect vine vigour. Leaf water potential measurements during the growth period were only made in 2012/13, but the series of measurements through the project could potentially indicate whether stress conditions were likely to result in large differences in leaf water potential between the vines on different rootstocks.

There was no strong relationship between rootstock vigour and Ψ_l (Figure 20), although comparing the vigour extremes, vines on Dog Ridge and Merbein 6262, suggested that high vigour vines had a less negative Ψ_l and low vigour vines a more negative Ψ_l. There was no interaction between rootstock and either season or phenological period.

When soil moisture was non-limiting Ψ_{pd} was also unaffected by rootstock (Figure 21a), but at periods of greater stress, the two highest vigour rootstocks were better able to maintain a higher Ψ_{pd} than the rest (Figure 21b, Figure 21c: flowering). However, veraison 2012/13 generated the hottest and driest weather that occurred at any time during the project's lifespan, resulting in Ψ_{pd} below -0.2 in all of the rootstocks and no difference between Dog Ridge/Ramsey and the other rootstocks (Figure 21c).

Overall, there was some evidence to support a hydraulic mechanism for differences in rootstock conferred vigour, but it was slight and was only measureable during periods of significant stress.

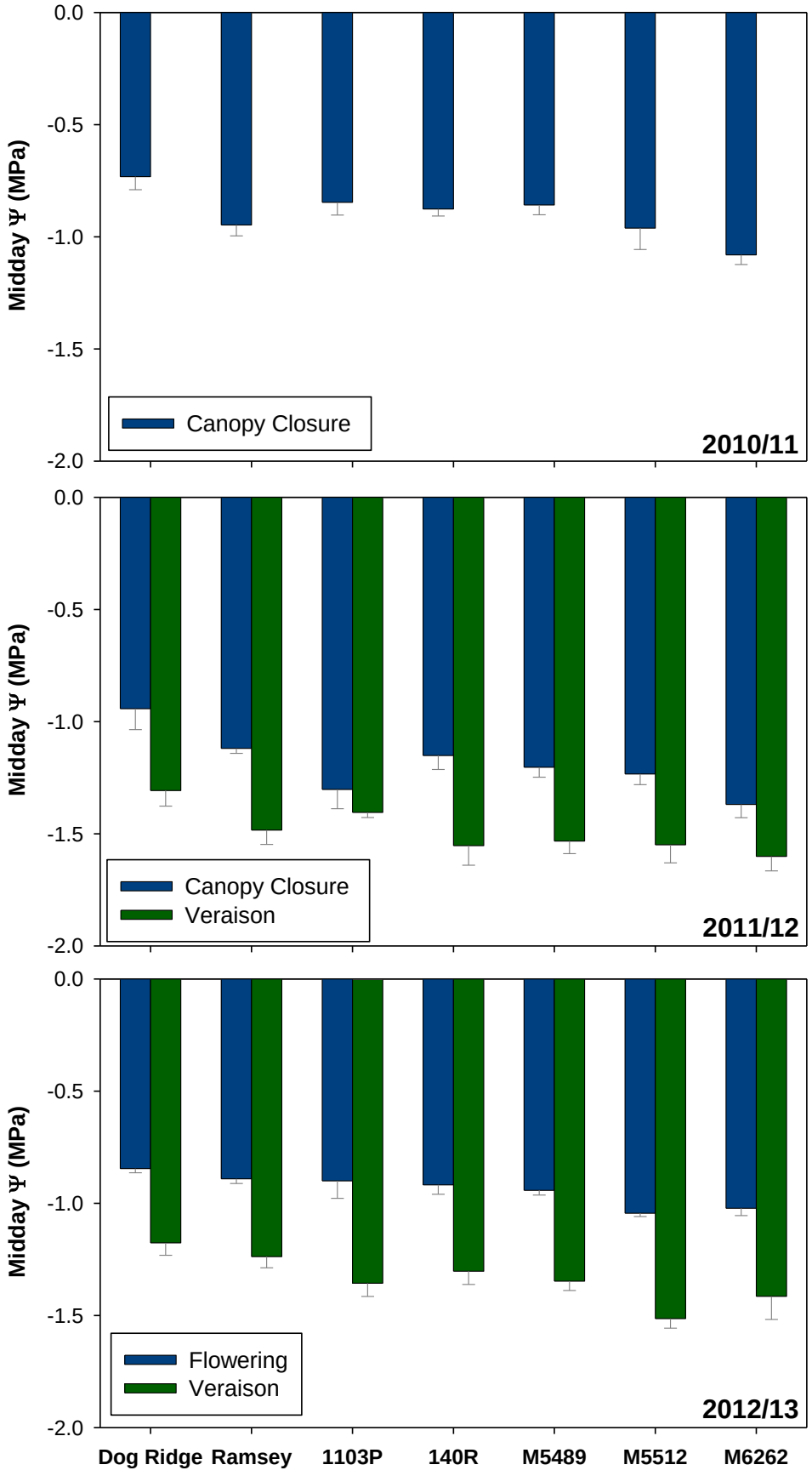


Figure 20. Midday leaf water potential of Shiraz scions on seven rootstocks at key phenological periods in the 2010/11 to 2012/13 seasons. Data are means -SE, n=6.

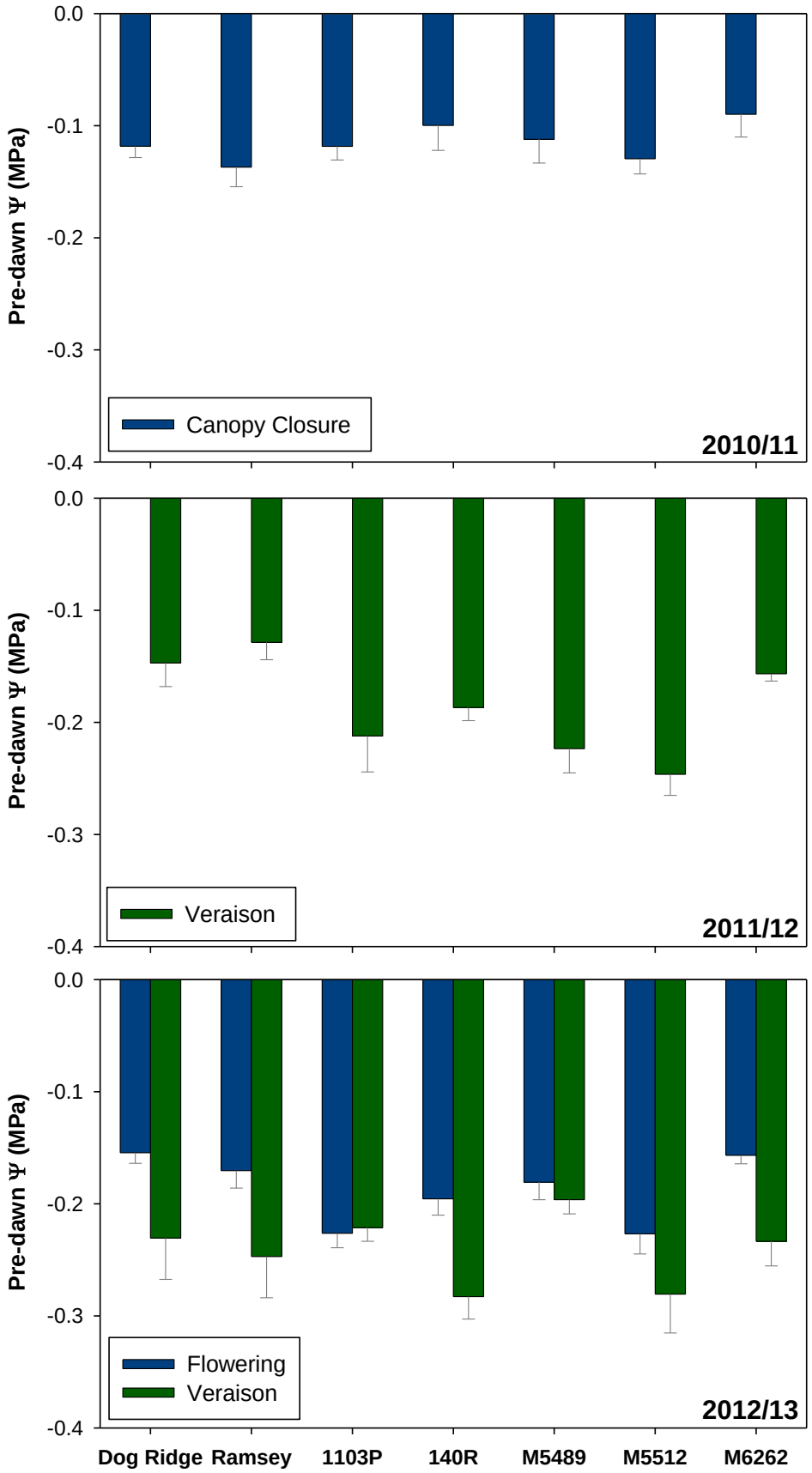


Figure 21. Pre-dawn leaf water potential of Shiraz scions on seven rootstocks at key phenological periods in the 2010/11 to 2012/13 seasons. Data are means -SE, n=6.

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Chapter 4

Rootstock impacts on scion drought and salinity tolerance

Background

The second component of the project was a set of glasshouse experiments utilised to manipulate soil moisture and soil salinity, whilst maintaining control over other environmental factors. These experiments enabled the physiology behind rootstock:scion interactions in response to water deficit to be examined, with the aim of extending the understanding of rootstock effects on the scion beyond what could be observed in the field experiment. Two of the glasshouse based experiments included exposing multiple rootstocks to drought and/or salinity stress with canopy gas exchange and growth assessed, together with sampling for the plant hormone abscisic acid (ABA), tissue elemental/ion and stable isotope analysis. This enabled an assessment of how drought and salt were affecting rootstock vigour and water-use.

Materials and methods

Both experiments were completed in the same glasshouse at the Waite Campus, Urrbrae, SA, with ambient light, daytime temperature limited to approximately 28°C and night-time temperature to approximately 20°C.

Experiment 1

One year old Shiraz vines grafted to 110 Richter, 140 Ruggeri, Ramsey and Schwarzmann rootstocks were purchased from Yalumba Nursery. These were maintained in the glasshouse and set out in a randomised block design, with two individuals of each rootstock in a block and nine blocks in total. One of these individuals was kept well irrigated as a control, whilst the second was provided with reduced irrigation. In both cases vines were irrigated every five days. Soil moisture was measured at least weekly in all pots using a TRASE TDR system (ICT International, Armidale, NSW). The reduced irrigation treatment was started on 23rd March 2011 and maintained until the vines were harvested between 10-20th May 2011.

Experiment 2

Dormant canes of 140 Ruggeri, Ramsey and Shiraz were obtained from the Riverland Vine Improvement Committee, Dog Ridge from SARDI and Merbein 5512 from Glenavon Vine Nursery, SA, rooted in a propagator then chip-bud grafted with Shiraz buds in February 2011. The vines were maintained in the glasshouse until use, being set out in a randomised block design with five blocks containing four replicates of each rootstock and Shiraz on own roots. Prior to the start of the experiment the vines were trained to a single shoot with 10-15 leaves. Treatments were well watered (WW), well watered saline (WS), deficit irrigation (DW) and deficit irrigation saline (DS), with one replicate of each treatment per rootstock per block. The two well watered treatments were irrigated to excess daily, whilst the two deficit treatments were irrigated to excess every five days, with a longer deficit period being used for the two irrigations prior to whole plant harvest. The saline treatments were irrigated with a 35 mM mixed chloride solution (Gong *et al.* 2011), whilst the non-saline treatments were irrigated using tap water. Treatments were started on 14th October 2011 and maintained until the vines were harvested from 28th November 2011. Soil moisture was monitored at 15 minute intervals using HS10 capacitance probes attached to an SMM data logger (ICT International, Armidale, NSW) in 30 pots, seven per treatment and encompassing at least one replicate of each rootstock/treatment combination.

Physiology measurements

Leaf function was assessed on a number of occasions in each experiment. Gas exchange was measured using a LiCor 6400 Photosynthesis Analyzer (John Morris Scientific, Kent Town, SA), stomatal conductance with a Delta-T porometer (MEA, Magill, SA) and midday leaf water potential (Ψ_l) with a Soil Moisture Equipment Plant Water Status Console (ICT International, Armidale, NSW). Xylem sap samples were taken following leaf water potential measurements in both experiments.

Each experiment was concluded with a whole plant harvest, with leaves, stem and roots being separated, oven dried and weighed.

Xylem sap was obtained from the cut end of petioles using a Scholander Pressure Bomb and analysed for abscisic acid (ABA) content using the method of Speirs *et al.* (2013).

Results and discussion

The two glasshouse experiments were similar, but differed in some of the rootstocks used, duration of experiment, the addition of saline treatments in experiment 2 and degree of water stress at harvest. Although the water deficits applied in the two experiments were similar (Figure 22), towards the end of experiment 1 the soil moisture in the deficit treatment was somewhat higher than earlier in the experiment (data not shown), whereas in experiment 2 the vines experienced a prolonged dry period before their final irrigation (Figure 22b).

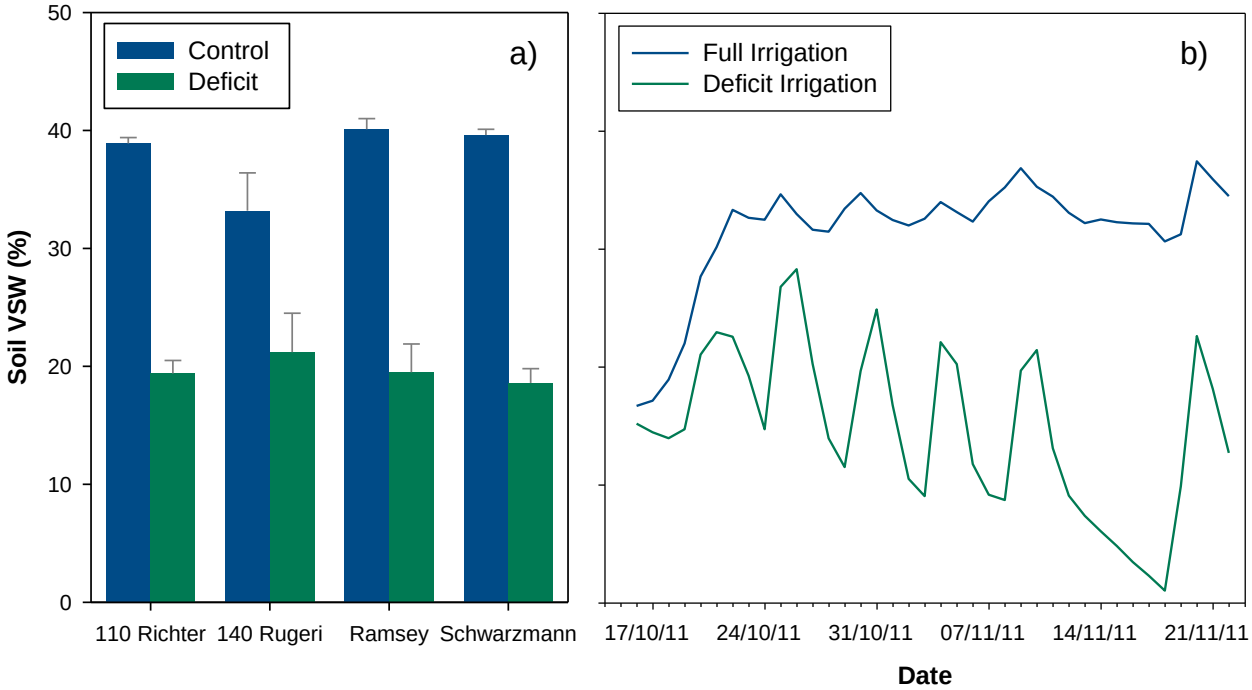


Figure 22. Volumetric soil moisture of control and deficit irrigation vines in a) experiment 1, mean of three time points measured by TDR, and b) experiment 2, mean of seven replicates measured by capacitance.

The difference in the extent of the water deficit between the two experiments was evident in the Ψ_l measurements, with the mean for the well watered control in experiment 1 being -0.63±0.09

MPa and -0.81±0.16 MPa for the deficit treatment, with no effect of rootstock. In experiment 2, there was also no significant effect of rootstock and, at the first measurement after the treatments were started (26/10/11), only a marginal effect of water deficit. There was a much larger effect prior to the harvest, when the deficit irrigated, non-saline treatment was much lower than for the other treatments (Figure 23). The impact of the deficit saline treatment on Ψ_l was much less, but still significantly lower than the well watered control. The well-watered saline treatment also had a slightly lower leaf water potential following the onset of the treatment.

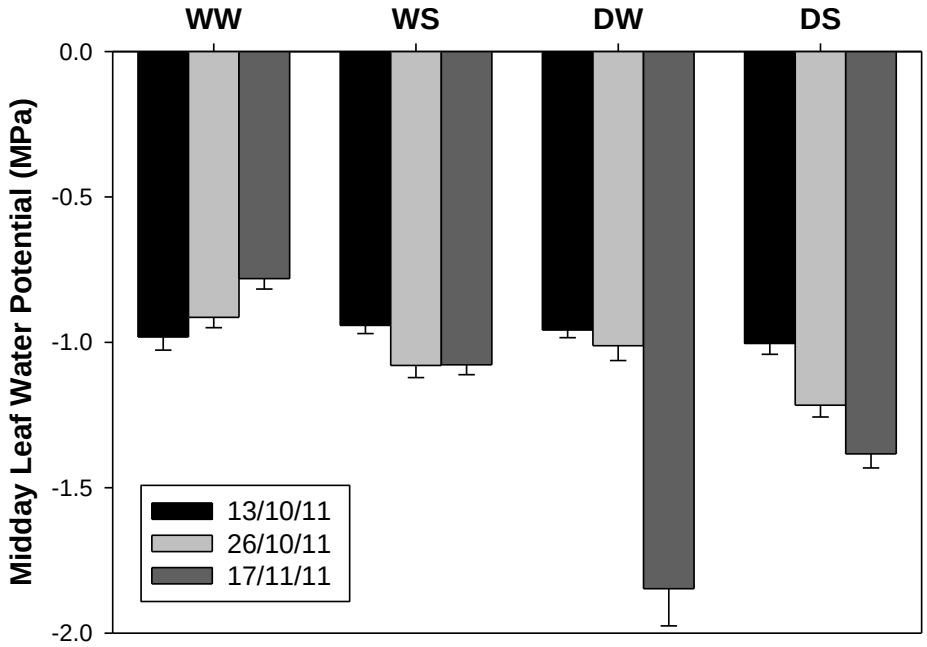


Figure 23. Mean midday leaf water potential of Shiraz scions on four rootstocks or own roots that were fully-irrigated (WW), fully irrigated with saline water (WS), deficit irrigated (DW) or deficit irrigated with saline water (DS). Bars are means of 25 replicates - SE.

The effect of the non-saline deficit treatment on Ψ_l was typical of that seen in grapevines, and Shiraz in particular, including field irrigation treatments (e.g. Girona *et al.* 2006, Maroco *et al.* 2002). Plants exposed to saline soil water typically exhibit reduced leaf water potential due to the increased osmotic potential of the soil solution (Walker *et al.* 1981). Both the saline treatments had lower leaf water potential, but the effect was only slight in the well watered saline treatment and, although the reduction was larger in the deficit saline treatment, these vines had a higher leaf water potential than those receiving the non-saline deficit treatment. The lesser effect of the deficit saline treatment could have been due to higher soil moisture content as a result of lower vine water use, associated with lower transpiration. Unfortunately, although the soil moisture sensors were designed to be effective at the conductivity of the saline irrigations and the pots were watered to excess, preliminary analysis of leachate (data not shown) suggests that the soil solution conductivity was much higher than the irrigations and above that at which the soil moisture sensors could operate accurately.

Although rootstock differences were not evident in leaf water potential this may not reflect field performance, as differences between rootstocks in their ability to take up water in the field may be due more to changes in root system topology than differences in physiology. In even a fairly large pot, there is limited opportunity for roots to explore different soil regions, with only root density

able to be varied (see below). Consequently, midday leaf water potential in the pot experiments was likely to reflect soil water potential. However, although there was no statistically significant interaction between rootstock and irrigation treatment in either experiment, leaf water potential of Dog Ridge was generally more negative and that of Merbein 5512 less negative than the other rootstocks in experiment 2, with both being significantly different from the other rootstocks when a post-hoc means comparison was carried out.

Rootstocks have the potential to affect vine water use and water use efficiency by imparting changes in vine morphology, such as canopy size, or via changes in physiology. Although there was little difference in leaf water potential between the rootstocks, stomatal conductance of the scion varied significantly with rootstock in both experiments. In experiment 1, scions of well watered Ramsey and Schwarzmann rootstocks had significantly lower conductance than those of 110 Richter or 140 Ruggeri, whereas only Schwarzmann maintained this difference under deficit irrigation (Figure 24).

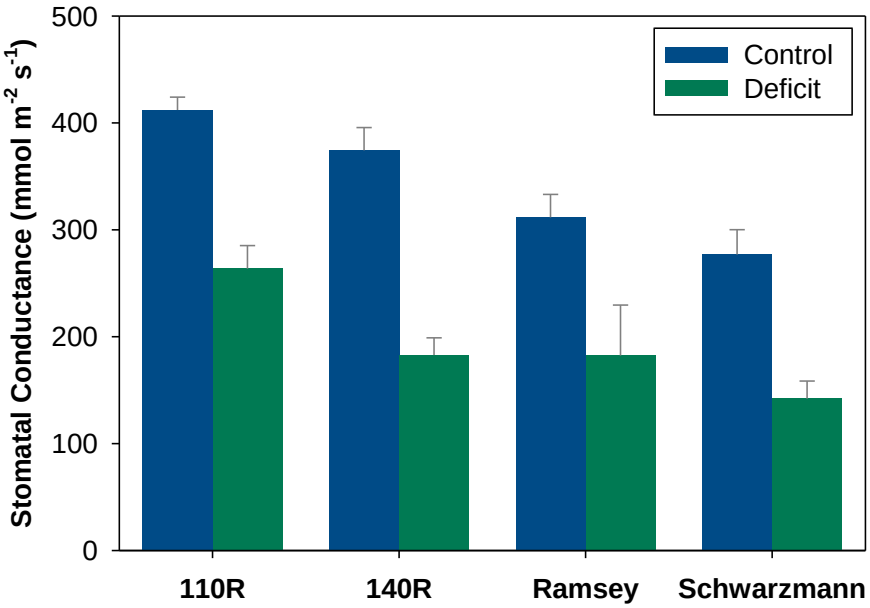


Figure 24. Stomatal conductance of Shiraz scions on 110 Richter (110R), 140 Ruggeri (140R), Ramsey and Schwarzmann rootstocks under full or deficit irrigation regimes. Data are means of eight replicates, each being a mean of three measurement times, + SE.

The response of rootstocks to sustained drought was also examined over the duration of the experiment using $\delta^{13}C$ isotope analysis (Figure 25). The stomatal conductance differences observed in the short-term gas-exchange measurements were also reflected in the isotope analysis. Application of water stress increased $\delta^{13}C$ across all rootstocks, indicative of increased uptake of $^{13}CO_2$ under stress conditions. Decreased stomatal conductance measured in Ramsey and Schwarzmann grafted vines was consistent with the higher $\delta^{13}C$ measurements compared to 110 Richter and 140 Ruggeri grafted vines. The component of $\delta^{13}C$ due to biological discrimination against carbon-13 by Rubisco has been shown to be negatively correlated with WUE in a wide range of plants including grapevines (de Souza *et al.* 2005; Gibberd *et al.* 2001).

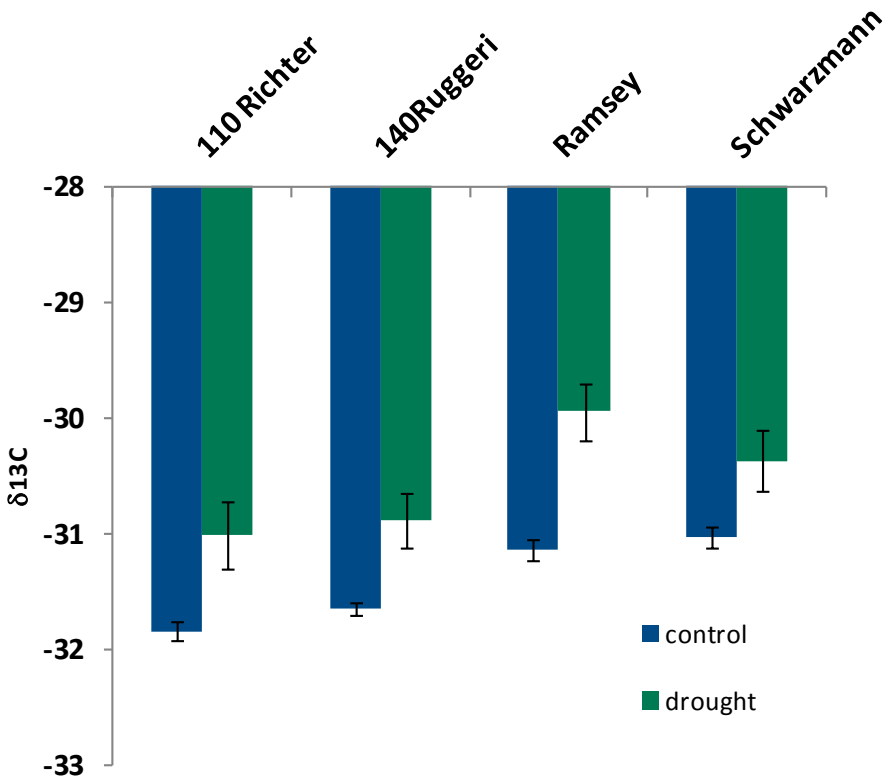


Figure 25. $\delta^{13}C$ isotope analysis of Shiraz leaves from scions on 110 Richter, 140 Ruggeri, Ramsey and Schwarzmann rootstocks under full or deficit irrigation regimes. Data are means of six replicates, taken at harvest, ±SE.

In the second experiment, some degree of water stress was present even in the fully-irrigated vines and this increased over time. As would be expected from the literature (e.g. Costa *et al.* 2012, Correia *et al.* 1995) and the previous experiment, stomatal conductance fell in all the deficit irrigated vines as the soil dried out (Figure 26). However, deficit irrigated Merbein 5512 vines had significantly higher conductance than any of the other rootstocks on the last two measurement dates (see below), although the rates in all the vines (including Merbein 5512) were extremely low at the final measurement. There were also differences between rootstocks in the response to saline irrigation when deficit irrigated, with Ramsey and 140 Ruggeri vines having higher stomatal conductance on the penultimate measurement date, viz. 187 and 214 mmol m^{-2} s^{-1} respectively cf. approximately 65 mmol m^{-2} s^{-1} in the other rootstocks, but only Ramsey maintained a difference at the final measurement, viz. 49 mmol m^{-2} s^{-1} cf. approximately 12 mmol m^{-2} s^{-1} in the other rootstocks.

Stomatal conductance typically decreases with increasing salinity, even in a well watered situation, reflecting the increasing osmotic stress in the rootzone. This stress is independent of drought stress, so salinity in combination with water deficit could be expected to exacerbate the fall in stomatal conductance. In this study the saline treatments both reduced stomatal conductance and there was no interaction with irrigation volume (Figure 26). Consequently, deficit irrigated vines receiving saline water did indeed have lower stomatal conductance rates than deficit vines irrigated with non-saline water, although by the end of the experiment all deficit irrigated vines had stomata that were effectively fully closed, so no further effect of salinity was possible.

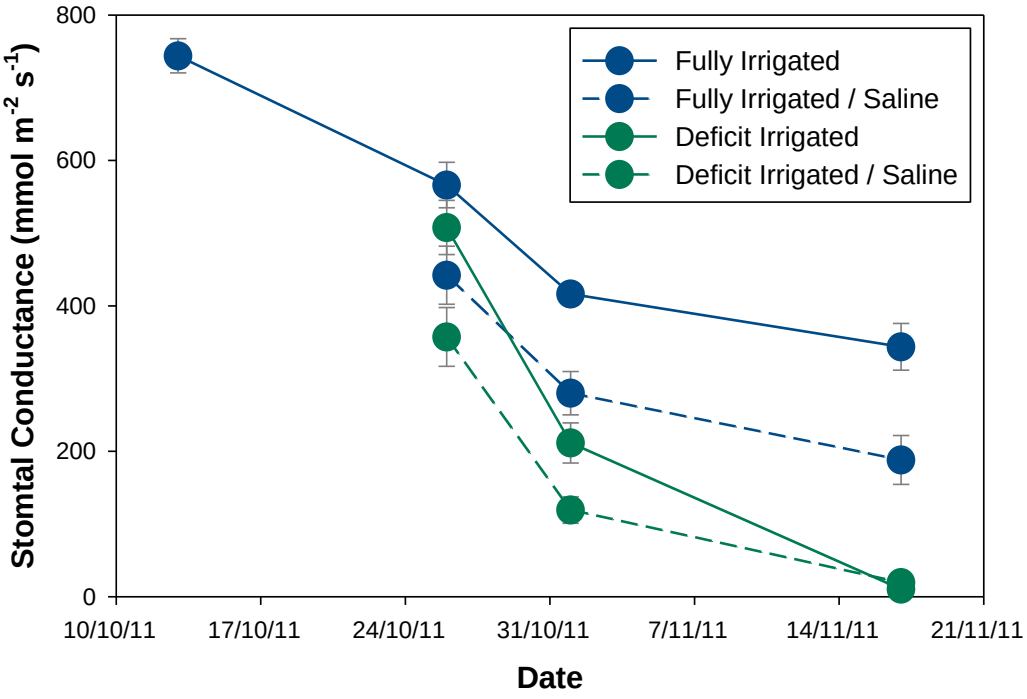


Figure 26. Mean stomatal conductance of well watered (blue) or deficit (green) irrigated Shiraz scions on four rootstocks or own roots with either fresh water (solid lines) or saline water (dashed lines). Points are means of 25 replicates ±SE.

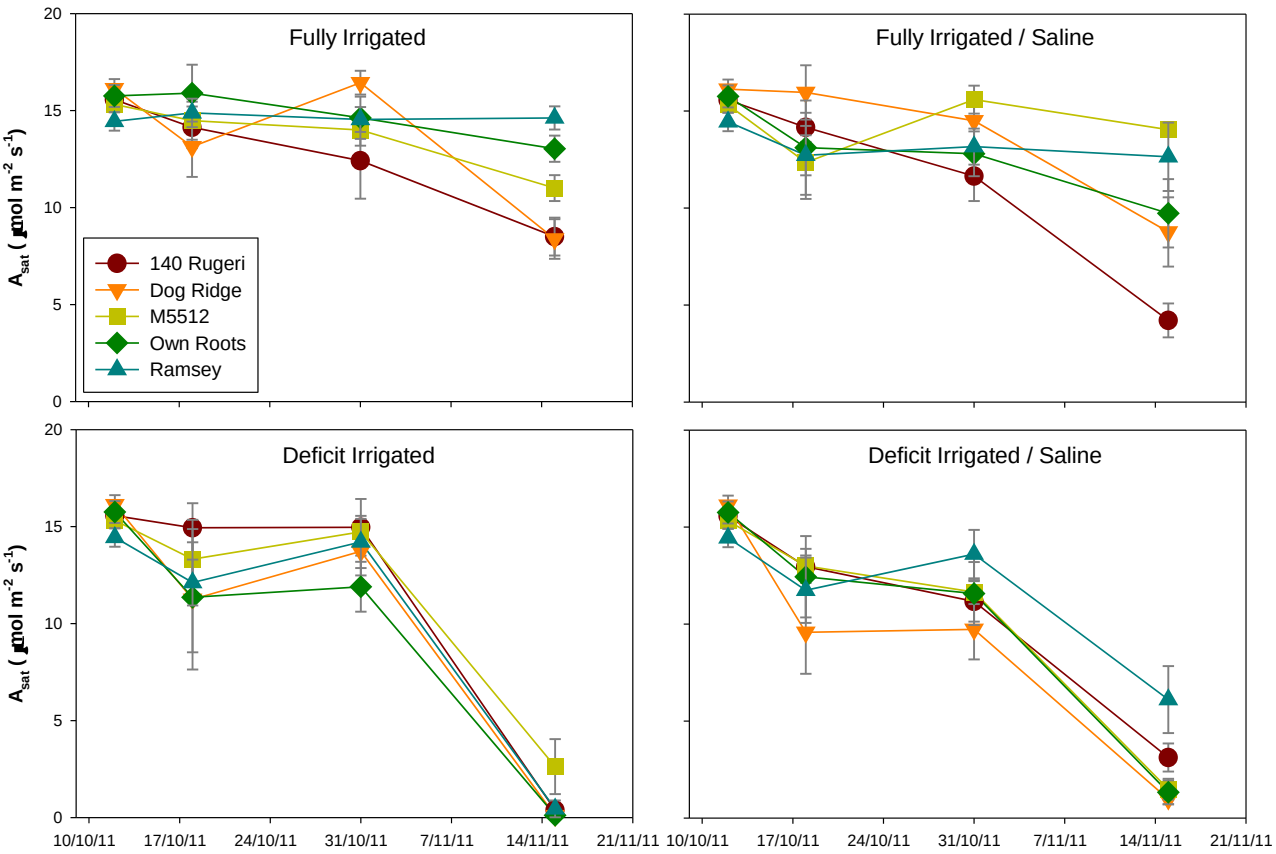


Figure 27. Assimilation under saturating light (A_{sat}) of Shiraz scions on four rootstocks and own roots when a) fully irrigated, b) fully irrigated with saline water, c) deficit irrigated, or d) deficit irrigated with saline water. Points are means of four to eight measurements ± SE.

As previously discussed (Chapter 3), changes in stomatal conductance will also have an impact on photosynthesis. Indeed, as conductance values measured by the gas exchange system (data not shown) were similar to those measured using porometry (Figures 24 & 26), effects of the treatments on conductance were also observed in the measurements of photosynthetic rates. A_{sat} of well irrigated vines supplied with saline water were similar, except for 140 Ruggeri, which had approximately half the control photosynthetic rate when saline irrigated (Figure 27). Deficit irrigated vines had similar rates, irrespective of whether saline irrigated or not for the first sets of measurements following the imposition of treatments, but at the end of the experiments photosynthetic rates were higher in the saline irrigated vines, albeit still much lower than the well watered treatments. The interaction between rootstock and treatment was limited, with Merbein 5512 having higher photosynthesis when highly drought stressed than the other rootstocks and Ramsey having higher rates under combined drought and salinity stress.

The response of stomata to drought is thought to be largely driven by abscisic acid (ABA) in the xylem stream produced by the root system as the soil environment dries (Davies and Zhang 1991) and an increase in leaf ABA content has been observed for grapevines growing under salt stress (Downton and Loveys 1981). Xylem sap was collected prior to the end of both glasshouse experiments and analysed for ABA content. In the first experiment the soil moisture had recovered somewhat from a low earlier in the experiment and this was reflected in the leaf physiology (see above) and in the ABA results (Table 2). Although xylem sap ABA in the deficit irrigated vines was significantly higher than controls for all the rootstocks it was below the values that might be expected to result in stomatal closure.

Table 2. ABA concentration of xylem sap from Shiraz scions on four rootstocks and fully (control) or deficit irrigated. Data are means of nine or more replicates ± SE.

Rootstock	**Control (ng ml^{-1})**		**Deficit (ng ml^{-1})**	
	Mean	**SE**	**Mean**	**SE**
110 Richter	43.2	6.1	92.8	13.4
140 Ruggeri	37.2	3.5	65.0	6.7
Ramsey	45.2	5.8	77.0	12.8
Schwarzmann	53.7	9.0	75.7	14.6

In contrast, the deficit irrigation treatments had a much larger impact on xylem sap ABA in experiment 2, with concentrations being two orders of magnitude higher in the non-saline deficit treatment than the control (Figure 28, DW vs WW). Xylem sap ABA concentrations in both saline treatments (WS & DS) were also higher than the control (note the log scale in Figure 28), but in the deficit saline treatment xylem sap ABA was much lower than in the non-saline deficit treatment. Differences in ABA between the rootstocks were evident in both deficit treatments, but not the well-watered saline treatment, the latter also being below the threshold (approx. 100 ng ml^{-1}, Collins *et al.* unpublished) expected to have an impact on stomatal conductance.

Although the stomatal conductance of the saline deficit irrigated vines was close to zero at the time the ABA samples were taken, the 140 Ruggeri and Ramsey rootstocks had significantly higher conductance than the other rootstocks in that treatment at the previous measurement date. These same vines also had lower xylem sap ABA at harvest, suggesting that the prior difference in conductance may indeed have been driven by differences in ABA. In the non-saline deficit treatment, the Merbein 5512 vines had a higher conductance than the other rootstocks and they also had lower ABA, half that of the other rootstocks (see below).

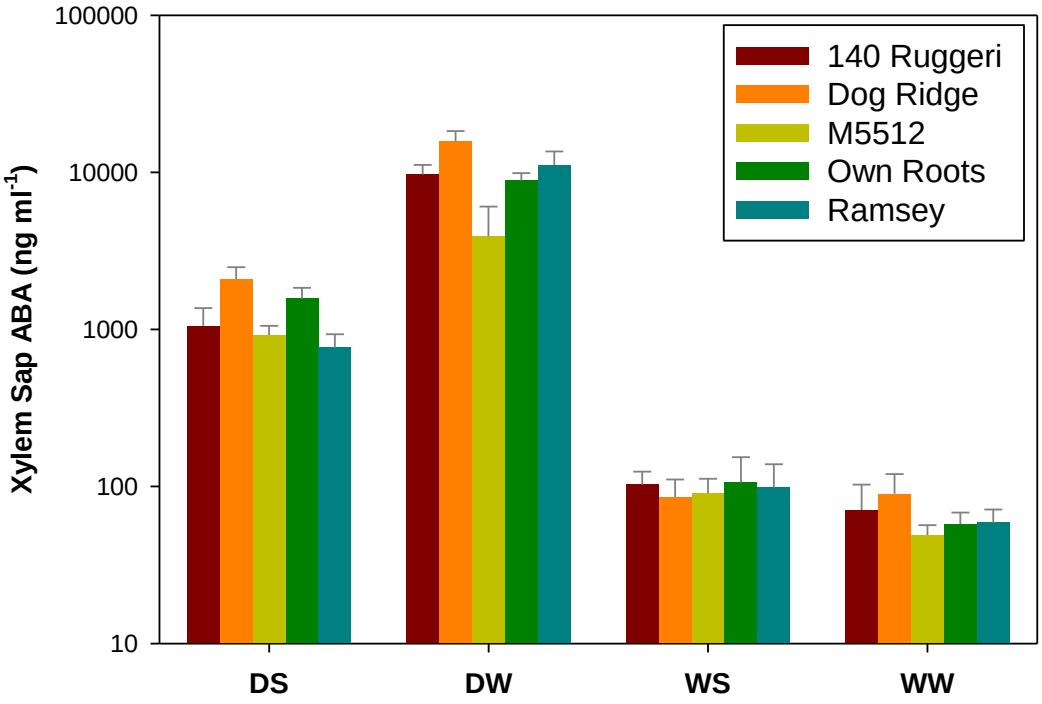


Figure 28. Xylem sap ABA concentration of Shiraz scions on four rootstocks and own roots that were fully-irrigated (WW), fully irrigated with saline water (WS), deficit irrigated (DW) or deficit irrigated with saline water (DS). Bars are means of five replicates + SE. Note that the y axis is on a log10 scale.

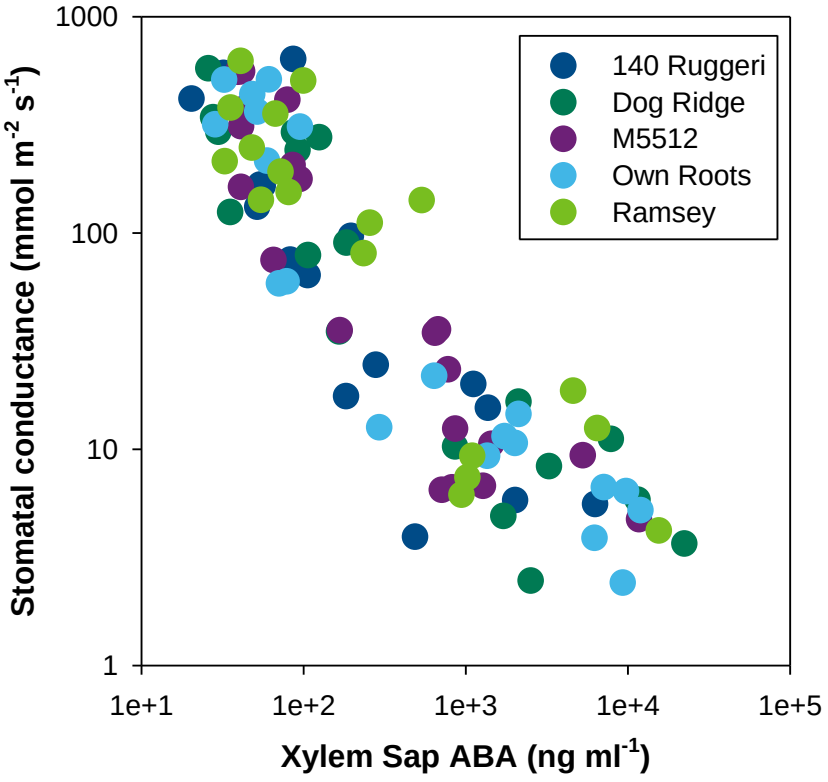


Figure 29. Stomatal conductance of individual replicates from all treatments as a function of petiole xylem sap ABA concentration in Shiraz scions on four rootstocks and own roots, subject to irrigation and saline treatments (not marked).

The ABA concentrations in the non-saline, deficit irrigated, vines were all extremely high, though at the equivalent of approximately 380 µmoles m^{-3}, not outside of previously published data (Dodd *et al.* 1996). These results were commensurate with the extremely low stomatal conductance values recorded at the same time, but may well have included apoplastic ABA. Notably, the Merbein 5512 vines also had lower ABA than Dog Ridge and own roots in the saline deficit treatment, despite there being no difference in conductance.

There was no evidence of differences in soil moisture between the Merbein 5512 and other vines, although there were only 1-2 vines of each rootstock instrumented per treatment. The soil moisture of the two monitored non-saline deficit Merbein 5512 vines was actually lower than the average of the other pots receiving the same irrigation at the time of ABA sampling, 6.7% (n=2) cf. 8.2% (n=4). If the differences in ABA between rootstocks were not due to differences in soil moisture it appears that the rootstocks are producing different ABA concentrations in response to the same soil moisture. Such differences have previously been inferred by Iacono *et al.* (1998), through observed differences in shoot ABA between different *Vitis* genotypes. If a rootstock is consistently transporting less, or more, ABA to a scion there is the possibility that ABA metabolism in the leaf may be down, or up-, regulated as a consequence. Figure 29 plots the xylem sap ABA data against the stomatal conductance results taken on the same day. Although this is a log:log plot and therefore visually reduces the impression of the variance present, it is clear that rootstock does not influence the relationship between the two parameters. Therefore, it is unlikely that scion response to ABA was strongly modulated by any long-term differences in ABA production between rootstocks.

Ultimately, the ability of a rootstock to maximise the drought and/or salinity tolerance of the grafted vine is through productivity. Whilst working with mature, fruiting, vines in a controlled environment is impractical, plant growth was used as a surrogate of productivity in these experiments. Further, if rootstock conferred vigour is present at an early stage after grafting these data could also be used to inform on any interaction between rootstock and drought and salinity effects on vigour.

Indeed, significant differences in vine growth between rootstock types were seen in both experiments. When well watered, in the first experiment, Ramsey, as in the field, had a higher growth rate than the other rootstocks (Figure 30) and in the second experiment Dog Ridge, also as seen in the field, had the highest growth rate (Figure 31a). In contrast, Ramsey had the lowest growth in the second experiment, not significantly different from Merbein 5512.

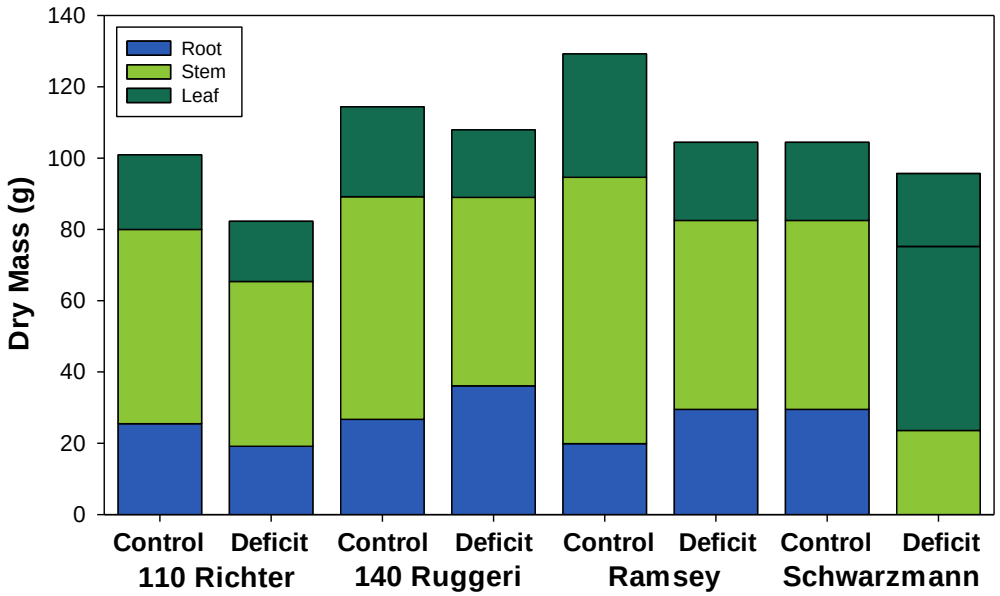


Figure 30. Whole vine dry weight, apportioned into leaf, stem and root weight of Shiraz scions on four rootstocks under full or deficit irrigation regimes in experiment 1. Data are means of nine replicates.

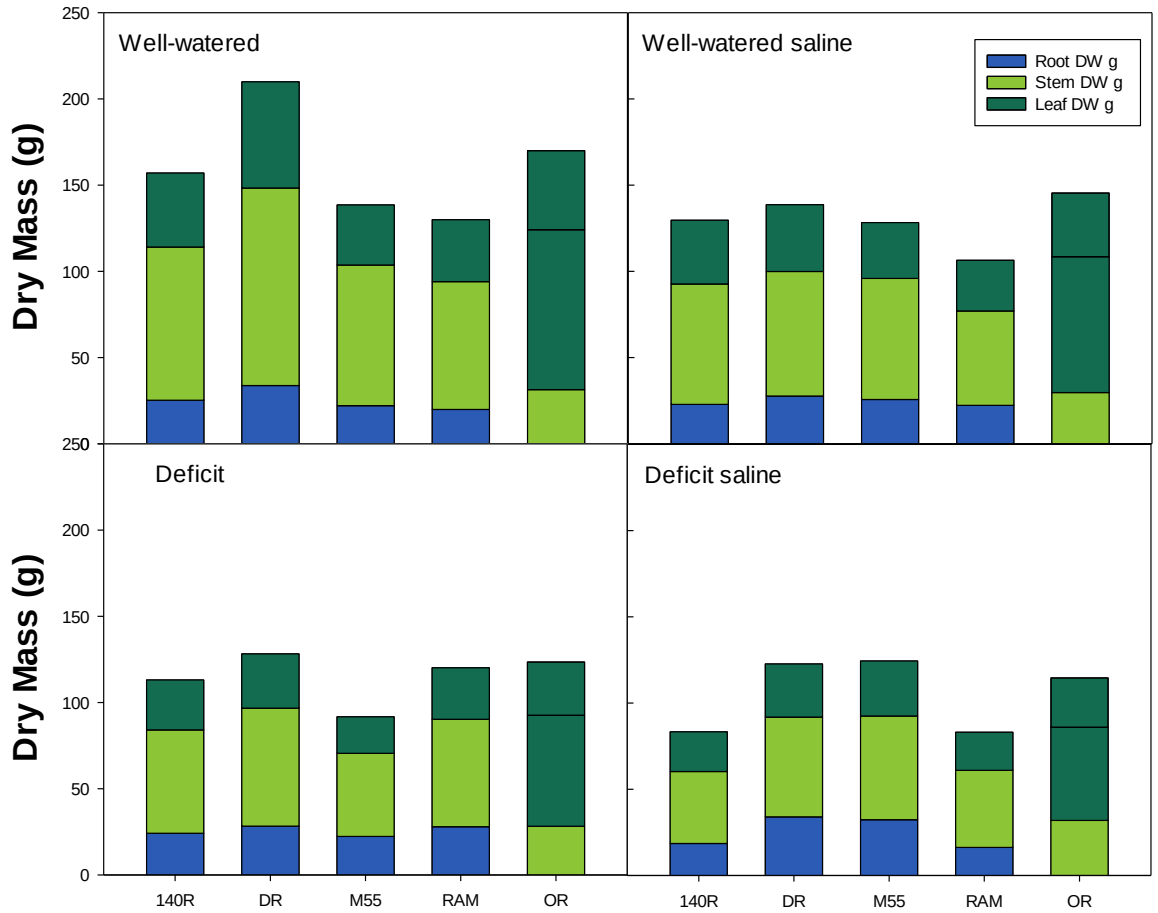


Figure 31. Whole vine dry weight, apportioned into leaf, stem and root weight of Shiraz scions on four rootstocks and own-roots under well-watered, well-watered saline, deficit and deficit saline irrigation regimes in experiment 2. Rootstocks used were 140 Ruggeri (140R), Dog Ridge (DR), Merbein 5512 (M55), Ramsey (RAM) and own-roots (OR). Data are means of six replicates.

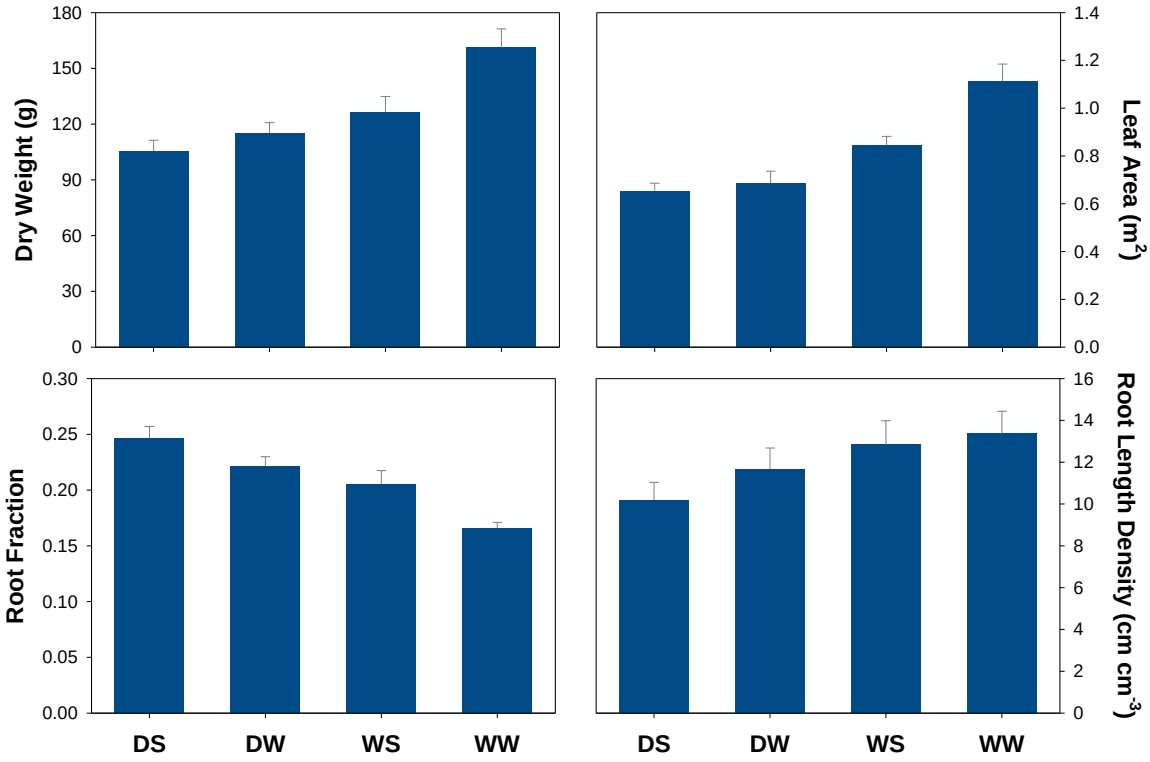


Figure 32. Mean growth responses of Shiraz scions on four rootstocks or own roots to full-irrigation (WW), full irrigation with saline water (WS), deficit irrigation (DW) or deficit irrigation with saline water (DS): a) whole plant dry weight, b) leaf area, c) root fraction and d) root length density. Bars are means of 25 replicates + SE.

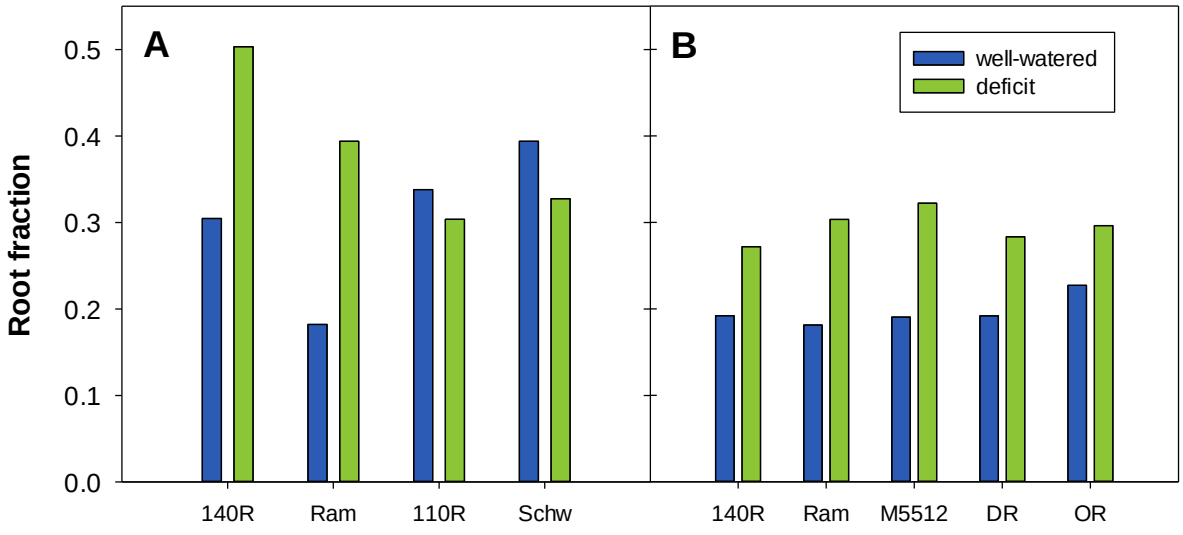


Figure 33. Root fractions of Shiraz scions grafted onto rootstocks in experiment 1 (A) and experiment 2 (B) under well-watered and deficit irrigation. Rootstocks were 140 Ruggeri (140R), Ramsey (Ram), 110 Richter (110R), Schwarzmann (Schw), Merbein 5512 (M5512), Dog Ridge (DR) and own-rooted (OR).

Despite the limited length of the experiments, being designed to examine physiological responses

rather than growth rate responses, the biomass of deficit irrigated vines was less than that of well-watered vines in all rootstocks and both experiments. The impact of deficit irrigation in experiment 1 was between 6% and 21% (140 Ruggeri and 110 Richter respectively), but between 8% and 39% (Ramsey and Dog Ridge respectively) in experiment 2 (Figure 31). However, the different irrigation regimes in the two experiments had inconsistent results for the two rootstocks common to both, with only a slight effect on 140 Ruggeri in experiment 1, but a much larger effect in experiment 2, but the reverse occurring in Ramsey.

Across all the rootstocks, saline irrigation also resulted in a measureable reduction in biomass, irrespective of irrigation level (Figure 32a). Both treatments generated an increase in root fraction (the proportion of plant biomass in the root system), with the effects of deficit and salt being additive (Figures 32 & 33). An increase in root fraction when field grown vines are drought stressed would have the effect of increasing access to soil moisture, relative to shoot biomass, that would otherwise have been unavailable and is a response commonly observed in woody plants (Kozlowski and Pallardy 2002), which has been directly linked with drought survival (Lopez *et al.* 2009).

There were also some differences between rootstocks in root responses. In experiment 1, Ramsey had a marginally higher absolute root biomass under deficit irrigation than when well-watered ($p=0.061$), whereas Schwarzmann had a significantly lower root biomass. In experiment 2, none of the differences in root biomass were significant, but root fraction was higher in all rootstocks in both experiments except 110 Richter and Schwarzmann in experiment 1.

Chapter 5

The role of hydraulic conductivity in driving rootstock conferred vigour

Background

It is well established in the literature (Bauerle *et al.* 2008; Gambetta *et al.* 2012; Gibberd *et al.* 2001) and from the results of our field and glasshouse experiments that significant differences in vigour, which occur between rootstocks, can have important implications for vine water-use and potentially their drought tolerance. However, the mechanism whereby rootstocks generate differences in vigour is still poorly understood. A role for hydraulic conductivity of the graft union, root system or trunk has been suggested, but the literature is largely limited, conflicting or inconclusive. Three glasshouse experiments were used to investigate the relationship between vine vigour and hydraulic conductivity, the two described in the previous chapter and a third experiment, designed to specifically address the involvement of hydraulics in rootstock conferred vigour. As plant size may have influenced the relationship between vigour and hydraulic conductivity in the first two experiments (see Chapter 4), the final experiment investigated how the dynamics of this relationship changed as vine size increased.

Methods and methods

The first two experiments are described in the previous chapter.

Experiment 3

The experiment was completed in the same glasshouse at the Waite Campus, Urrbrae, SA, with ambient light, daytime temperature limited to approximately 28°C and night-time temperature to approximately 20°C. Dormant canes of Dog Ridge and 1103 Paulsen were obtained from Riverland Vine Improvement Committee, and canes of M6262 and Shiraz from Cleggetts (Langhorne Creek) were all rooted in a propagator then chip-bud grafted with Shiraz buds in February 2012. The initial harvest took place when bud-break occurred in the newly grafted vines (5th March) in order to establish a baseline measurement for root hydraulic conductance. The vines were arranged in the glasshouse in a randomised block design with four blocks and eight replicates of each rootstock in each block. As the vines grew, three further harvests were made on 19th March, 16th April and 13th May 2012. All vines were watered daily to excess, maintaining a wet soil profile and fertigation occurred weekly. At each harvest eight vines per rootstock were harvested, with the leaves and roots of four vines being snap-frozen in liquid nitrogen for later aquaporin gene analysis. A representative root sample was taken for WinRhizo analysis of root length before the remaining leaves and roots were oven dried.

Hydraulic conductivity

An HPFM system (ICT International, Armidale, NSW) was used to measure whole root system hydraulic conductivity on at least four plants per rootstock/treatment combination in experiments 1 & 2. In experiment 3 four vines were used to measure whole root system root hydraulic conductivity before the whole plant harvest of leaves, stem and roots occurred. Measurements of root hydraulic conductivity were made immediately after the removal of the top of the vine, with each measurement being repeated in triplicate.

Aquaporin gene expression

Aquaporin activity was measured in well-watered and deficit water treatment vines during

experiment 2 according to the method in Vandeleur *et al.* (2009) with minor modifications to the primer sequences. Aquaporin activity was measured in five replicate samples per treatment.

Results and discussion

Root hydraulic conductance was examined as an explanatory variable for differences in growth rate in the well watered plants. Baurle *et al.* (2011) found that low vigour apple rootstocks had a lower hydraulic conductance than high vigour rootstocks when well watered and, in *Citrus*, Rodriguez-Gamir *et al* (2010) observed that scion canopy size, root hydraulic conductance and whole plant water use were all closely correlated. The latter study also found a link between hydraulic conductance and xylem vessel size, with vessel size itself having previously been linked with vigour (Trifilo *et al.* 2007, Tombesi *et al.* 2010).

In all of our experiments, whole root system hydraulic conductance was strongly correlated with plant size (experiments 1 & 2: Figure 34), as would be expected to enable the root system to function. Hydraulic conductance is typically expressed as specific to another plant parameter, commonly trunk cross-sectional area, canopy area or root length to elucidate differences between genotypes or environmental conditions. In our experiments, trunk cross-sectional area wasn't measured, but root length and total leaf area were determined. When the specific data (expressed on a root length or leaf area basis, Table 3) were plotted against plant size as individual vines there was little correlation (data not shown). However, when the data were expressed as a rootstock mean, strong correlations with plant dry mass were present for root length specific hydraulic conductance (Figure 35a) and, to a lesser extent leaf area specific hydraulic conductance (data not shown) in experiment 1, but not experiment 2 (Figure 35b).

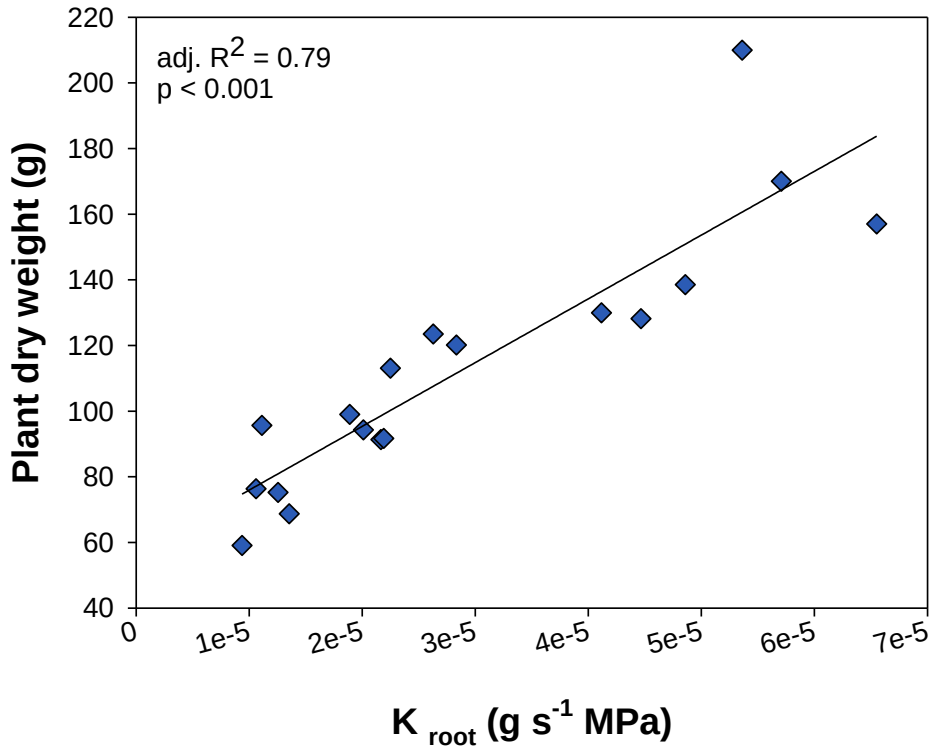


Figure 34. Relationship between plant dry weight and root hydraulic conductance. Combined data from experiments 1 & 2, each point is a mean of approximately four measurements.

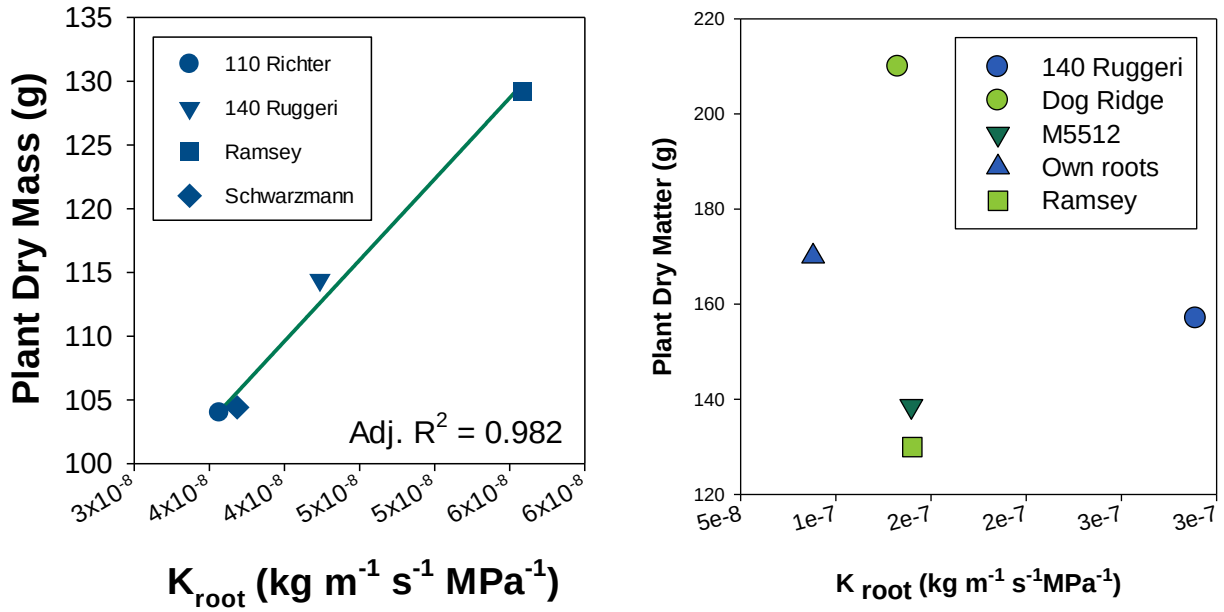


Figure 35. The relationship between whole plant mass and root length specific hydraulic conductivity in Shiraz scions on a) four rootstocks in experiment 1 and b) four rootstocks plus own roots in experiment 2. Each point is a mean of at least four well watered vines.

A possible reason for this is that both plant size and root hydraulic conductivity were significantly increased in experiment 2 compared to experiment 1 (Figure 31 cf Figure 30), so possibly the root volume was limited by pot-size or other confounding factors. To investigate this further, the relationship between vine size and root hydraulic conductance was examined as vines went from bud-burst through to 1-2 m canes in experiment 3.

Despite choosing three rootstocks that generally are known to have low (M6262), moderate (1103 Paulsen) and high (Dog Ridge) vigour the growth of both roots and shoots of the Shiraz grafted vines were very similar across the three month growing period (Figure 36). The exception to this was Dog Ridge at the final harvest which had significantly larger root mass than the other two rootstocks. Across all rootstocks, both whole root and root length specific hydraulic conductivity decreased as vine size increased (Fig. 37).

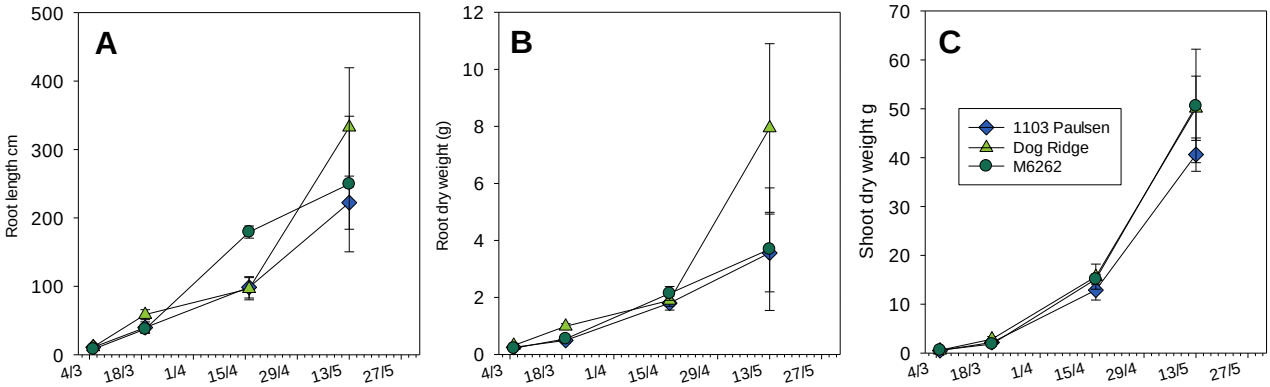


Figure 36. Growth of well-watered Shiraz vines grafted onto 1103 Paulsen, Dog Ridge and Merbein 6262 rootstocks (experiment 3). Variables measured include root length (A), root (B) and shoot dry weight (C). Values are means of four vines ±SE.

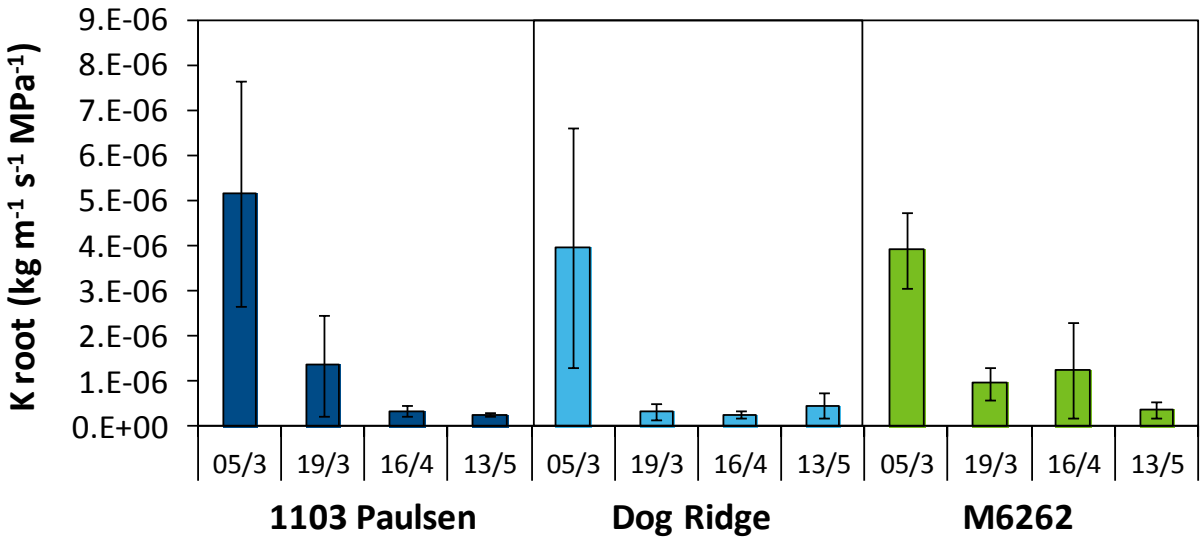


Figure 37. Changes in root length specific hydraulic conductance of well-watered Shiraz grafted onto three rootstocks, 1103 Paulsen, Dog Ridge and M6262 in experiment 3. Bars are means of four vines ±SE.

Due to the strong ontogenetic effect (reduction in specific hydraulic conductance with vine age), plant dry weight was highly negatively correlated with root hydraulic conductivity when expressed on a root length, root and shoot weight specific basis, when the whole experiment was examined together. The correlations were exponential and the coefficient of determination (R^2) was 0.88, 0.69 and 0.66 for shoot dry mass specific, root length specific and root dry mass specific hydraulic conductivity, respectively. This suggested that differences in hydraulic conductivity between rootstocks may not be a driver of plant size, however, when rootstock means at a single timepoint were plotted in the same way as for experiment 1 (Figure 35a) there were highly significant correlations (Figure 38). The end harvest was used as that was the only timepoint where there was a difference in total plant biomass.

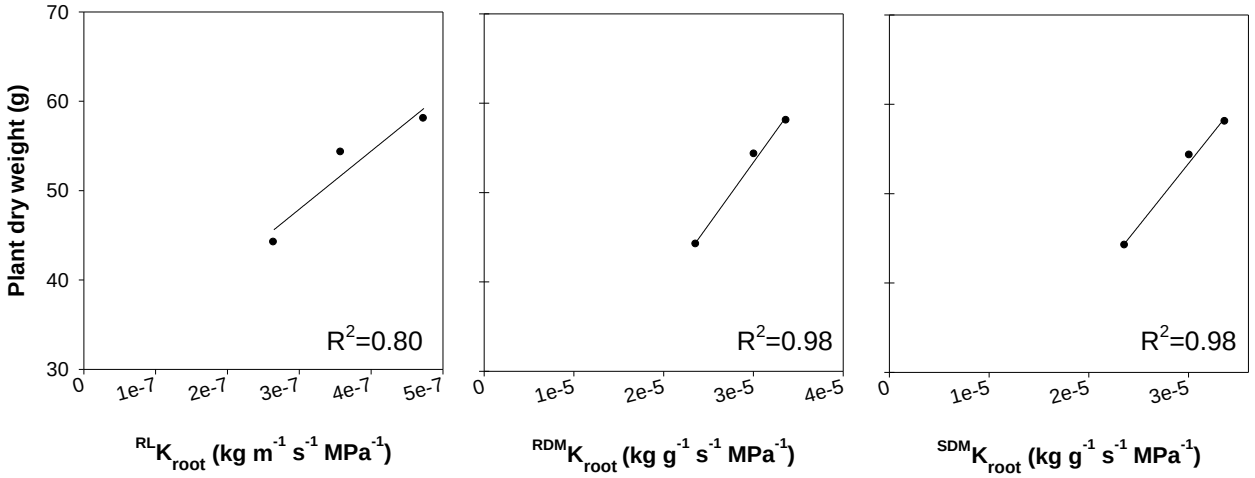


Figure 38. Relationship between whole plant mass and root hydraulic conductivity at the final harvest in experiment 3, expressed as a) root length specific ($^{RL}K_{root}$), b) root dry weight specific ($^{RDM}K_{root}$) and c) shoot dry weight specific ($^{SDM}K_{root}$) for three rootstocks. Values are the means of four vines.

The decrease in root length specific hydraulic conductivity with age was in contrast to the rootstock effect and suggests that the differences in hydraulic conductance between rootstocks were not due to differences in plant size, but, rather, that those differences between rootstocks in plant size were due to the differences in hydraulic conductance.

In the first two experiments abiotic stress factors that might affect rootstock conferred traits were included; water deficit in experiment 1 and both water deficit and salinity in experiment 2 (see Chapter 4). In both experiments water deficit led to large decreases in root hydraulic conductivity (Figures 39 and 40), the only instance where this wasn't a significant drop was for Dog Ridge in experiment 2. A decrease in hydraulic conductance in response to drought is often observed (e.g. Nobel and Cui 1992) and although partially attributable to differences in plant size as a result of the applied stress, the decrease in root hydraulic conductance was much larger than any effect on biomass, suggesting that the decrease was, at least partially, under metabolic control. In tobacco, reductions in hydraulic conductivity were matched by reductions in activity of genes encoding aquaporin proteins (Mahdieh *et al.* 2008) and Lovisolo *et al.* (2008) found that almost half of the root hydraulic conductance of *Vitis* hybrids under water stress was metabolically dependant. In contrast, root hydraulic conductivity doubled in vines on the 1103 Paulsen rootstock when exposed to drought in the field (Alsina *et al.* 2011) and this difference could not be explained by physical changes in the xylem structure or by hydraulic conductivity of individual root segments.

The difference between irrigation regimes in the relationship between root hydraulic conductivity and plant size indicated that 35% and 57% of the decrease in hydraulic conductivity, for experiments 1 & 2 respectively, was due to factors other than growth differences (Figure 41).

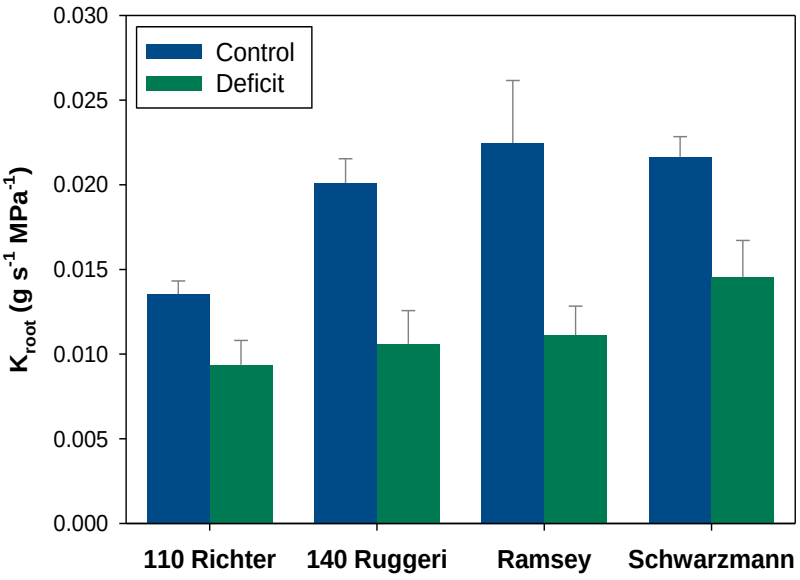


Figure 39. Whole root system hydraulic conductance of four rootstocks grown with full or deficit irrigation in Experiment 1. Bars are means of at least six replicates + SE.

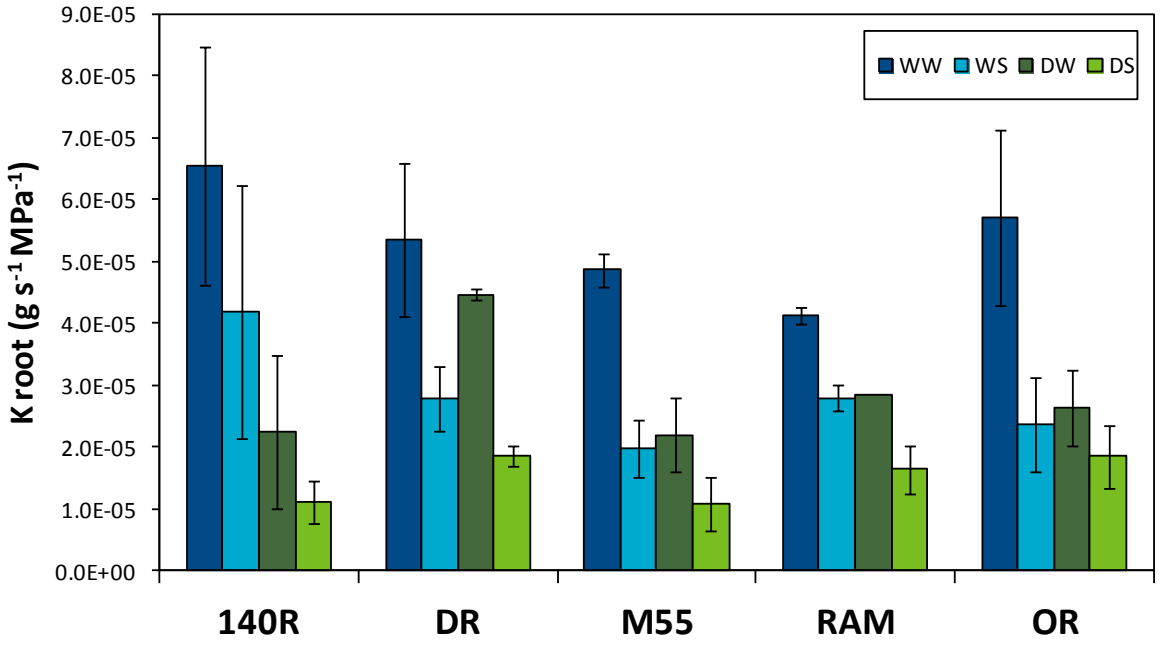


Figure 40. Whole root system hydraulic conductance of four rootstocks (140 Ruggeri, Dog Ridge, M5512, Ramsey) and own-rooted (OR) Shiraz grown with full (WW and WS) or deficit irrigation (DW, DS) in Experiment 2. Vines were irrigated with regular (WW, DW) or saline water (WS, DS). Bars are means of at least four replicates + SE.

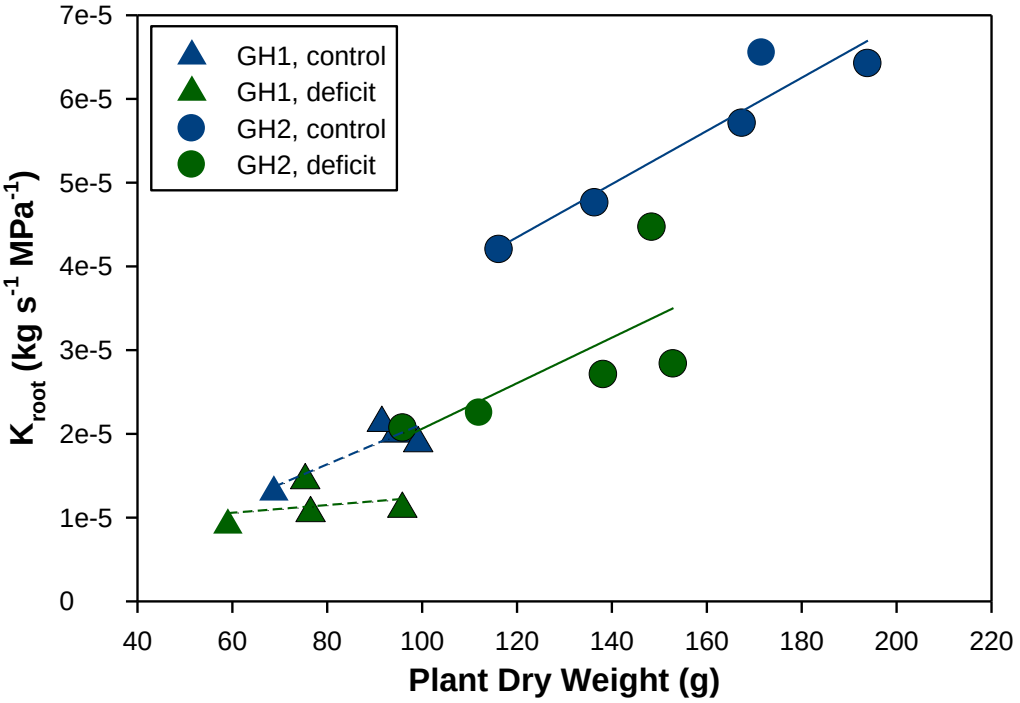


Figure 41. Root hydraulic conductance as a function of plant dry weight in experiments 1 & 2.

The effect of rootstock and/or water deficit on hydraulic conductance in this project could have been due to physiological changes such as aquaporin expression and cavitation or physical changes such as xylem structure or root system size/topology. Hydraulic conductance and canopy transpiration are highly coordinated (Meinzer and Grantz 1991) so the effect of water deficit on

leaf g_s and area could result in reduced hydraulic conductance, whether via the above mentioned or some other mechanism. Table 3 provides the root hydraulic conductance expressed per unit root length and per unit leaf area for experiment 1. Neither fully explains the depression seen in the deficit irrigated vines and this was also the case for deficit vines in experiment 2 (data not shown). In the case of the leaf area specific data, the relative effect of deficit irrigation was somewhat less, approximately 72% of the control compared with 60% of control on a whole root system basis. On a root length basis the change in relative effect was rootstock specific, with a larger difference between deficit and control in 140 Ruggeri and Ramsey, but no difference at all in Schwarzmann. Consequently, it appears that, in general, the effects of deficit irrigation on root hydraulic conductivity were not due to changes in growth following water deficit, but other factors such as aquaporin activity.

Table 3. Whole root system hydraulic conductance expressed per unit root length and per unit leaf area (Experiment 1). Data are means of at least six replicates ± SE.

Rootstock	**Root Length Specific (kg cm^{-1} s^{-1} MPa^{-1})**				**Leaf Area Specific (kg m^{-2} s^{-1} MPa^{-1})**			
	Control		**Deficit**		**Control**		**Deficit**	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
110 Richter	$3.6*10^{-8}$	$4.9*10^{-9}$	$2.4*10^{-8}$	$3.8*10^{-9}$	$2.6*10^{-5}$	$2.3*10^{-6}$	$2.2*10^{-5}$	$3.5*10^{-6}$
140 Ruggeri	$4.2*10^{-8}$	$6.0*10^{-9}$	$2.0*10^{-8}$	$3.8*10^{-9}$	$3.2*10^{-5}$	$2.9*10^{-6}$	$2.3*10^{-5}$	$4.0*10^{-6}$
Ramsey	$5.6*10^{-8}$	$7.5*10^{-9}$	$2.6*10^{-8}$	$5.6*10^{-9}$	$3.4*10^{-5}$	$6.9*10^{-6}$	$2.0*10^{-5}$	$3.3*10^{-6}$
Schwarzmann	$3.7*10^{-8}$	$3.9*10^{-9}$	$4.4*10^{-8}$	$8.0*10^{-9}$	$4.4*10^{-5}$	$3.2*10^{-6}$	$3.3*10^{-5}$	$6.5*10^{-6}$

There is strong experimental evidence that aquaporins are key drivers of plant water relations (Tyerman *et al.* 2002). Aquaporins can account for 35 to 80% of root hydraulic conductance (North *et al.* 2004) and are actively involved in recovery of root conductivity after water stress (Lovisolo *et al.* 2008). Under both well-watered and drought conditions, genes PIP1.1 and PIP2.2 were the most highly expressed across all rootstocks (Table 4). There were much larger increases in relative expression in these genes than the other PIP genes studied. Other studies have found that PIP2 aquaporin activity contributes more to water transport activity than PIP1 genes (Secchi *et al.* 2007) and in all rootstocks the expression of PIP2s were significantly higher than PIP1s. PIP1s are suggested to play a complementary role with PIP2s in water transport activity. According to observations by Vandeleur *et al.* (2009), grapevine PIP2.2 requires co-expression of PIP1.1 to be fully activated as a water channel. A similar observation has been made in other studies (Fetter *et al.* 2004).

Table 4: Impact of water-stress on aquaporin PIP gene activity for individual rootstocks. Data are means of five replicates (Experiment 2). Different letters indicate significant differences between well-watered (WW) and deficit water (DW) treatments as measured by ANOVA.

Rootstock	**PIP1.1**		**PIP1.4**		**PIP2.1**		**PIP2.2**		**PIP2.3**	
	WW	***DW***	***WW***	***DW***	***WW***	***DW***	***WW***	***DW***	***WW***	***DW***
M5512	28876^{a}	27939^{a}	1633^{a}	2061^{a}	2222^{a}	1656^{a}	41644^{a}	34786^{a}	3704^{a}	10425^{a}
Ramsey	26963^{a}	18728^{a}	1950^{a}	3175^{a}	1387^{a}	1067^{a}	48613^{a}	29915^{b}	8205^{a}	21113^{a}
Dog Ridge	20988^{a}	20390^{a}	917^{a}	1211^{a}	791^{a}	807^{a}	30306^{a}	33216^{a}	3493^{a}	11516^{a}
140 Ruggeri	15739^{a}	13826^{a}	1677^{a}	2099^{a}	810^{a}	1269^{a}	31530^{a}	25037^{a}	1323^{a}	5813^{a}

With the exception of PIP2.2 in Ramsey vines, aquaporin gene activity was not affected by water-stress, however there was a considerable amount of variability in expression levels between samples. In grapevines, a variable aquaporin response to water stress has been observed in previous studies (Vandeleur *et al.* 2009) with some grapevine scions (Chardonnay) up-regulating

PIP1 and PIP2 genes in response to water-stress and others (Grenache) showing no aquaporin response to application of water-deficit. In other grapevine aquaporin studies, over-expression of PIP2.4N gene enhanced water transport under well-watered conditions, but played a minor role under drought conditions, presumably because other signals such as ABA, take over the control of water flow (Perrone *et al.* 2012).

Aquaporin activity varied significantly between rootstocks with expression levels of PIP 1.4, PIP2.1, PIP2.2 and PIP2.3 being different and the expression level varying from 1.8 fold (PIP 2.2) to 6.2 fold (PIP 2.3) differences across the rootstocks (Table 5). Other studies have also found significant differences in aquaporin activity between grapevine rootstocks. Gambetta *et al.* (2012) found under well-watered conditions PIP gene activity varied across rootstocks that differed in vigour. Increased expression of several PIP genes was consistently associated with high vigour compared with lower levels of expression in smaller, low vigour rootstocks (Gambetta *et al.* 2012). Total leaf area increased with rootstock vigour, which in turn was positively correlated with fine root hydraulic conductivity and increased root surface area (Gambetta *et al.* 2012). The results suggest that the increased leaf area and transpiration requirement in high vigour vines was matched by increases in root hydraulic conductivity via increased aquaporin activity. This pattern of increased PIP expression in high vigour rootstocks was in contrast to our results.

Table 5: Range of expression of individual PIP (aquaporin) genes across rootstocks in Exp.2.

Gene	Rootstock effect	Range of expression
PIP1;1	NS	1.8 fold
PIP1;4	P = 0.000	2.4 fold
PIP2;1	p = 0.013	3.1 fold
PIP2;2	p = 0.051	1.8 fold
PIP2;3	p = 0.005	6.2 fold

The relationships between aquaporin activity and plant growth varied across the different PIP genes. Overall, PIP gene expression was inversely correlated with root hydraulic conductivity and in the case of PIP2.2 with plant size. For example, expression levels of PIP1.4 and PIP2.3 were negatively correlated with leaf area specific root hydraulic conductivity and PIP2.2 with plant dry weight (Fig. 42). High vigour plants with nearly twice the leaf area had the lowest levels of aquaporin activity and *vice versa.* Expression of PIP2.2 and PIP2.3 was also highly negatively correlated with root hydraulic conductivity with smaller vines having higher root hydraulic conductivity both generally and when expressed specifically as a function of root length (Fig. 43). The growth limitation observed in lower vigour rootstocks was associated with the up-regulation of both PIP1s and PIP2s potentially as a compensation for low root hydraulic conductivity and reduced root mass, possibly helping to alleviate limitations to whole plant growth. Studies of aquaporin gene activities in olives, using dwarfing and vigorous rootstocks, also found an up-regulation of PIP1s and PIP2s in dwarfing clones that had limited root growth (Lovisolo *et al.* 2007). In that study the higher expression of PIP genes in the lower vigour plants increased root specific conductance, partially compensating for smaller roots and preventing potential limitation to water transport.

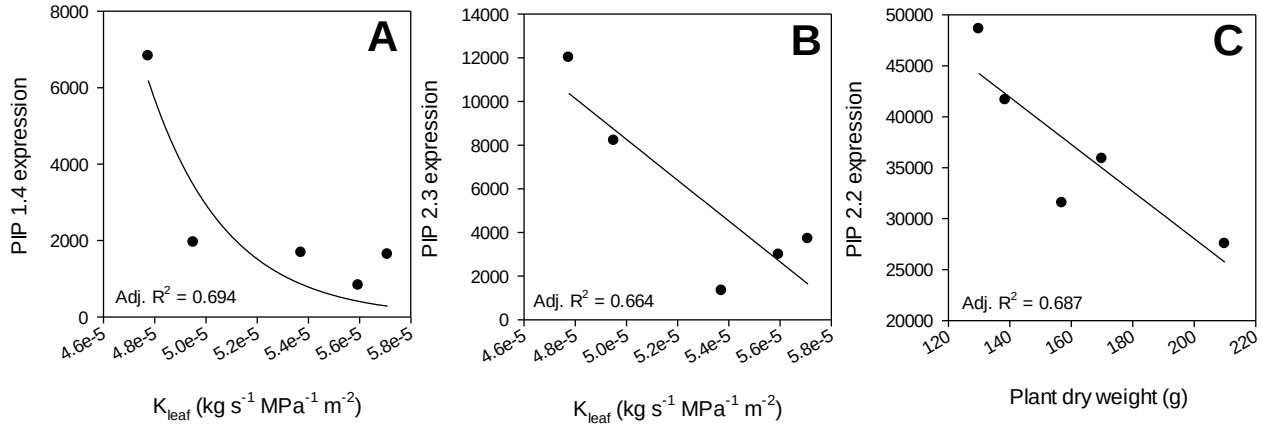


Figure 42. Relationship between individual aquaporin PIP gene activity and (A, B) leaf area specific hydraulic conductivity and (C) plant dry weight for well-watered Shiraz on own roots and four rootstocks (Experiment 2). Each point is a mean of five vines.

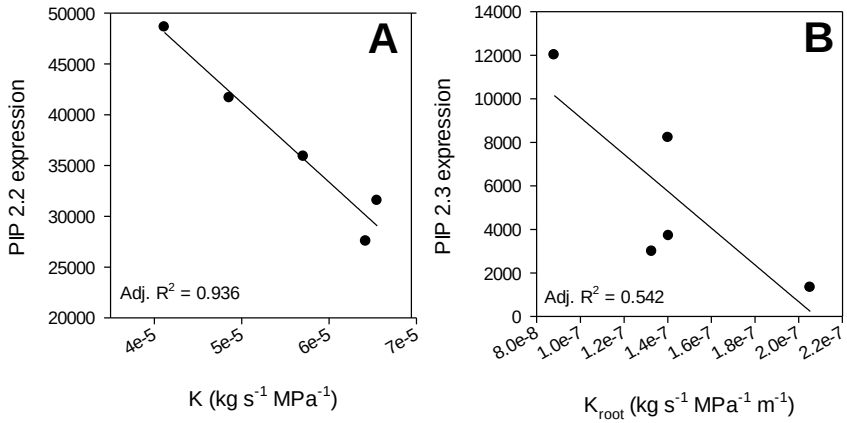


Figure 43. Relationship between (A) PIP 2.2 gene activity and root hydraulic conductivity and (B) PIP 2.3 gene activity and root length specific hydraulic conductivity for well-watered Shiraz on own roots and four rootstocks (Experiment 2). Each point is a mean of five vines.

Several studies in other woody perennials have observed that shoot growth is determined by the hydraulic conductance of the root (Lovisolo *et al.* 2007; Nardini *et al.* 2006) highlighting the importance of root aquaporin regulation in controlling whole plant growth. Of course, the hydraulic limitation that seems to be limiting growth in low vigour vines could also be due to other factors such as xylem vessel size and the compatibility of the graft union. The increased activity of PIP1 and PIP2 genes in roots of vines with low vigour may be a response mechanism to over-come limitations of this sort rather than a driving factor. It is hoped that the future results of the aquaporin analysis for experiment 3 will further inform this discussion.

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Chapter 6

Conclusions and recommendations

The relationship between vine vigour, water use and water use efficiency

Using yield per unit leaf area as a surrogate of harvest index, Figure 11 demonstrated a difference of 60% between rootstocks in the amount of crop that was produced per unit area of canopy. However, there was a yield penalty associated with the efficiency gain, with Merbein 5512, the rootstock with the highest yield per unit canopy, producing 21% less fruit over four seasons than Dog Ridge, the rootstock with the lowest yield per unit canopy. The vigour data (LAI and pruning weights) suggested that the seven rootstocks studied in the field fell into three broad vigour groupings. Figure 44 provides a summary of the field data based on those groupings. Yield and water use both increased with increasing vigour, but the increase in yield was much less than the increase in water use, consequently water use efficiency (crop water use index) decreased with increasing vigour.

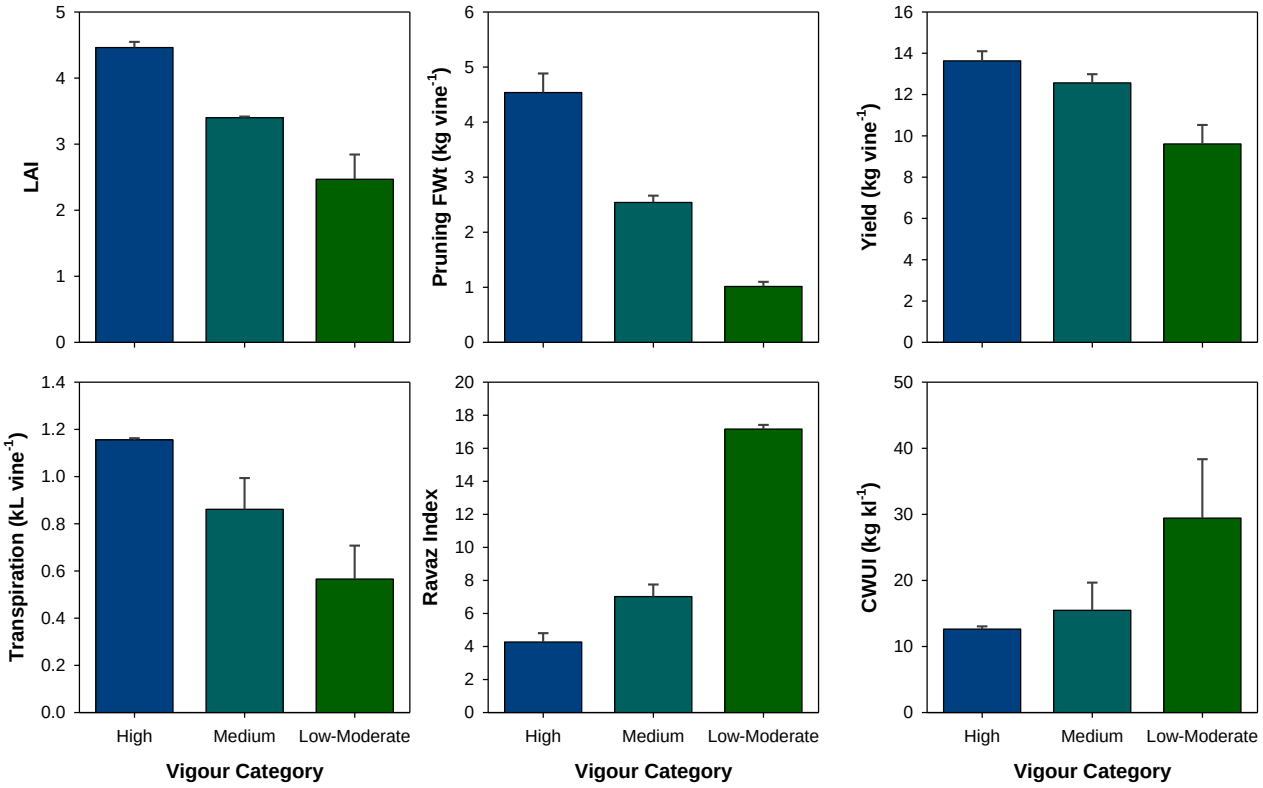


Figure 44. Summary of results from mature Shiraz scions grafted onto seven rootstocks at the CSIRO Sunraysia field station. Each bar is a mean of 2-3 rootstocks, with each rootstock datum being a mean of 3-4 seasons.

The potential for improvements in winegrape water use efficiency through the judicious use of low to moderate vigour rootstocks is evident. It was outside the scope of this project, but by altering planting densities in conjunction with appropriate irrigation schedules based on the reduced water use of the low to moderate vigour rootstocks, it is entirely possible that yield per hectare may be maintained whilst continuing to reduce irrigation water use. We therefore recommend that trials to optimise, not only water use efficiency, but yield per unit of water applied per unit of land should be undertaken using low-moderate vigour rootstocks in higher planting densities. Further,

as water use efficiency and vigour are clearly strongly linked, work to elucidate the mechanism whereby rootstocks confer vigour is essential for future breeding programs to provide a range of material for high and low production scenarios.

Assessment of rootstock drought tolerance

Field experiments using deficit irrigation in unrestricted rooting volumes do not distinguish between drought tolerance, the ability to survive reduced water availability, and drought avoidance, the ability to find an alternative water source. In contrast, glasshouse experiments using deficit irrigation typically do not allow drought avoidance to occur due to the spatial restrictions of the pot environment and thus test drought tolerance only. The mature field trial utilised in this project was not suitable to apply both low and high irrigation scenarios at the same time, due to the number of vines available of each rootstock. However, during the final two seasons, irrigation was intentionally applied at below ET_c (Table 1) and below the local norm for a mature Shiraz block, for example around 7 to 7.5 ML ha^{-1} (Stevens *et al.* 2010). Consequently, the results from the 2011/12 and 2012/13 seasons represent production under a water limitation. Unfortunately the preceding season, 2010/11, was the wettest season on record for the Sunraysia district, with a single rain event producing more than twice the seasonal average rainfall, and the season before that, 2009/10, was also the season that the irrigation method was changed from overhead sprinkler to drip, so any conclusions about differential rootstock responses to water limitation would be confounded by other factors.

The more detailed assessment of rootstock drought tolerance was provided by the glasshouse experiments, two of which examined both the response to drought and the underlying physiology. The range of rootstocks available for examination was limited, with only one of the Merbein series rootstocks being used in one of the experiments that included a deficit irrigation treatment. However, six different rootstocks were utilised across the two experiments, together with own rooted vines, and two common rootstocks were utilised in both experiments to provide continuity. These experiments demonstrated that rootstocks can impart significant variation to a range of the responses that occur during a period of water deficit. The response of the two common rootstocks varied between the experiments, particularly in growth, suggesting that the severity of drought (the first experiment being a milder water stress) may also interact with the different responses imparted by rootstock choice.

The results of the Merbein 5512 rootstock in the glasshouse, which had the highest yield per unit canopy area and the highest CWUI in the field, were of particular interest as ABA production in response to a soil water deficit was reduced compared with the other rootstocks and consequently stomatal conductance was higher (Figure 28). This did not appear to be due to differences in soil moisture and was the opposite response to what might be expected from a drought tolerant vine as it would potentially lead to greater water loss per unit area of leaf. However, it does demonstrate fundamental differences in physiology between rootstocks that could potentially be exploited by specific irrigation regimes.

Inter-relationships between rootstock CWUI, drought tolerance and 'salt tolerance'

The water use efficiency of Merbein 5512 in the field under limited irrigation and the maintenance of leaf function under water deficit of the same rootstock in the glasshouse, suggested that CWUI and drought tolerance could be conferred by the same rootstock. However, the field situation represented a mild stress at most, whereas the second glasshouse experiment was much more severe. Salinity tolerance was also examined in the latter experiment and, in terms of minimum impact on growth, Merbein 5512 performed better than any of the other rootstocks examined.

Furthermore, the combined drought and salinity treatment had no greater impact on Merbein 5512 than either stress alone. Consequently, there is potential for a rootstock to be both drought and salt tolerant.

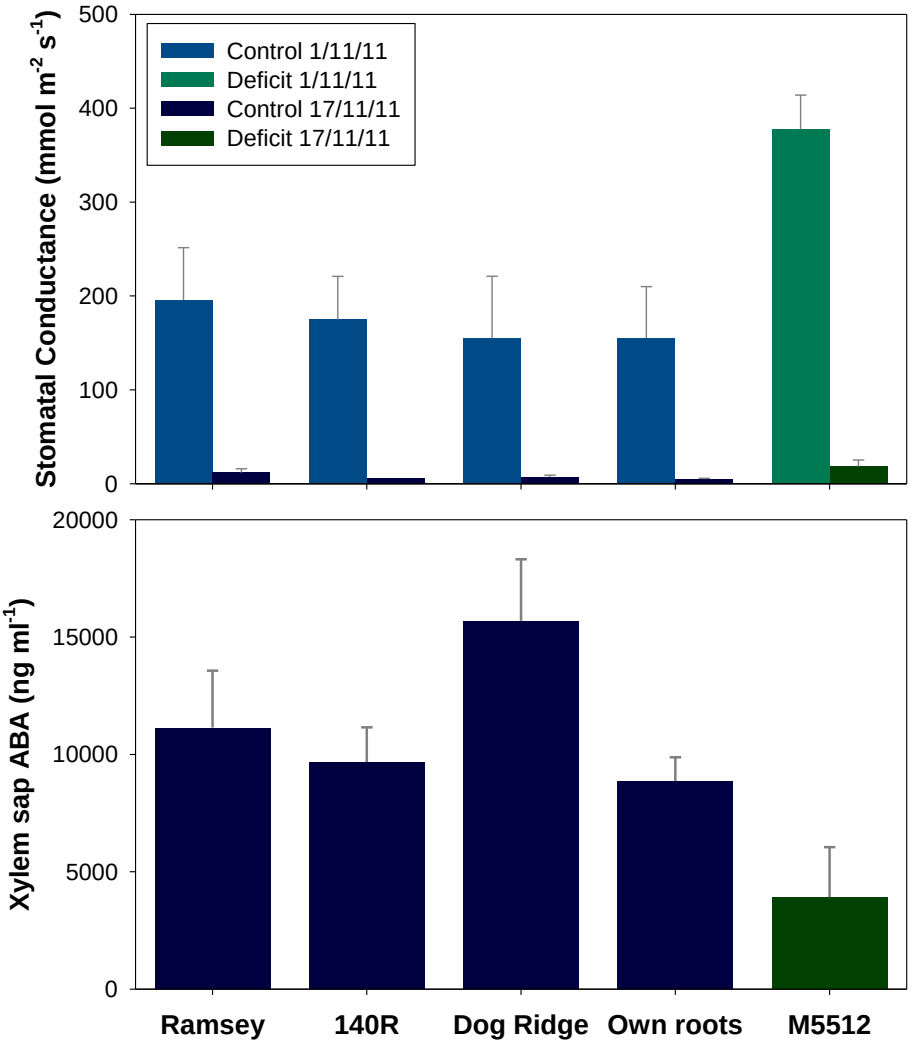


Figure 45. Stomatal conductance during drought stress and at harvest (a) and xylem sap ABA concentration at plant harvest (b) of Shiraz scions on four rootstocks and own roots. Bars are means of at least five replicates + SE.

Further work to determine the breadth of response to combined abiotic stresses amongst a wide range of rootstock genotypes would be valuable in confirming whether these traits are independent.

Conclusions

Rootstocks impart a significant range of drought tolerance, salt tolerance and vigour. The physiology underlying these relationships is starting to be understood and such understanding is expected to be further advanced in future projects. In summary:

- Yield has a positive relationship with canopy area, but the yield gain becomes smaller as canopy size gets larger.
- Yield/canopy area is higher in low to moderate vigour rootstocks than higher vigour

rootstocks.

- Crop water use index (an indicator of water use efficiency) is higher in low to moderate vigour rootstocks.
- Scion response to ABA is not strongly modulated by differences in rootstock ABA production.
- Rootstock conferred vigour is highly and positively correlated with root length specific hydraulic conductivity in a glasshouse environment.
- Aquaporin encoding genes are more highly expressed in rootstocks that limit vigour, suggesting a physical mechanism for vigour control.
- There is potential for a rootstock to be both drought and salt tolerant.

Appendix 1

Project aims and performance targets

Outputs and Activities 2009-10

	Output	Target Date	Activities
a	Field performance of vines grafted on targeted rootstocks and managed under deficit irrigation determined	30/06/2010	• Irrigation treatments (well watered and deficit) imposed • Maximum canopy size measured • Berry samples collected at harvest for berry size and basic fruit composition (TSS, pH, acidity, K, Na, Cl) • Yield and its components determined at harvest • Pruning weights measured • Surrogate for harvest index determined
b	Effect of rootstock on scion physiology determined for field vines managed under deficit irrigation	30/06/2010	• Soil moisture monitoring equipment installed and utilised over the season. • Transpiration determined using sap flow techniques • Basic physiological parameters (eg. assimilation, stomatal conductance) measured at key phenological stages • Samples collected from various plant tissues for carbohydrate analyses. • Leaf samples collected for stable isotope and Na, Cl analyses
c	Relationships between yield, harvest index, crop water use index, drought and salt tolerance across targeted rootstocks established	30/06/2010	• Field data collated and analyses implemented from season 1 • Preliminary relationships between key parameters determined
d	Drought tolerance of targeted rootstock genotypes determined under glasshouse conditions	30/06/2010	• Intensive glasshouse pot trials established with a range of rootstock genotypes with varying levels of drought stress • Basic physiological parameters (eg. water use, assimilation, stomatal conductance) measured • Vines harvested to assess biomass, root development and architecture • Samples collected from various plant parts for carbohydrate and stable isotope analyses

Outputs and Activities 2010-11

	Output	Target Date	Activities
a	Field performance of vines grafted on targeted rootstocks and managed under deficit irrigation determined	30/06/2011	• Irrigation treatments (well watered and deficit) retained • Maximum canopy size measured • Berry samples collected at harvest for berry size and basic fruit composition (TSS, pH, acidity, Na, Cl) • Yield and its components determined at harvest • Pruning weights measured • Surrogate for harvest index determined
b	Effect of rootstock on scion physiology determined for field vines managed under deficit irrigation	30/06/2011	• Soil moisture monitored over the season • Transpiration determined using sap flow techniques • Basic physiological parameters (eg. assimilation, stomatal conductance) measured at key phenological stages • Samples collected from various plant tissues for carbohydrate analyses. Analyses completed for season 1 samples. • Leaf samples collected for stable isotope and Na, Cl analyses. Analyses completed for season 1 samples.
c	Relationships between yield, harvest index, crop water use index, drought and salt tolerance across targeted rootstocks established	30/06/2011	• Data collated and analysed from seasons 1 and 2 • Relationships between key parameters determined
d	Drought (and salt) tolerance of targeted rootstock genotypes determined under glasshouse conditions	30/06/2011	• Intensive glasshouse pot trials established with a range of rootstock genotypes with varying levels of drought and salt stress. Treatments/techniques modified based on results from season 1. • Basic physiological parameters (eg. water use, assimilation, stomatal conductance) measured • Vines harvested to assess biomass, root development and architecture • Samples collected from various plant parts for carbohydrate, Na, Cl and stable isotope analyses. Analyses from year 1 completed.
e	Preliminary links between field and glasshouse performance established	30/06/2011	• Field and glasshouse data used in analyses to relationships for key variables

Outputs and Activities 2011-12

	Output	Target Date	Activities
a	Field performance of vines grafted on targeted rootstocks and managed under deficit irrigation determined	30/06/2012	• Irrigation treatments imposed and modified based on outputs from previous seasons (if deemed applicable). • Maximum canopy size measured • Berry samples collected at harvest berry size and basic fruit composition (TSS, pH, acidity, K, Na, Cl) • Yield and its components determined at harvest • Pruning weights measured • Surrogate for harvest index determined
b	Effect of rootstock on scion physiology determined for field vines managed under deficit irrigation	30/06/2012	• Soil moisture monitored over the season • Transpiration determined using sap flow techniques • Limited measurement of physiological parameters (eg. assimilation, stomatal conductance) at key phenological stages • Samples collected from various plant tissues for carbohydrate analyses and analysed • Leaf samples collected for Na, Cl and stable isotopes analysed
c	Targeted rootstock comparison under glasshouse conditions	30/12/2012	• Targeted glasshouse pot trial established with a range of rootstock genotypes. Treatments/techniques modified based on results from season 2. • Basic physiological parameters (eg. water use, assimilation, stomatal conductance) measured • Vines harvested to assess biomass, root development and architecture • Samples collected from various plant parts for carbohydrate, Na, Cl and stable isotope analyses. Analyses from year 1 completed.
d	Interim Report for DAFF Climate Change program	30/03/2012	• Interim Report outlining project progress including preliminary recommendation on most promising rootstock identified with drought tolerance, salt tolerance, water use efficiency characteristics submitted to the GWRDC by 30/03/12.

Outputs and Activities 2012-13

	Output	Target Date	Activities
a	Field performance of vines grafted on targeted rootstocks and managed under deficit irrigation determined	30/06/2013	• Irrigation treatments imposed and modified based on outputs from previous seasons (if deemed applicable). • Maximum canopy size measured • Berry samples collected at harvest berry size and basic fruit composition (TSS, pH, acidity, K, Na, Cl) • Yield and its components determined at harvest • Pruning weights measured • Surrogate for harvest index determined
b	Effect of rootstock on scion physiology determined for field vines managed under deficit irrigation	30/06/2013	• Soil moisture monitored over the season • Transpiration determined using sap flow techniques • Basic physiological parameters (eg. assimilation, stomatal conductance) measured at key phenological stages • Samples collected from various plant tissues for carbohydrate analyses and analysed • Leaf samples collected for Na, Cl and stable isotopes analysed
c	Root growth and architecture determined for targeted rootstocks managed under deficit irrigation	30/06/2013	• Field studies conducted to determine root architecture, distribution and biomass using soil pit techniques
d	Targeted rootstock comparisons under glasshouse conditions	30/6/2013	• Targeted glasshouse pot trial established with a range of rootstock genotypes. Treatments/techniques modified based on results from seasons 2 & 3. • Basic physiological parameters (eg. water use, assimilation, stomatal conductance) measured • Vines harvested to assess biomass, root development and architecture • Samples collected from various plant parts for carbohydrate, Na, Cl and stable isotope analyses. Analyses from year 1 completed.
e	Links between glasshouse and field studies established in relation to measurement of vigour, drought tolerance, water use efficiency and salt tolerance	30/6/2013	• Data analysed to establish links between field and glasshouse performance for key parameters.

f	Timeframe for adoption of best available combinations of drought tolerant, salt tolerant and water use efficient rootstocks developed for the post project period, 2013-2015.	30/6/2013	• Strategies for a structured program for release for wider evaluation under commercial conditions developed.
	Final report delivered to GWRDC.	30/6/2013	• All data analysed, collated and documented in final report. • Recommendations delivered to industry. • Future research opportunities/directions identified.

Appendix 2

Communication

Communication to industry and the scientific community has been an integral part of this project. Results have been presented at a range of industry and scientific meetings throughout the life of the project,.

Refereed publications

2012 Edwards, E.J., Collins, M.J., Boettcher, A., Clingeleffer, P.R. and Walker, R.R. The role of rootstocks in grapevine water use efficiency: impacts on transpiration, stomatal control and yield efficiency. *Acta Horticulturae.* In press.

Scientific Conferences

2011 Clingeleffer, P.R., Smith, B.P., Edwards, E.J., Collins, M.J., Morales, N., and Walker, R.R. Rootstocks, a tool to manipulate vine growth characteristics, fruit composition and wine quality attributes, water use efficiency and drought tolerance. 17th GiESCO, Asti, Italy, August 2011.

2012 Edwards, E.J., Collins, M.J., Clingeleffer, P.R., Walker, R.R. The role of rootstocks in grapevine water use efficiency: impacts on transpiration, stomatal control and yield efficiency. 7th International Symposium on Irrigation of Horticultural Crops, Geisenheim, Germany, July 2012.

2012 Collins, M.J., Edwards, E.J., Boettcher, A., Clingeleffer, P.R. and Walker, R.R. The role of root hydraulic conductivity in determining shoot growth of grapevines under well-watered and drought conditions. COMBIO, Adelaide, Australia, September 2012.

2013 Edwards, E.J., Boettcher, A., Collins, M.J., Clingeleffer, P.R. and Walker, R.R. The role of rootstock conferred vigour in determining crop water use efficiency. IX International Symposium on Grapevine Physiology and Biotechnology, La Serena, Chile, April 2013.

2013 Collins, M.J., Boettcher, A., Edwards, E.J., Clingeleffer, P.R. and Walker, R.R. The role of hydraulic conductivity in determining rootstock conferred vigour. IX International Symposium on Grapevine Physiology and Biotechnology, La Serena, Chile, April 2013.

2013 Collins, M.J., Edwards, E.J., Boettcher, A., Clingeleffer, P.R. and Walker, R.R. Linking rootstock effects on vigour, yield and water-use. 18th GiESCO, Porto, Portugal, July 2013.

2013 Edwards, E.J., Boettcher, A., Collins, M.J., Clingeleffer, P.R. and Walker, R.R. Rootstocks and water use efficiency I: the role of conferred vigour in determining crop water use index. 15th Australian Wine Industry Technical Conference, Sydney, Australia, July 2013.

2013 Collins, M.J., Edwards, E.J., Boettcher, A., Clingeleffer, P.R. and Walker, R.R. Rootstocks and water use efficiency II: the role of hydraulic conductivity in determining conferred vigour. 15th Australian Wine Industry Technical Conference, Sydney, Australia, July 2013.

Industry workshops and presentations

2012 Edwards, E.J., Collins, M.J., Loveys, B.R., Clingeleffer, P.R. and Walker, R.R. Root to shoot signals: unlocking the potential. HAL Showcase, Sydney 15/3/12.

2013 Collins, M.J., Edwards, E.J., Boettcher, A., Clingeleffer, P.R. and Walker, R.R. Linking rootstock

effects on vigour, yield and water-use. Workshop 40 ‘Rootstock to meet vineyard challenges’, 15th Australian Wine Industry Technical Conference, Sydney, Australia, 18/7/2013.

Appendix 3

Intellectual property

The information generated by this project will enter the public domain and will be accessible for future reference.

Appendix 4

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Appendix 5

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Appendix 6

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