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Wine Australia

New rootstocks for Australian conditions



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New rootstocks for Australian conditions

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Front cover: Canopy reponse to short term drought during ripening at Padthaway in 2017. Left: Maintenance of a full canopy. Right: Significant canopy loss with marginal leaf burn and over-exposure of bunches.

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1. Abstract

Field trial evaluation has identified three new rootstocks that maintain productivity in replant situations and produce fruit with acceptable fruit composition and are resistant to selected root knot nematode and phylloxera isolates. The efficacy of early rootstock screening techniques was demonstrated for traits including plant growth, conductance, leaf temperature and potassium measured in the nursery. As a first step for developing durable, resistant rootstocks, we have mapped new root knot nematode and phylloxera resistance loci in *V. cinerea* and identified molecular markers linked to these traits. Vigour, conferred by rootstocks was stable across sites and is under strong genetic control. Trials with advanced rootstock selections have been established in collaboration with industry in different regions with a range of varieties.

2. Executive summary

Currently, the wine industry is reliant on rootstocks bred and selected overseas for conditions that may not be the same as those in Australia. Breeding and selecting new locally adapted rootstocks offers the potential to have a positive impact on vine performance and wine quality while addressing the issues of sustainability and risk management.

A major component of this project was the evaluation of existing trials planted with advanced CSIRO rootstock selections. Three new rootstock selections (C114, C113 and C20) have been identified for release and adoption by industry. These new rootstocks maintain productivity in replant situations in hot climates, have performed well when irrigated with moderately saline water in Padthaway and are resistant to selected root knot nematode and phylloxera isolates. They produce fruit with acceptable fruit composition. It should be noted that 140 Ruggeri and 1103 Paulsen performed poorly in the hot climate replant situation. Furthermore, a number of rootstocks, still under evaluation, which maintain productivity under deficit irrigation and when irrigated with moderately saline water in Padthaway show potential. Other trials, still under evaluation, involve the salt tolerant C7 selection grafted with Pinot Gris and Shiraz at Padthaway. Except for its chloride exclusion capability, the growth and fruit composition of C7 has closely mimicked that of own roots, compared to 140 Ruggeri or Ramsey.

Significant rootstock x scion interactions and rootstock x region interactions have been found from field trials with Chardonnay, Cabernet Sauvignon and Shiraz in a hot climate and Shiraz in Padthaway. This indicates that a suite of rootstocks is required to meet industry requirements and that no single rootstock will be suited to all varieties and regions. The establishment of trials with advanced rootstock selections, in collaboration with regional groups and interested growers has been undertaken to extend industry engagement and provide further information on adaptability to region and specific varietal information to enhance knowledge and foster adoption.

To test the efficacy of previous screening procedures, vine growth characteristics, fruit composition and the mineral status of grafted vines under evaluation has been linked to traits measured in previous studies. Useful traits were trunk diameter, root architecture (i.e. root type, diameter, number and angle) and measurements in the nursery plants under well-watered and deficit conditions of growth traits (shoot length, pruning weight),

physiological traits (conductance, leaf temperature and transpiration efficiency) and leaf mineral elements, in particular potassium (K).

While the three new selections possess resistance to certain root knot nematode species and phylloxera genotypes, there is a pressing need to develop rootstocks with more durable resistance to a broad-spectrum of root knot nematode and phylloxera genotypes. As a first step in achieving this aim we have identified new sources of resistance and developed molecular markers that will ultimately be used to combine these new resistance traits with existing resistance loci in new rootstock breeding lines. In this project, we successfully mapped the *Vitis cinerea* C2-50 root knot nematode resistance locus, *MJR1*, which provides complete resistance to two major species of root knot nematode in Australia, *Meloidogyne javanica* and *M. arenaria*. The *V. cinerea* C2-50 phylloxera resistant locus, *RDV2*, was also mapped and molecular markers were identified. The *RDV2* locus provides good resistance to the two major phylloxera genotypes in Australia: G1 and G4.

The project has demonstrated large impacts of rootstock genotype on vine growth characteristics which have been linked to fruit and wine composition. In particular, strong genetic control of conferred vigour (pruning weight) by rootstock genotype was stable across different environments. In general, it was associated with negative effects on fruit composition (high pH, malate and K and in some instances with reduced berry anthocyanins and wine colour). With the exception of malate, which was closely linked to pruning weight, all other growth and fruit composition parameters were more impacted by environmental conditions rather than by rootstock. The project has also demonstrated that rootstocks with large berries and large bunches generally had poor fruit composition.

Over the course of the project there has been significant interaction with industry with presentations to industry groups, wine companies and growers, supported by tasting of wines produced from new rootstock selections. Mother vine plantings of new selections have been established to ensure that adoption is not limited by the supply of propagation material.

The valued input of Dr Kevin Powell (DEDJTR) who undertook the phylloxera screening, Marica Mazza, Bec Spencer and Peter Rogers (DEDJTR) and John Gledhill and Michael Coode (AWRI-WIC winemaking services) for small-scale wine making and the Cornell

University Biotechnology Resource Center (BRC) for genotype-by-sequencing and the financial support of Wine Australia and CSIRO is gratefully acknowledged.

3. **Background**

Major drivers for rootstock development from an Australian perspective (Walker and Clingeleffer, 2009 and references therein) are tolerance of root knot nematodes (Cousins and Walker, 2002, Smith et al. 2014) and phylloxera (Korosi et al., 2011), appropriate rootstock vigour, reduced potassium uptake to reduce grape juice pH and acid adjustment during winemaking (Mpelasoka et al., 2003, Walker and Clingeleffer 2009, 2016), and tolerance of abiotic stresses (Clingeleffer et al. 2011, Williams, 2010, Walker et al. 2014).

A 2012 review of the grapevine rootstock breeding program (project GWR 1009) concluded that rootstock breeding for Australian conditions is a valuable asset that should be supported (Whiting, 2012). Whiting (2012) pointed out that the large expansion of the Australian plantings in the 1990s will soon require replanting, with rootstocks playing an important role. Respondents to the survey associated with the 2012 review indicated that they would like more information on local performance of rootstocks and most identified issues with 'practically all rootstocks' (Whiting, 2012). Commercial experiences and research results have shown that the widely adopted high vigour, nematode tolerant rootstock varieties contribute to negative impacts on wine quality associated with high potassium uptake, high pH and malate levels, which require tartaric acid supplements in winemaking for pH adjustment, and reduced colour in berries and poor spectral properties in red wine (Walker and Clingeleffer 2016). This has produced a generally negative attitude by industry to rootstocks, which together with their higher cost has discouraged adoption.

Recently, it has been observed that local populations of root knot nematodes in Australia have caused expression of vine decline for rootstocks considered tolerant or resistant (Walker and Cox, 2011a, b). While it is clear that some rootstocks do not have sufficient resistance against populations of root knot nematodes in some regions of Australia, it remains to be discovered why these same rootstocks provide adequate resistance in other regions.

In addition to molecular markers and early screening methods, breeding efficiency can be increased by utilisation of estimates of trait heritability to calculate breeding values of rootstock genotypes. For instance, Smith et al. (2013) identified genotypes in the CSIRO

collection that have a greater probability of transmitting the trait of high root strike to their progeny.

The grapevine rootstock '1103 Paulsen' is now the most popular choice for new plantings of grapevines, however this rootstock has been observed to have problems in replant situations in some areas when root knot nematodes are present. This rootstock shares a lineage with most of the rootstocks used in Australia. There is a potential for mass failings of new plantings across Australia, as the cause of this problem is still not fully understood. A better understanding of this problem is warranted, with results incorporated into a targeted rootstock breeding program.

Rootstocks with low potassium uptake offer a solution to problems of high juice and wine pH and associated negative impacts on wine quality (Clingeleffer 1996, Mpelasoka et al., 2003, Walker and Clingeleffer 2009, 2016, Smith et al. 2014).

The most widely used rootstocks in Australia, which represent 88% of sales, each have at least one major viticultural flaw. These rootstocks varieties are '1103 Paulsen' (susceptible to Australian endemic root knot nematodes), 'Ramsey' (high vigour, poor fruit colour, and high juice pH), '101-14' (drought susceptible), and '140 Ruggeri' (low root strike and relatively poor graftability).

In the previous project CSP 0503, a *Vitis cinerea* accession that transmits resistance to root knot nematodes as a single dominant gene was identified. Glasshouse-based early screening methods for identification of grapevine genotypes with root knot nematode resistance and high mineral element discrimination, e.g. lower potassium and chloride uptake were developed. Additionally, deficit irrigation treatments were applied to a nursery block of rootstock genotypes grafted with Shiraz. Genotypes were selected that had minimal decrease in growth compared to well-watered controls (CSP 0503). These vines have been established in trials in Sunraysia, VIC and Padthaway, SA for evaluation under field conditions. These field trials were maintained in the previous project (CSP 1101) awaiting further evaluation to identify genotypes for commercial release, as well as to perform analysis of the efficacy of our early screening methods.

Additionally, previous projects CSP 0503 and CSP 1002 evaluated CSIRO rootstock

germplasm material and selected seven rootstocks for further evaluation as grafted vines in field trials. Project CSP 0503 selected five rootstocks that are near final decision for commercial release, pending analysis of field trials which were maintained in CSP 1101. Project CSP 1002 identified two genotypes with superior chloride and sodium exclusion ability. These genotypes have been established at field sites in Padthaway. Further analysis of all these field trials is required before a final decision can be made on which genotypes should be released for commercial use.

Currently, the industry is reliant on rootstocks bred and selected overseas for conditions that may not be the same as those in Australia. Breeding and selecting new locally adapted rootstocks offers the potential to have a positive impact on vine performance and wine quality while addressing the issues of sustainability and risk management. This project continued development of rapid screening techniques to select rootstocks with appropriate characteristics, and where gaps were identified, undertake targeted selection. It undertook the evaluation of trials established in previous projects with genotypes meeting key selection criteria with the aim to deliver new rootstocks for industry. Knowledge and techniques developed in previous rootstock projects were used to identify additional promising rootstock genotypes from CSIRO breeding lines at various stages of development. Genetic markers for nematode and phylloxera resistance were developed from existing test populations to assist in the early selection and screening of rootstock genotypes for field evaluation.

4. Project objectives and planned outputs

The primary objectives were:

- Strengthen interaction with industry on rootstock development, evaluation, and knowledge exchange to define pathways to delivery. We will enhance engagement of industry partners and facilitate an open exchange. Barriers to entry of new rootstocks will be identified and pathways for delivery formulated to overcome these impediments.
- Evaluation of existing field trials and assess the efficacy of rapid screening techniques. There are currently four rootstock field trials that are being maintained by funding from GWRDC (CSP 1101), as well as two trials recently established (CSP 1002). We will evaluate vine performance, fruit quality, and wine attributes. This analysis will be used to determine which advanced selections should be released commercially. Additionally, vine performance in these trials will be used to test the efficacy of early screening assay for resistance to root knot nematodes, potassium uptake, and salt exclusion. Further evaluation and development of screening methods is proposed in CSP 1302.
- Develop molecular markers for targeted selection of new rootstocks. A population of *Vitis cinerea* x *Vitis vinifera* has been identified as an important tool to develop markers for key rootstock traits. We will identify molecular markers associated with root knot nematode and phylloxera resistance. Based on our previous finding of the genetic control of phylloxera resistance, we believe that resistance is controlled by complimentary dominant genes in our mapping population. We will test this hypothesis and potentially identify additional molecular markers for phylloxera resistance.
- Establish more small-scale regional rootstock trials. A small group of rootstocks developed through previous projects and new genotypes that will be selected in this current project, will be tested in as many warm and cool viticultural areas regions as possible, encompassing a range of pest pressures, soil types, irrigation regimes, and abiotic stresses.

Planned Project Outputs

Outputs and Activities 2013–14

Year 1	Output	Target Date	Activities
a	Documented information on optimal pathways to deliver new rootstocks and enhance uptake.	1/11/2013	Establish industry reference group including grape growers and winemakers and other key industry members. Industry reference group engaged in workshop on “Future Rootstocks” to identify barriers to adoption and optimum pathways for delivery.
b	Data from field performance of rootstocks in season 2014.	1/06/2014	Collect data from 6 rootstock field trials in Sunraysia and Padthaway for harvest fruit quality and dormant vine pruning weight (vigour), and from at least 3 selected trials, the collection of tissues for analysis of salt exclusion, mineral nutrient status, and targeted small-scale winemaking for fruit from reduced irrigation treatments (at least 25 wines).
c	Genetic linkage map of V. cinerea X V. vinifera test population.	1/06/2014	Extract DNA from V. cinerea X V. vinifera test population (up to 96 lines) for Genotyping by Sequencing.
d	Rootstock nursery trials of advanced, near to release rootstock material.	1/06/2014	Propagation of at least 5 grapevine genotypes and establishment of nursery pre-release propagation trials with 2 selected nurseries.
e	At least 25 wines from fruit from experimental rootstock trials in season 2014.	1/06/2014	Fruit from select experimental rootstock and commercial rootstock varieties (at least 25 wines) made into wine for sensory and compositional analysis and assessment by industry.
f	Data on phylloxera and root knot nematode resistance of V. cinerea X V. vinifera test population obtained in glasshouse trials.	1/06/2014	A minimum of 80 individual genotypes from V. cinerea X V. vinifera test population propagated and phenotyped in glasshouse screens for traits of phylloxera and root knot nematode resistance in support of development of molecular markers of these traits.

Outputs and Activities 2014–15

Year 2	Output	Target Date	Activities
a	Regional rootstock trials of advanced, near to release rootstock material.	31/12/2014	New regional rootstock trials established with at least 4 near to release selections and other promising selections with industry collaborators.
b	Data from field performance of rootstocks in season 2015.	1/06/2015	Collect data from 6 rootstock field trials in Sunraysia and Padthaway for harvest fruit quality and dormant vine pruning weight (vigour), as well as from at least 3 selected trials the collection of tissues for analysis of salt exclusion, mineral nutrient status, and response to reduced irrigation treatment. Small-scale wine lots produced from selected experimental material from 2015 season (at least 25 wines). Sensory, compositional analysis and industry assessment of at 25 wines completed for wines made from selected experimental material from 2014 season (output 1e).
c	Data on the propagation characteristics of near to release selections.	1/06/2015	Collect and collate data on growth habit and propagation characteristics of six near-to-release experimental rootstock mothervines being grown under testing agreement by nurseries as well as from mothervines in the CSIRO germplasm collection (i.e. at 4 locations).
d	Communication of information about new grapevine rootstocks and technologies.	1/06/2015	Extend information and act on feedback from “Future Rootstocks” workshop to identify barriers to adoption of new rootstocks and viticultural practices and identify optimum delivery pathways.

e	Test populations phenotyped for phylloxera and root knot nematode resistance.	1/06/2015	A minimum of 80 and 100 individual genotypes from test population propagated and phenotyped in glasshouse screening for traits of phylloxera and root knot nematode resistance, respectively, in support of development of molecular markers of these traits.
f	Progress report including a review of the project and suggested variation.	30/06/2015	Discussion to be held with GWRDC to evaluate progress and modify outputs/activities where necessary for remainder of the project (Stop/go review point).

Outputs and Activities 2015–16

Year 3	Output	Target Date	Activities
a	Field trials of new rootstocks.	31/12/2015	Create experimental design for high density planting of at least 6 new rootstocks, organize material for propagation and establish field trials.
b	Data from field performance of rootstocks in season 2016.	30/06/2016	Collect data from 6 rootstock field trials in Sunraysia and Padthaway for harvest fruit quality and dormant vine pruning weight (vigour), as well as from at least 3 selected trials the collection of tissues for analysis of salt exclusion, mineral nutrient status, and response to reduced irrigation treatment. Small-scale wine lots produced from selected experimental material from 2016 season (at least 25 wines). Sensory, compositional analysis and industry assessment completed for wines made in 2015.
c	Decision based on results of field trials, glasshouse screening assays, and propagation traits made on commercial release of experimental rootstocks.	30/06/2016	Collect data on growth habit and propagation characteristics of six near to release experimental rootstock mothervines being grown under testing agreement by nurseries as well as mothervines in the CSIRO germplasm collection. Decisions for commercial release made based on results of field trials, glasshouse screening assays, and propagation traits. PBR applications and DUS trials established for selections identified for release.
d	Commercial availability of new rootstocks and methodologies.	30/06/2016	Develop infrastructure for delivery of new rootstocks including licensing arrangements and extend information on viticultural techniques with input from industry stakeholders.
e	Key genotypes phenotyped for phylloxera and root knot nematode resistance.	30/06/2016	A minimum of 100 individual genotypes from test population propagated and phenotyped in glasshouse screening for traits of phylloxera and root knot nematode resistance in support of development of molecular markers of these traits. DNA extraction and deep sequencing of all genotypes in the V. cinerea X V. vinifera test population completed.
f	Molecular markers associated with phylloxera and root knot nematode resistance.	30/06/2016	Compile phenotypic data of segregation of resistance to phylloxera and root knot nematodes for genetic mapping.

Outputs and Activities 2016–17

Year 4	Output	Target Date	Activities
a	Communication of recommendations for appropriate rootstock choices based on soil structure, scion variety, irrigation system, and average temperature of a region.	30/06/2017	Analyse data from all rootstock trials to identify appropriate rootstock genotypes for a range of scenarios based on soil structure, scion variety, irrigation system, and average temperature of a region. Sensory, compositional analysis and industry assessment completed for wines made in 2016.
b	Molecular markers identified for resistance to phylloxera and root knot nematodes.	30/06/2017	Integrate compiled phenotypic data of segregation of resistance to phylloxera and root knot nematodes with genotype x sequencing information to identify markers linked to nematode and phylloxera resistance.
c	New screening methods for rapid selection of rootstocks with complex traits of interest including resistance to phylloxera and nematodes.	30/06/2017	Analyse data from grapevine rootstock field trials on performance of vines selected through best available early screening methods for drought response, mineral nutrient uptake and salt exclusion to determine the efficacy of early screening methods.
d	Rootstock germplasm containing selections with desirable traits.	30/06/2017	Marker assisted selection of germplasm for pyramiding of rootstock traits.
e	Mothervines of new promising rootstock genotypes.	30/06/2017	Evaluation of rootstock trials to make final round of selection of promising genotypes.

5. Method

1. Rootstock trial evaluation

The performance of new rootstock genotypes, established in replicated trials in previous projects (CSP 0503) was assessed in this study. The information was used to identify new rootstocks with potential for release to industry, identify new rootstocks for further evaluation and to verify early screening methods, also undertaken in previous projects.

a) Trials established with 'near to release' selections included Chardonnay (clone I10V5), Cabernet Sauvignon (clone 22-4) and Shiraz (clone PT23) grafted on 26 new rootstock genotypes and three commercial rootstocks (1103 Paulsen, 140 Ruggeri, and Ramsey), planted at CSIRO Irymple in 2005 in a randomised block design with five replicates. The new rootstock genotypes had been selected for low potassium uptake of ungrafted vines, high rate of root strike and grafting success, and moderate to high vigour of ungrafted vines. The vines, trained on a two-wire vertical trellis as a quadri-lateral cordon system were hand spur-pruned, until conversion to simulated mechanical hedging in winter 2016. A similar Shiraz (commercial clone) trial was also established in a commercial vineyard in Padthaway in 2009 with the same suite of new rootstock genotypes with the commercial rootstocks, 101-14 Mgt, 140 Ruggeri, 1103 Paulsen, and Ramsey as controls in a replicated block design with three replicates. The vines, trained on a 0.9 m T-trellis were hand spur-pruned. First crops were produced in 2012. In this project, attention was focussed on five of the 26 rootstock genotypes, selected for their performance in the previous project (CSP 0503) and their root knot nematode and phylloxera resistance to selected isolates.

b) A Shiraz (clone PT23) rootstock trial was established in November 2010 to evaluate the field performance of 55 of the most promising drought tolerant selected, under nursery conditions, from 310 genotypes in the previous project (CSP 0503). In addition to the 55 rootstock genotypes grafted with Shiraz (clone PT23), six commercial rootstocks (140 Ruggeri, 1103 Paulsen, Merbein 5489Φ, Merbein 5512Φ and Merbein 6262Φ and Schwarzman) and own rooted Shiraz were included. Vines were arranged as single vine replicates in a randomised complete block design in four adjoining blocks. Seventeen genotypes were selected on the basis of high rate of root strike, vigour potential, potassium uptake

and performance in the rootstock nursery under a low irrigation treatment and nematode resistance. The remaining 37 genotypes were selected from a single family (F86) to evaluate the genetic component of drought tolerance. Two of the four blocks were irrigated with approximately 5.5 ML/ha (control) with the other two blocks receiving 2.5 ML/ha (deficit). The vines, trained on a two-wire vertical trellis as a quadri-lateral cordon system were hand spur-pruned, until conversion to simulated mechanical hedging in winter 2016. Leaf condition based on leaf burn and loss was also assessed in autumn 2017 to determine the effects of the irrigation treatments on senescence.

c) A Shiraz (clone PT23) rootstock trial was established in a commercial vineyard in Padthaway, South Australia in November 2009. The trial consisted of 52 experimental rootstocks from a single family (F86) grafted with Shiraz (clone PT23), along with a further eight promising selections, seven commercial rootstocks (101-14 Mgt, 140 Ruggeri, 1103 Paulsen, Dog Ridge, Merbein 5489Φ, Merbein 5512Φ and Merbein 6262Φ), and own rooted Shiraz. Vines were arranged as five adjoining blocks of single vine replicates in a randomised complete block design. This family was selected on the basis of normal distribution pattern of root strike and vigour potential, root knot nematode resistance in both parents, potassium uptake and average response in vigour reduction to reduced irrigation in a nursery. In the 2017 there was a problem with the irrigation system, leading to a period of severe stress during berry development in early February, leading to severe leave loss of some vines. Hence, symptoms of leaf burn and leaf loss were assessed prior to harvest to identify rootstocks with potential short term drought tolerance.

d) Two further trials, involving best salt tolerant selections established as part of CSP 1002 (Walker et al. 2014) on a commercial vineyard in Padthaway were also evaluated in this project. The first, a Shiraz trial (clone AC-728189) was planted in 2010 with replacements planted in 2011. It included two CSIRO selections (C5 and C7) and 140 Ruggeri, established as a replicated block (12 replicates) design with single vine plots. The second trial with Pinot Gris included the CSIRO salt tolerant selection C7, 140 Ruggeri and Ramsey was planted in 2012 with replacements planted in 2013. Both trials were trained to develop bilateral cordons

on a single fruiting wire at 100 cm from the ground with provision for vertical shoot positioning. Row and vine spacing was 3.5 m and 1.8 m, respectively.

The evaluation of the above trials included:

- Collection of berry samples prior to harvest to determine berry weight and for fruit composition analyses.
- Collection of yield and bunch number, allowing calculation of all yield component data including bunch weight and berries per bunch.
- Collection of pruning weight data and calculation of the Ravaz Index (yield/ pruning weight) as a measure of vine balance and a surrogate for carbon efficiency (using pruning weight as an indicator of canopy size) and water use efficiency (using pruning weight as an indicator of canopy size and hence transpiration).
- Analysis of fruit composition including juice Total Soluble Solids (TSS) by refractometer, pH and titratable acidity by titration (Metrohm Auto-titrator), organic acids (malate and tartrate), yeast assimilable nitrogen (YAN) by FTIR (Oenphos), mineral composition using ICP and total berry anthocyanin and phenolic substances using spectrophotometry.
- Targeted small-scale winemaking, using services provided by DEDJTR in Irymple Victoria and AWRI-WIC in Adelaide.
- Analysis of small-scale wines at bottling for pH, titratable acidity, residual sugar and post-bottling assessment of spectral properties (colour density, hue, total anthocyanins, ionised anthocyanins and total phenolic substances).

2. Assessment of the predictive value of simple traits measured previously (CSP 0503) with grafted vine performance in field trials

This component of the project aimed to test the efficacy of early screening assays undertaken in the previous project (CSP 0503) for vine growth characteristics, mineral element composition, in particular potassium uptake and response to drought and deficit irrigation. For this purpose, the study focussed on the performance of genotypes from F86 (a cross involving *V. cineria*, *V. champini*, *V. riparia* and *V. rupestris)* grafted with Shiraz established in trials b, c listed above and the *V. cineria* x Riesling test population (F34)

which was genotyped by next generation sequencing as part of this project (see below) and also included in abiotic stress screening studies, established as part of CSP 1302.

Traits measured in the previous project (CSP 0503, Clingeleffer and Smith 2011) for the ungrafted seedlings of both test populations which were planted in 2002 and established on a 1.0 m high bi-lateral cordon included:

- visual assessment of leaf loss across the season after one or two seasons without irrigation in 2008/09 and 2009/10.
- mid-summer canopy height measured in 2007.
- trunk girth measured in 2007
- dormant pruning weight measured in winter 2007 and 2008, after one or two seasons of drought in 2009 and 2010 respectively, and after recovery from drought in 2011.
- assessment of root architecture after removal of vines in winter 2011, including root number, root size, root angle and root type.

In addition, for the F86 test population, data were collected from a replicated trial established in 2006 under field nursery conditions (Clingeleffer and Smith 2011). The data included:

- transpiration efficiency determined by carbon isotope discrimination in mature leaf of ungrafted rootstock genotypes in 2007
- shoot length of ungrafted rootstock genotypes in 2007
- shoot length of grafted rootstock genotypes 2008
- dormant pruning weight of grafted rootstock genotypes with a control and deficit irrigation in 2009.
- stomatal conductance, leaf temperature and temperature differential from ambient determined with a Delta T porometer for control and deficit irrigation in 2009.
- mineral status of dried petioles of nursery grown vines determined by ICP for ungrafted plants (2007), control and deficit irrigation (2009).

To provide an indication of the efficacy of screening using the above traits, correlation and multilinear regression techniques were used to establish links between the above traits and grafted vine performance and fruit composition.

3. Targeted selection to address gaps using molecular markers

SNP based genetic linkage maps of *Vitis cinerea* populations were generated utilising next

generation sequencing technology in collaboration with Cornell University/USDA-ARS. This map was used to generate molecular markers associated with resistance to root knot nematodes and phylloxera (in collaboration with DPI Victoria). See results section for more detail on the methodologies used in these studies.

4. New trials established with rootstocks for final stage of evaluation

New rootstock trials were established over the course of the project with the 'near to release' selections under assessment in the various field trials listed above. The propagation and grafting was undertaken by the commercial nurseries, licenced to propagate CSIRO rootstocks. This provided opportunities to assess propagation characteristics under commercial conditions of the new selections.

6. Results/Discussion

1. Conduct a workshop to define delivery pathways for new rootstocks

A 'Future Rootstocks Workshop' was held on 25 November 2013 with members of the industry reference group, Wine Australia and CSIRO researchers with follow-up meetings with WA in June 2015 and the reference group in November 2015. Barriers to adoption of rootstocks were identified. These included:

1. A recognition that the lack of vineyard development had been an impediment to recent adoption of rootstocks.
2. A recognition that negative attitudes from winemakers and company personnel had also negatively impacted the adoption of rootstocks in some regions.
3. Recognition of requirements for more specific regional and varietal information from growers and winemakers.
4. The lack of sufficient mother vine material to meet demands for propagation, including for the new CSIRO low-moderate vigour rootstocks released in the 2005, particularly in South Australia.

CSIRO has been actively engaged with industry to address these issues and improve communication on the benefits of rootstocks and to provide updates on the past and current research (see Communications Appendix below). Input has also been provided into development of the Wine Australia web-based 'Rootstock Selector Tool' and the Wine Australia cost benefit analysis which showed the benefits of rootstock adoption. Mechanisms for the release of new rootstocks were significant discussion points for the meetings identified above. There was general agreement that the current process, developed for earlier rootstock releases in the CSIRO program, involving Plant Breeders Rights (PBR) protection and propagation by licensed nurseries was appropriate. To facilitate rapid adoption, once a decision is made to release a new rootstock, CSIRO has already established a PBR comparator trial to establish Distinctness, Uniformity and Stability for the most promising selections. Furthermore, CSIRO in consultation with licensed nurseries has ensured that mother vine plantings have been established so that adoption of the new rootstocks is not impeded by the unavailability of material. Once a decision is made to release a new rootstock variety, information leaflets will be developed

containing key information on scion compatibility and impact of the new rootstock on scion performance and fruit and wine composition.

To overcome the impediments associated with lack of regional and specific varietal information for new rootstocks, demonstration trials have been established for the more advanced selections across a range of climates, varieties and soil types to showcase the benefits of the new rootstocks in collaboration with regional groups and interested growers. These trials have included the range of advanced 'near to release' selections evaluated in this project (see later results section for details). The collaboration with the Mornington Peninsula Technical Group, which has involved the establishment of two sites with Pinot Noir on different soil types is considered to be a model for involvement with industry in meeting the need for regional information once new rootstocks are released from the CSIRO rootstock development program.

2. Evaluation of rootstock trials and early screening methods

2.1. 'Near to release' selections: Irymple site

The trial was established, in a replant situation following own rooted Sultanas, in 2006 with 26 rootstock selections and three commercial rootstocks (Ramsey, 140 Ruggeri and 1103 Paulsen) grafted with Chardonnay, Cabernet Sauvignon and Shiraz. The replicated trial, established as part of CSP 0503 was designed to:

- assess the performance of the three scion varieties grafted on the 26 selections
- enable interactions between rootstock genotype and scion variety to be determined
- test the efficacy of early screening procedures conducted in CSP 99/2 and CSP 0503.

The original 26 genotypes were selected based on screens conducted with ungrafted vines for potassium uptake, vigour, response to short term drought, root architecture, rooting and grafting. Six of the selections, covering a range in conferred vigour were identified with potential for release in the previous project CSP 0503 ('near to release' selections) having root knot nematode and phylloxera resistance, good propagation and performance characteristics (Clingeleffer and Smith 2011). Their performance has been monitored in this study, seasons 2013-2017.

Sampling dates, prior to harvest for each variety in the 'near to release trials' are provided in Table 1. The vines were sampled when the mean maturity level (TSS) across the rootstock genotypes was approximately 24 ^{o}Brix. The results show that the harvest in season 2017 was delayed for all varieties, compared to the other seasons.

Table 2.1. Sampling dates for the three varieties, seasons 2013-17.

Season	***Chardonnay***	***Cabernet Sauvignon***	***Shiraz***
2013	30 Jan.	05 Mar.	04 Feb.
2014	28 Jan.	27 Feb.	11 Feb.
2015	27 Jan.	23 Feb.	19 Feb.
2016	28 Jan.	18 Feb.	15 Feb.
2017	10 Feb.	10 Mar.	10 Mar.

2.1.1. Chardonnay

A. Yield and its components, pruning weight and Ravaz index

Yield and yield component data, collected over five seasons (2013-17) for Chardonnay grafted on 'near to release' selections and standard rootstocks is presented in Table 2.1.1.1. GLM, ANOVA analysis of the data showed significant effects of Block, Rootstock and Year for almost all variables. However, there were no significant rootstock and year interactions, except a weak interaction with bunch weight and the calculated Ravaz index. Hence, only the means of rootstocks and seasons are presented in the absence of interactions.

There were significant effects of season on yield and its components, pruning weight and the Ravaz index of Chardonnay (Table 2.1.1.1). Highest yield and bunch number were recorded in 2017, post conversion to simulated mechanical hedging in winter 2016. Season 2015 had the lowest yield, bunch weight and berries per bunch. Largest and smallest berries were recorded in 2013 and 2017 respectively. The highest and lowest pruning weights were recorded in 2013 and 2016 respectively. Despite the conversion to light mechanical hedging, pruning weights in 2017 were high. The Ravaz index, an indicator of vine balance and a surrogate for carbon efficiency and water use efficiency, was highest in in 2014 and lowest in 2015.

The significant effects of rootstock on yield and growth characteristics of Chardonnay indicate that rootstocks could be classified into four groups, based on conferred vigour (pruning weight) and performance (Table 2.1.1.1). The highest vigour rootstocks, the standards Ramsey and 1103 Paulsen and the CSIRO selections, C113 and C114 had the highest bunch numbers and produced the highest yield. The high to moderate vigour rootstocks, 140 Ruggeri and C112 had significantly fewer bunches and lower yields than the high vigour rootstocks but higher yields and bunch numbers than the moderate vigour rootstock, C20. The CSIRO selection C29, which had lower pruning weight than all the other rootstocks, not only produced the lowest yield but had significantly lower bunch numbers, bunch weight and berry weight. The CSIRO selection C20 had significantly larger bunches and berries than all other rootstocks. There was no effect of rootstock on berries per bunch, indicating that flowers per inflorescence and/or fruit set was not impacted by the rootstock genotype. Vine balance, based on the Ravaz index, was largely

unaffected by rootstock except for the low vigour, low yielding C29 selection which had a very high value.

Table 2.1.1.1. Mean yield, bunches per vine, bunch weight, berry weight, berries per bunch, pruning weight and Ravaz index of Chardonnay grafted on five CSIRO rootstock selections and three standard rootstocks over five seasons (2013-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***Yield (kg/vine)***	***Bunches/ vine***	***Bunch weight (g)***	***Berry weight (g)***	***Berries/ bunch***	***Pruning weight (kg/vine)***	***Ravaz Index***
Ramsey	29.4a	309.5a	97.3b	1.18ab	82.5a	2.57a	11.8cd
1103P	29.5a	285a	108.9ab	1.22ab	90.5a	2.53a	12.4bc
140R	25.0b	254bc	101.9b	1.20ab	85.0a	2.22b	12.2cd
C112	22.9b	245bc	96.4b	1.23ab	79.0a	2.32b	11.2cd
C113	28.4a	302a	97.1b	1.19ab	81.4a	2.88a	9.9d
C114	25.7ab	251bc	103.6ab	1.23ab	84.7a	2.78a	9.5d
C20	19.0c	179d	117.6a	1.29a	90.1a	1.49c	13.9bc
C29	13.2d	172d	79.7c	1.12b	81.4a	0.62d	25.0a
Year							
2013	22.6c	159c	145.2a	1.39a	104.3a	2.44a	10.1c
2014	25.6b	244b	103.5b	1.11c	93.3 a	1.81b	16.5a
2015	18.2d	235b	76.5c	1.28b	59.8b	2.08ab	10.7c
2016	20.8c	251b	83.9c	1.12c	85.4 a	1.78b	14.8b
2017	32.4a	338a	95.3b	1.10c	87.1a	2.33a	15.5ab
Significance							
Block[a]	***	***	**	ns	*	***	ns
Rootstock	***	***	***	**	ns	***	***
Year	***	***	***	***	***	***	***
R x Y[b]	ns	ns	*	ns	ns	ns	***

[a] *, **, *** represent significance values, p< 0.05, 0.01, 0.001 respectively.

[b] RxY indicates the rootstock x season interaction.

Based on significant correlations, the main driver of yield across blocks, seasons and rootstocks was bunch number (Table 2.1.1.2). There was no significant association between berry weight and yield. There was also a strong association between yield and pruning weight. However, because node number, budburst and shoot fruitfulness were not recorded in this study the underlying causes for this relationship cannot be elucidated. A highly significant multilinear regression (p<0.001) indicated that 94.5% of yield variation could be accounted for by inclusion of bunches and bunch weight in the model.

Alternatively, if berries per bunch and berry weight are included in the model, with bunch weight removed then the regression ($p<0.001$) accounts for 87.1 % of the yield variation with bunches, berry weight and berries per bunch all significant factors ($p<0.001$). Negative correlations between bunch number and bunch weight indicate that vines carrying more bunches produced smaller bunches with smaller berries. Positive correlations indicate that differences in berry weight and berries per bunch both contributed to bunch weight variability. The negative association between yield and Ravaz index indicates that pruning weight has a stronger influence on the ratio than yield.

Table 2.1.1.2. Correlation matrix, describing linear relationships between yield component variables, pruning weight and the Ravaz index for Chardonnay across the full data set including block, season and rootstock (n=200). Values in bold indicate significant correlations determined using the stringent Bonferroni analysis ($p<0.05$).

	Yield	***Bunches***	***Bunch weight***	***Berry weight***	***Berries per bunch***	***Pruning weight***
Bunches	**0.83**					
Bunch weight	**0.29**	**-0.24**				
Berry weight	-0.19	**-0.35**	**0.35**			
Berries/ bunch	**0.25**	-0.05	**0.53**	**-0.45**		
Pruning weight	**0.60**	**0.63**	-0.03	-0.04	-0.06	
Ravaz index	**-0.40**	**-0.36**	-0.07	-0.08	-0.09	**-0.65**

To isolate rootstock effects, correlation analyses were also conducted using only the rootstock means to explore relationships between yield determinants and growth characteristics across the rootstock genotypes with variation associated with block and season removed. The results presented in Table 2.1.1.3 indicate that the main determinant of yield across the rootstock genotypes was bunch number, accounting for 90% of the variation. There was also a strong association between yield and pruning weight. However, as discussed above, the underlying causes for this relationship cannot be elucidated. A highly significant multilinear regression ($p<0.001$) indicted that 98.5% of yield variation across the rootstock genotypes could be accounted for by inclusion of bunches and bunch weight in the model. Alternatively, if berries per bunch and berry weight are included, with bunch weight removed then the regression ($p<0.001$) accounts for 98.1% of the variation across rootstocks with bunches, berry weight and berries per bunch all significant. Across the rootstock genotypes, the strong positive relationships

between bunch weight and berry weight and berries per bunch indicate that differences in berry weight and berries per bunch both contributed to differences in bunch weight. The very strong negative association between pruning weight and Ravaz index indicates that pruning weight (four-fold differences) has a stronger influence on the ratio than yield (two-fold differences).

Table 2.1.1.3. Correlation matrix across rootstock means (n=9) to describe the effect of rootstock on Chardonnay with environmental variables removed. Values in bold indicate significant correlations ($p<0.1$).

	Yield	***Bunches***	***Bunch weight***	***Berry weight***	***Berries per bunch***	***Pruning weight***
Bunches	**0.95**					
Bunch weight	0.35	0.05				
Berry weight	0.21	-0.00	**0.83**			
Berries/ bunch	-0.03	-0.27	**0.60**	0.11		
Pruning weight	**0.93**	**0.90**	0.29	0.35	-0.28	
Ravaz index	**-0.85**	**-0.74**	-0.54	**-0.59**	0.06	**-0.93**

B. Fruit composition

Berry juice composition data collected over five seasons (2013-17) for Chardonnay grafted on 'near to release' selections and standard rootstocks is presented in Table 2.1.1.4. GLM, ANOVA analysis of the data showed significant effects of Block, Rootstock and Year for all variables. The results for seasons indicate that there were significant differences in maturity when samples were collected, ranging from a mean of 28 ^{o}Brix in 2013 to 22.3 ^{o}Brix in 2017. To test the effect associated with the differences in maturity, TSS was included as a covariate in the ANOVA analysis. While there were significant effects of TSS as a covariate on pH and YAN, the effect on rootstock and season means was small (i.e. 0.01 for pH, 13 mg/L for YAN), hence the data are not shown. Except for TSS, there were no significant rootstock and year interactions. Hence, only the means of rootstocks and seasons are presented in the absence of interactions.

In the case of TSS, the interaction between rootstock and season showed that lowest TSS values were recorded with, C29 in 2013 (26.5 ^{o}Brix), C114 in 2014 (24.8 ^{o}Brix) and 2016 (22.5 ^{o}Brix), C20 (21.6 ^{o}Brix) in 2015 and 1103 Paulsen (19.2 ^{o}Brix) in 2017. Similarly, highest TSS values were recorded by C20 (29 ^{o}Brix) in 2013, C113 (25.5 ^{o}Brix) in 2015 and C29 in 2014, 2016 and 2017 (i.e. 26.9, 24.8, 26.7 respectively). Overall, the effects of

rootstock on TSS were relatively small (range 23.9 - 25.8 ^{o}Brix) with C29 having the highest TSS while 1103 Paulsen had the lowest TSS.

The results show significant effects of season on all berry juice variables (Table 2.1.1.4). These included juice pH (3.78 to 4.38 in 2017 and 2013 respectively), TA (3.39 to 5.97 g/L in 2013 and 2017 respectively), malate (3.67 to 5.15 g/L in 2016 and 2014 respectively), tartrate (3.82 to 6.87 g/L in 2017 and 2014 respectively) and YAN (204 to 286 g/L in 2015 and 2017 respectively).

Rootstocks had significant effects on all juice compositional variables, except tartrate (Table 2.1.1.4). Across the rootstocks, mean pH ranged from 3.91 to 4.13 with selection C20 having the lowest pH and C112 and C113 the highest values. The standard rootstocks, Ramsey, 1103 Paulsen and 140 Ruggeri, C114 and C29 all had similar pH levels. Rootstock effects on TA were relatively small (4.03 - 4.96 g/L) with C20 and Ramsey having the highest levels and C29 the lowest level. Selection C29 had lower levels of malate than all other rootstocks. YAN values ranged from 139 - 292 g/L with the standard rootstocks, C112 and C113 having the highest levels and C29 the lowest level.

Table 2.1.1.4. Mean juice TSS, pH, TA, Malate, Tartrate, YAN, AAN, and ammonia of Chardonnay grafted on five CSIRO rootstock selections and three standard rootstocks over five seasons (2013-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***TSS (ºBrix)***	***pH***	***TA (g/L)***	***Malate (g/L)***	***Tartrate (g/L)***	***YAN (mg/L)***
Ramsey	24.3cb	4.03b	4.80a	4.55a	4.84a	279a
1103P	23.9cb	4.02b	4.74ab	4.58a	5.00a	280a
140R	25.2ab	4.04b	4.50ab	4.40a	5.09a	270a
C112	25.4ab	4.13a	4.62ab	4.68a	5.22a	247ab
C113	24.8ab	4.11a	4.66ab	4.62a	4.98a	292a
C114	24.7ab	3.99b	4.75ab	4.30a	5.02a	232b
C20	24.0cb	3.91c	4.96a	4.51a	4.92a	221b
C29	25.8a	4.05ab	4.03b	3.66b	5.04a	179c
Year						
2013	28.0a	4.38a	3.39d	3.79cd	4.50c	225c
2014	25.9b	3.92c	5.14b	5.15a	5.06c	239bc
2015	24.3c	4.12b	3.81d	4.14c	4.91c	204c
2016	23.3cd	3.92c	4.52c	3.67d	6.57a	266ab
2017	22.3d	3.78d	5.97a	4.85b	3.82d	286a
Significance						
Block[a]	**	*	**	**	*	***
Rootstock	**	***	***	***	ns	***
Year	***	***	***	***	***	***
R x Y[b]	***	ns	ns	ns	ns	ns
TSS covariate	-	**	ns	ns	ns	***

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively.

[b] RxY indicates the rootstock x season interaction.

C**. Relationships between rootstock growth characteristics and fruit composition**

Correlation analyses were undertaken to explore the effect of rootstock genotype on relationships between vine growth characteristics and berry juice composition (Table 2.1.1.5). Significant correlations indicate that both yield and bunch number across the rootstock genotypes was positively associated with juice malate and YAN. Bunch weight was negatively correlated with TSS and pH but positively correlated with TA. Berry weight was negatively associated with TSS and positively associated TA and malate. Berries per bunch was negatively associated with pH and tartrate. Rootstock conferred vigour (pruning weight) was positively associated with higher TA, malate and YAN. Vine balance as measured by the Ravaz index, was negatively associated with TA, malate and YAN.

Table 2.1.1.5. Correlation matrix across rootstock means (n=9) describing relationships between rootstock growth characteristics and fruit composition of Chardonnay with environmental variables removed. Values in bold indicate significant correlations, $p<0.1$.

	Yield	***Bunches***	***Bunch weight***	***Berry weight***	***Berries per bunch***	***Pruning weight***	***Ravaz index***
TSS	-0.53	-0.32	**-0.81**	**-0.60**	-0.43	-0.40	0.48
pH	0.23	0.48	**-0.65**	-0.47	**-0.70**	0.33	-0.13
TA	0.56	0.45	**0.61**	**0.81**	-0.21	**0.67**	**-0.71**
Malate	**0.63**	**0.62**	0.36	**0.62**	-0.44	**0.76**	**-0.74**
Tartrate	-0.08	0.04	-0.24	0.13	**-0.74**	0.18	-0.09
YAN	**0.88**	**0.91**	0.24	0.25	-0.33	**0.87**	**-0.71**

Multilinear regression techniques were used to identify the best combinations of growth traits with strong predictive value for Chardonnay juice composition measured across the rootstock genotypes. They showed that a significant proportion of the variability in berry composition across the rootstock genotypes could be accounted for based on adjusted R^2 values including:

- 61% (p=0.008) of TSS by bunch weight (-ve)
- 57% (p=0.033) of pH by bunch number (+ve) and bunch weight (-ve)
- 80% (p=0.003) of TA by bunch number and berry weight (both +ve)
- 76% (p=0.006) of TA by berry and pruning weight (both +ve)
- 87% (p=0.004) of malate by yield (-ve), bunch number and berry weight (both +ve)
- 76% (p=0.017) of malate by pruning weight (+ve)
- 48% (p=0.024) of tartrate by berries per bunch (-ve)
- 79% (p=0.001) of YAN by bunch number (+ve)
- 73% (p=0.002) of YAN by pruning weight (+ve)

D. Small-scale winemaking

Single ferment Chardonnay wines were produced from most rootstocks in 2014 with the intent to identify potential fermentation issues, assess if pH adjustment from tartaric acid addition led to unbalanced wines and to provide wine samples for tasting by industry. Formal sensory assessments were not conducted although preferences were recorded during internal assessments. The fruit for winemaking was harvested on the 29/01/2014. The must data show some differences in fruit maturity (Table 2.1.1.6). Of the standard

rootstocks, Ramsey had a lower level of TSS, lower pH and higher acid than 140 Ruggeri. The CSIRO selection C20 had the lowest TSS, pH and highest TA. While the must of C113 and C114 both had high TSS, the pH of C114 was much lower than the pH of C113. Tartaric acid additions of 1.0 g/L were used for pH adjustment of the rootstock musts prior to fermentation, with exception that 1.5 g/L was added to the C113 must and no additions were required for C20. Following pH adjustment, at the commencement of fermentation, must pH ranged from 3.49 for C20 to 3.6 for 140 Ruggeri while TA values were all similar, ranging from 6.1 to 6.4 g/L. pH was further adjusted where required during racking. At bottling all wines had similar levels of TA, although Ramsey and C20 had the lowest and highest values, i.e. 5.6 and 6.4 g/L respectively (Table 2.1.1.6). Wine pH values were all similar, except for C20 which had a much lower pH. Except for Ramsey and C20, all wines had some residual sugar, in particular the wine of C114. There were only minor differences between the wines in free sulfur (29-34 ppm), total sulfur (130-149 ppm) and volatile acidity (0.37-0.42%). In the informal tastings, C114 wine, described as having rich, peach and sweet characters was preferred, possibly due to the high level of residual sugar (Table 2.1.1.6). The least preferred wines were from 140 Ruggeri, described as coarse and phenolic and C29, described as indistinct and yeasty. Of those remaining, Ramsey wines were described has having a pineapple, citrus but bitter character, C20 wines were described as having good acid and citrus characters while C113 wines were described as having fruity, sweet characters.

Table 2.1.1.6. Must, wine data and preference rankings for Chardonnay wines produced from two standard rootstocks (Ramsey and 140 Ruggeri) and four CSIRO selections, season 2014.

	Must data			***Bottling data***				***Preference***
	TSS (°Brix)	**pH**	**TA** (g/L)	**pH**	**TA** (g/L)	**Alc.** (%)	**Resid. sugar** (%)	
Ramsey	22.6	3.67	5.95	3.52	5.56	14.0	0	****
140R	23.3	3.76	5.39	3.52	5.85	14.3	1.0	***
C113	23.6	3.84	5.17	3.54	6.07	14.6	2.3	****
C114	23.5	3.72	5.07	3.49	5.91	14.0	4.4	*****
C20	20.8	3.49	6.29	3.41	6.36	13.1	0	****
C29	22.8	3.78	5.05	3.56	6.04	14.0	2.3	***

E. Discussion re Chardonnay and performance of CSIRO 'near to release' rootstocks

The results over five seasons show significant effects of rootstock genotype on the vine performance and fruit composition of Chardonnay, associated with conferred vine vigour. In the high vigour category, the CSIRO selections C113 and C114 may provide alternative rootstocks to the standard rootstocks Ramsey and 1103 Paulsen for Chardonnay production in hot climates as their productivity, fruit composition and wine quality attributes were similar, at least when compared with Ramsey wines. C112 may provide an alternative rootstock for Chardonnay in the high to moderate vigour category if wine attributes were acceptable. The moderate vigour rootstock selection C20, which had high Ravaz index values and was able to adequately mature the crop may be a useful alternative for enhanced water use efficiency, for establishment in high density plantings to improve vineyard productivity and sustainability and also for use in sites with high vigour potential. The low vigour, low yielding selection C29, shows little promise for Chardonnay production in hot climates.

The results clearly show that the main effects of rootstock on Chardonnay performance were on conferred vigour (pruning weight), which was strongly linked to yield and bunch number, the latter being the main driver of yield, TA, malate and YAN. It is interesting to note that variables associated with bunch development (bunch weight, berries per bunch and berry weight) were not linked to pruning weight or yield, an indication that the effect of rootstock on these variables was independent of conferred vigour and yield. This is important for fruit composition as negative correlations with TSS would indicate that rootstocks producing larger bunches with larger berries would have delayed maturation.

Further study of the underpinning physiology leading to the relationships with inherent vine vigour and fruit composition (TA, malate and YAN) and the effects of bunch weight, berry weight and berries per bunch on maturation are required.

It is also important to note that the effects of rootstock on conferred vigour, crop development and fruit composition of Chardonnay were consistent across seasons, as shown by the lack of a significant rootstock x season interaction. Further study is also required to monitor whether the large increase in yield in 2017 (50%) following conversion to mechanical hedging can be maintained in future seasons by all rootstocks and to assess the longer term impacts on both growth and fruit composition. Furthermore, the underpinning causes for the significant block effects on both growth characteristics and fruit composition of grafted Chardonnay warrant investigation.

2.1.2. Cabernet Sauvignon

A. Yield and its components, pruning weight and Ravaz index

Yield and yield component data, collected over five seasons (2013-17) for Cabernet Sauvignon grafted on 'near to release' selections and standard rootstocks are presented in Table 2.1.2.1. GLM, ANOVA analysis of the data showed significant effects of Block, Rootstock and Year for most variables. However, there were no significant rootstock and year interactions. Hence, only the means of rootstocks and seasons are presented, in the absence of interactions.

There were significant effects of season on yield and its components, pruning weight and the Ravaz index of Cabernet Sauvignon (Table 2.1.2.1). Highest yield and bunch number were recorded in 2017, post conversion to simulated mechanical hedging in winter 2016. Season 2016 had the lowest yield, bunch weight and berries per bunch. Largest and smallest berries were recorded in 2017 and 2016, respectively. The highest and lowest pruning weights were recorded in 2013 and 2016 respectively, despite the conversion to light mechanical hedging. The Ravaz index, an indicator of vine balance and a surrogate for carbon efficiency and water use efficiency, was very low across all rootstocks due to the high inherent vigour and low yields of Cabernet Sauvignon. The Ravaz index was highest in 2017, post conversion to light mechanical hedging, due to the large yield in that season and lowest in 2013.

The significant effects of rootstock on yield and growth characteristics of Cabernet Sauvignon indicate that rootstocks could be classified into two groups, based on yield and to a lesser degree conferred vigour (pruning weight) (Table 2.1.2.1.). The highest yields and bunch numbers were produced by Ramsey, C113, C114 and C20. While Ramsey, C113, C114 had high pruning weights, the growth of C20 was much lower. In contrast, both 1103 Paulsen and C112 which had high pruning weights produced low bunch numbers and yield. The moderate vigour 140 Ruggeri rootstock had low bunch number and low yield. The low vigour, C29 selection had low bunch numbers and low yield. The higher yielding rootstock genotypes Ramsey, C113, C114 and C20 produced the largest bunches with more berries per bunch. It is not possible from this study to determine if this response was due to the effect of rootstock on flowers per inflorescence and/or fruit set. Vine balance, based on the Ravaz index, was very low across most rootstocks due to both the high inherent vigour of Cabernet Sauvignon and the low the yields recorded. Overall

there was a four-fold difference in the Ravaz index with C29 and C112 having the highest and lowest values, respectively. The higher Ravaz index of selection C20 associated with its low vigour compared to the other higher yielding rootstocks, was closer to optimum levels of around 8 described for spur pruned Cabernet Sauvignon (Dokoozlian et al. 2011). These results indicate significant potential through the use of rootstocks to improve the vine balance of Cabernet Sauvignon grown in irrigated hot climate vineyards and enhance fruit composition and wine quality attributes.

Table 2.1.2.1. Mean yield, bunches per vine, bunch weight, berry weight, berries per bunch, pruning weight and Ravaz index of Cabernet Sauvignon grafted on five CSIRO rootstock selections and three standard rootstocks over five seasons (2013-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***Yield (kg/vine)***	***Bunches/ vine***	***Bunch weight (g)***	***Berry weight (g)***	***Berries/ bunch***	***Pruning weight (kg/vine)[c]***	***Ravaz Index***
Ramsey	18.0a	235a	74.0a	1.04a	71.2b	8.48a	2.44c
1103 Paulsen	12.9b	175bc	68.8b	1.02a	67.5bc	7.08a	1.95c
140 Ruggeri	11.8b	176bc	64.3b	1.02a	63.5c	5.35bc	3.21bc
C112	10.3b	158c	59.7b	0.97a	60.4bc	7.94a	1.47c
C113	18.7a	246a	75.6a	1.03a	73.5ab	7.63a	2.51c
C114	18.4a	231ab	80.4a	1.06a	76.4ab	7.12ab	2.95c
C20	17.7a	221ab	81.3a	1.05a	77.7ab	4.35cd	4.61ab
C29	10.4b	155c	69.1b	1.04a	65.6c	2.00d	6.11a
Year							
2013	13.7b	146d	90.5a	1.04a	86.80a	7.09a	2.14c
2014	16.3b	199bc	79.2 b	1.02ab	77.70ab	5.45b	3.89b
2015	13.0b	177cd	64.2c	1.06a	60.28c	6.87a	2.40bc
2016	10.1c	213b	45.6d	0.97b	47.06d	5.10b	2.78bc
2017	24.1a	279a	84.7ab	1.05a	81.60a	5.81b	5.64a
Significance[a]							
Block	***	***	***	*	***	**	ns
Rootstock	***	***	***	ns	***	***	***
Year	***	***	***	***	***	***	***
R x Y[b]	ns	ns	ns	ns	ns	ns	ns

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively.

[b] RxY indicates the rootstock x season interaction.

Based on significant correlations, both bunch number and bunch weight associated with berries per bunch were significant contributors to yield variability across blocks, seasons and rootstocks (Table 2.1.2.2). There were no significant associations between berry weight or pruning weight and yield. A highly significant multilinear regression ($p<0.001$) indicated that 96% of yield variation could be accounted for by inclusion of bunches and bunch weight in the model. Positive correlations between bunch number and bunch weight indicate that vines carrying more bunches also produced larger bunches, a very different result to that reported for Chardonnay above. The Ravaz index was more impacted by the strong negative association with pruning weight than the positive association with yield.

Table 2.1.2.2. Correlation matrix, describing linear relationships between yield component variables, pruning weight and the Ravaz index of Cabernet Sauvignon across the full data set including block, season and rootstock (n=200). Values in bold indicate significant correlations determined using the stringent Bonferroni analysis ($p<0.006$).

	Yield	***Bunches***	***Bunch weight***	***Berry weight***	***Berries per bunch***	***Pruning weight***
Bunches	**0.86**					
Bunch weight	**0.66**	**0.24**				
Berry weight	0.20	0.07	**0.35**			
Berries/ bunch	**0.64**	**0.24**	**0.95**	0.07		
Pruning weight	0.13	-0.01	0.11	0.07	0.10	
Ravaz index	**0.40**	**0.374**	**0.24**	0.05	**0.25**	**-0.70**

Correlation analyses were also conducted to explore relationships between yield determinants and growth characteristics across the rootstock genotypes (Table 2.1.2.3). Both bunch number and bunch weight, associated with berries per bunch, were significant contributors to yield variability across the rootstock genotypes. There was no association between yield and pruning weight. A highly significant multilinear regression ($p<0.001$) indicted that 99.0% of the yield variation across the rootstock genotypes could be accounted for by inclusion of bunches and bunch weight in the model. Across the rootstock genotypes, the strong positive relationships between bunch weight and berry weight and berries per bunch indicate that differences in berry weight and berries per bunch both contributed to differences in bunch weight. The very strong negative association between pruning weight and the Ravaz index (R^2 =85%) indicates that that

pruning weight (four-fold differences) had a stronger influence on the ratio than yield (two-fold differences).

Table 2.1.2.3. Correlation matrix across rootstock means (n=9) describing relationships between rootstock growth characteristics and fruit composition of Cabernet Sauvignon with environmental variables removed. Values in bold indicate significant correlations, $p<0.10$.

	Yield	***Bunches***	***Bunch weight***	***Berry weight***	***Berries per bunch***	***Pruning weight***
Bunches	**0.99**					
Bunch weight	**0.89**	**0.82**				
Berry weight	**0.59**	0.55	**0.76**			
Berries/ bunch	**0.92**	**0.84**	**0.99**	**0.66**		
Pruning weight	0.26	0.34	-0.15	-0.33	-0.08	
Ravaz index	0.06	-0.01	0.43	0.55	0.36	**-0.92**

B. Fruit composition

Berry juice composition data collected over five seasons (2013-17) for Cabernet Sauvignon grafted on 'near to release' selections and standard rootstocks is presented in Table 2.1.2.4. GLM, ANOVA analysis of the data showed significant effects of Block, Rootstock and Year for all variables. The results indicate that there were significant differences in maturity when samples were collected, ranging from a mean of 24 ^{o}Brix in 2016 to 26.8 ^{o}Brix in 2015. To test the effect associated with the differences in maturity, TSS was included as a covariate in the ANOVA analysis. There were significant effects of TSS as a covariate on all variables except pH and tartrate. Hence, adjusted means are presented where applicable (Table 2.1.2.4) to describe both season and rootstock effects. There were no significant rootstock and year interactions, hence, only the means of rootstocks and seasons are presented.

The results show significant effects of season on all berry juice variables (Table 2.1.2.4). These included juice pH (4.21 to 4.41 in 2017 and 2014 respectively), TA (3.01 to 4.03 g/L in 2014 and 2017 respectively), malate (3.42 to 6.24 g/L in 2017 and 2014 respectively), tartrate (4.75 to 6.24 g/L in 2013 and 2014 respectively) and YAN (159 to 232 g/L in 2016 and 2015 respectively), anthocyanin (0.87 to 1.36 mg/g in both 2014 and

2015 and 2016 respectively) and phenolics (1.33 to 1.68 a.u. in 2014 and 2016 respectively).

Rootstocks had significant effects on all juice compositional variables, except phenolic substances (Table 2.1.2.4). The effects of rootstock on TSS were relatively small (range 24.0-25.7 ^{o}Brix) with C29 having the highest and Ramsey the lowest values. Selections C112 and C113 had the highest pH followed by the standard rootstocks (Ramsey, 1103 Paulsen and 140 Ruggeri) and C114 while selections C20 and C29 had the lowest pH. Rootstock effects on TA, although significant were relatively small (3.45 - 4.02 g/L) with C112 having the highest level and C29 and C20 the lowest levels. There were significant effects of rootstock on malate (3.88 - 5.16 g/L) with selection C112 having the highest levels and selection C20 the lowest levels. The effect of rootstock on tartrate was small (5.00 - 5.98 g/L), with C20 having the lowest values. YAN values ranged from 164 - 226 g/L with the standard rootstocks, C112 and C113 having the highest levels and C29 the lowest level. The effect of rootstock on berry anthocyanin was small with Ramsey, 140 Ruggeri and selections C20 and C29 having the highest and C114 the lowest values.

Table 2.1.2.4. Mean juice TSS and adjusted means based on covariate analysis of pH, TA, Malate, Tartrate, YAN, anthocyanin and phenolic substances of Cabernet Sauvignon grafted on five CSIRO rootstock selections and three standard rootstocks over five seasons (2013-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***TSS ºBrix***	***pH***	***TA (g/L)***	***Mal. (g/L)***	***Tart. (g/L)***	***YAN (mg/L)***	***Anth. (mg/g)***	***Phen (a.u.)***
Ram.	24.0d	4.34b	3.81ab	4.70bc	5.57b	211ab	1.05a	1.53
1103P	24.9bc	4.36b	3.72b	4.74ab	5.63ab	210ab	1.01ab	1.47
140R	25.6a	4.32b	3.78ab	4.71bc	5.65ab	226a	1.04a	1.58
C112	25.2ab	4.42a	4.02a	5.16a	5.98a	213ab	1.00ab	1.44
C113	25.6a	4.40a	3.70b	4.86ab	5.65ab	207ab	0.99ab	1.45
C114	24.5cd	4.34b	3.61bc	4.54b	5.39bc	179bc	0.95b	1.45
C20	25.3ab	4.26c	3.46bc	3.98d	5.00c	172bc	1.06a	1.50
C29	25.7a	4.25c	3.51bc	4.31cd	5.43b	173bc	1.06a	1.51
Year								
2013	25.0b	4.23b	3.81ab	4.10b	4.75c	207b	-	-
2014	25.2b	4.41a	3.02c	6.26a	6.24a	173c	0.86c	1.32c
2015	26.8a	4.39a	4.06a	5.81a	5.47b	268a	0.79c	1.50b
2016	24.0c	4.39a	3.50b	3.23c	5.83b	137d	1.41a	1.72a
2017	24.4c	4.21b	3.98a	3.30c	5.15bc	189c	1.00b	1.42b
Sig.								
Block[a]	ns	*	ns	*	ns	**	ns	*
Rootstock	***	***	**	***	**	**	*	ns
Year	***	***	***	***	***	***	***	***
R x Y[b]	ns	ns	ns	ns	ns	ns	ns	ns
TSS covariate	-	ns	*	***	ns	***	***	*

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively.

[b] RxY indicates the rootstock x season interaction.

C. **Relationships between growth characteristics and fruit composition**

Correlation analyses were conducted using only the rootstock means to explore relationships between vine growth characteristics and berry juice composition across the rootstock genotypes with variation association with block and season removed (Table 2.1.2.5). Surprisingly, the results show little impact of growth characteristics of rootstocks on fruit composition, except for the significant positive correlations between pruning weight and TA and YAN. Although just not significant pruning weight was also positively linked to pH, malate and tartrate levels. Similarly, both yield and bunch number tended to be positively linked to TA, malate and tartrate. It is likely that the high inherent

vigour of Cabernet Sauvignon across all rootstocks may be masking many of the effects of individual rootstocks on fruit composition.

Table 2.1.2.5. Correlation matrix, describing linear relationships between growth characteristics (yield component variables, pruning weight and the Ravaz index) and berry juice composition of Cabernet Sauvignon across rootstock (n=9). Values in bold indicate significant correlations ($p<0.10$).

	Yield	*Bunches*	*Bunch weight*	*Berry weight*	*Berries per bunch*	*Pruning weight*	*Ravaz index*
TSS	-0.03	-0.03	-0.01	-0.22	0.06	-0.11	-0.01
pH	0.11	0.18	-0.10	0.03	-0.10	0.44	-0.43
TA	0.40	0.48	0.14	0.25	0.12	**0.60**	-0.42
Malate	0.35	0.43	0.09	0.12	0.09	**0.54**	-0.41
Tartrate	0.41	0.49	0.16	0.08	0.19	0.50	-0.34
YAN	0.23	0.36	-0.12	-0.02	-0.13	**0.70**	**-0.56**
anthocyanin	0.21	0.23	0.01	-0.32	0.10	0.37	-0.32
phenolics	0.31	0.38	0.12	-0.16	0.17	0.23	-0.10

Multilinear regression techniques were used to identify the best combinations of growth traits with strong predictive value for Cabernet Sauvignon juice and berry composition measured across the rootstock genotypes. As expected, based on the correlations presented in Table 2.1.2.6, very few multilinear regression models were significant. Those that were significant showed that a significant proportion of the variability in berry composition across the rootstock genotypes could be accounted for based on adjusted R^2 values including:-

- 44% ($p=0.042$) of TA by berry pruning weight and berry weight (both +ve)
- 42% ($p=0.034$) of YAN by pruning weight (+ve)

E. Small-scale winemaking

Single ferment Cabernet Sauvignon wines were produced from most rootstocks in 2014 with the intent to identify potential fermentation issues, assess whether pH adjustment from tartaric acid addition led to unbalanced wines; determine the impacts of rootstocks on spectral properties and to provide wine samples for tasting by industry. Formal sensory assessments were not conducted although preferences were recorded during internal assessments.

The fruit for winemaking was harvested 28/02/2014. The must data show some differences in fruit maturity (Table 2.1.2.6). 140 Ruggeri and C113 had the highest TSS and highest pH. Ramsey had the lowest TSS but a relatively high pH. Must TA levels were all similar. Tartaric acid additions of 2.5 - 3.0 g/L were used for pH adjustment of the rootstock musts prior to fermentation with minor additions during racking. At bottling all wines had similar levels of TA, except C114 which had the lowest value. Wine pH levels of all rootstocks was higher than desired maximum level of pH 3.6, except for C29. Further acid additions to reduce the pH would have made the wines too acidic with levels potentially exceeding 8.0 g/L. There were only minor differences between the wines in free sulfur (29-30 ppm), total sulfur (66-86 ppm) and volatile acidity (0.03-11%). In the informal tastings, C113 wine, described as having purple colour and rich, fruit characters, was preferred. (Table 2.1.2.6). The least preferred wines were from 140 Ruggeri, described as brown, stalky, hot characters (possible associated with the high alcohol) and C114 with stalky herbal characters. Wine of C29 with purple colour and rich herbal characters was ranked second highest. Ramsey wine was described as having red colour with fruity herbal characters. C20 wine was described as having purple colour with acidic and fruit character. There were only small differences in wine spectral properties with C20 and C114 having the highest and lowest colour densities respectively. C113 wine had the highest hue, most likely associated with its high pH. Wines from 140 Ruggeri, C114 and C20 had the highest anthocyanin while Ramsey, C113 and C29 had the lowest levels of anthocyanin. Wines from C113 and C114 tended to have lower levels of ionised anthocyanins than the other rootstocks. Wines from 140 Ruggeri had the highest level of phenolic substances while Ramsey and C114 had the lowest levels of phenolic substances.

Table 2.1.2.6. Must, wine data, spectral properties (colour density, hue, anthocyanin and phenolic substances) and preference rankings for Cabernet Sauvignon wines produced from two standard rootstocks (Ramsey and 140 Ruggeri) and four CSIRO selections, season 2014.

	Must data			***Bottling data***			***Spectral properties***					***Pref.***
	TSS	**pH**	**TA**	**pH**	**TA**	**Alc.**	CD	Hue	A	IA	Phen	
	(°Brix)		(g/L)		(g/L)	(%)					.	
Ram.	25.0	4.12	3.96	3.63	7.64	14.5	7.86	0.68	310	42.3	32.3	***
140R	26.1	4.16	3.79	3.65	7.96	15.6	8.15	0.71	334	42.4	37.4	**
C113	26.8	4.19	3.91	3.67	7.70	15.0	7.78	0.73	309	38.5	35.0	*****
C114	25.7	4.08	4.16	3.62	6.62	14.4	7.09	0.70	330	40.0	32.5	**
C20	25.4	4.04	4.17	3.62	7.64	15.0	8.29	0.68	332	44.6	35.5	***
C29	25.2	4.08	3.93	3.60	7.43	14.6	7.86	0.70	313	44.0	35.2	****

F. Discussion re Cabernet Sauvignon and performance of CSIRO 'near to release' rootstocks

The results over five seasons show significant effects of rootstock genotype on the vine performance and fruit composition of Cabernet Sauvignon. Conferred vigour of almost all rootstock genotypes was very high leading to very low Ravaz index values, indicatiing that Cabernet Sauvignon demonstrated inherently low water and carbon use efficiency. High conferred vigour of the CSIRO selections C112, C113 and C114 was similar to Ramsey and 1103 Paulsen rootstocks, an indication that they may provide alternative rootstocks for Cabernet Sauvignon production in hot climates as their productivity, fruit composition and wine quality attributes were similar. The moderate vigour rootstock selection C20 had high Ravaz index values, was able to adequately mature the crop and may be a useful alternative to the lower yielding 140 Ruggeri rootstock. It may be useful for enhanced water use efficiency and for establishment in high density plantings to improve vineyard productivity and sustainability and also for use in sites with high vigour potential. The low vigour, low yielding selection C29, shows little promise for Cabernet Sauvignon production in hot climates.

In this study with Cabernet Sauvignon, bunch number was the main driver of yield, although other developmental factors (i.e. bunch weight, berry weight and berries per bunch) also contributed to rootstock effects on yield. However, there were no significant relationships between conferred vigour and any of the factors involved in crop development across the rootstock genotypes. The moderate vigour C20 was among the highest yielding whereas the yields of 1103 Paulsen and 140 Ruggeri were low, due to low

bunch numbers. The underpinning causes for these effects associated with vine fruitfulness warrant investigation. In this study, conferred vigour was positively linked to TA, malate and YAN suggesting links with N nutrition and malic acid production which warrant investigation. In this high vigour situation, there were no other significant relationships between crop development and fruit composition variables.

It is also important to note that the effects of rootstock on conferred vigour, crop development and fruit composition of Cabernet Sauvignon were consistent across seasons, as shown by the lack of significant rootstock x season interactions. Further study is also required to monitor whether the large increase in yield in 2017 (two-fold) following conversion to mechanical hedging can be maintained in future seasons by all rootstocks and to assess the longer term impacts on both growth and fruit composition, particularly if enhancement in vine balance based on the Ravaz index can be maintained. Furthermore, the underpinning causes for the significant block effects on both growth characteristics and fruit composition warrant investigation.

2.1.3. Shiraz

A. Yield and its components, pruning weight and Ravaz index

Yield and yield component data, pruning weight and the Ravaz index, collected over five seasons (2013-17) for Shiraz grafted on 'near to release' selections and standard rootstocks is presented in Table 2.1.3.1. GLM, ANOVA analysis of the data showed significant effects of Block, Rootstock and Year for almost all variables. However, there were no significant rootstock and year interactions. Hence, only the means of rootstocks and seasons are presented in the absence of interactions.

There were significant effects of season on yield and its components, pruning weight and the Ravaz index of Shiraz (Table 2.1.3.1). Highest yield and bunch number were recorded in 2017, post conversion to simulated mechanical hedging in winter 2016. Season 2015 had the lowest yield. Bunch numbers were lowest in 2013. Largest and smallest bunches were produced in 2013 and 2016, respectively. Largest and smallest berries were recorded in 2013 and 2017, respectively. The highest and lowest berries per bunch were recorded in 2013 and 2015, respectively. The highest and lowest pruning weights were recorded in 2013 and 2016 respectively, despite the conversion to light mechanical hedging in 2017. There was more than a two-fold difference in the Ravaz index, an indicator of vine balance and a surrogate for carbon efficiency and water use efficiency, across the seasons being highest in in 2017 post conversion to mechanical hedging and lowest in 2013.

The significant effects of rootstock on yield and growth characteristics of Shiraz indicate that rootstocks could be classified into three groups, based on yield (Table 2.1.3.1). The highest yields and bunch numbers were produced by Ramsey and C114. Of the other rootstocks, 1103 Paulsen and C113 had the lowest yields. 1103 Paulsen and C20 had the lowest number of bunches. There was no effect of rootstock on bunch weight. Berry weights were highest with Ramsey and lowest with C20. Berries per bunch were highest with C20. There was highly significant effect of rootstock on conferred Shiraz vigour (pruning weight) with C114 and C112 having the highest vigour followed by Ramsey, 1103 Paulsen and C113. Shiraz grafted on 140 Ruggeri and C20 had the lowest vigour. There was more than a two-fold difference in the Ravaz index across the rootstocks with 140 Ruggeri and C20 having higher values than the other rootstocks. These results indicate significant potential of to use rootstocks to improve the vine balance of Shiraz

grown in irrigated hot climate vineyards and enhance fruit composition and wine quality attributes.

Table 2.1.3.1. Mean yield, bunches per vine, bunch weight, berry weight, berries per bunch, pruning weight and Ravaz index of Shiraz grafted on four CSIRO rootstock selections and three standard rootstocks over five seasons (2013-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***Yield (kg/vine)***	***Bunches/ vine***	***Bunch weight (g)***	***Berry weight (g)***	***Berries/ bunch***	***Pruning weight (kg/vine)[c]***	***Ravaz Index***
Ramsey	30.0ab	277b	110.3a	1.49a	84.8ab	5.44bc	5.69b
1103P	22.4c	205c	112.0a	1.33ab	87.7ab	4.58bc	5.14b
140R	25.6bc	259b	103.7a	1.31ab	81.6ab	3.63c	8.13a
C112	25.3bc	242b	107.3a	1.42ab	79.7ab	6.08a	4.61b
C113	23.4c	240b	102.0a	1.37ab	79.7ab	4.99bc	5.02b
C114	33.5a	313ab	102.0a	1.46ab	81.9ab	8.48a	4.20b
C20	24.1b	204c	124.1a	1.21b	97.2a	3.87c	7.26a
Year							
2013	24.4c	172d	144.2a	1.72a	104.1a	6.80a	3.89c
2014	30.5b	281b	110.8b	1.31cb	85.1b	5.46b	6.24b
2015	21.2d	199c	106.6b	1.44b	74.1c	5.31b	4.49c
2016	25.2c	279b	90.7c	1.22cd	74.8c	3.66c	7.65a
2017	35.2a	374a	94.8c	1.17d	81.4b	4.37	8.77a
Significance							
Block[a]	*	ns	**	ns	*	**	ns
Rootstock	***	***	ns	**	*	***	***
Year	***	***	***	***	***	***	***
R x Y[b]	ns	ns	ns	ns	ns	ns	ns

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively.

[b] RxY indicates the rootstock x season interaction.

Based on significant correlations, bunch number was the main driver of Shiraz yield across blocks, seasons and rootstocks (Table 2.1.3.2). There were no significant associations between bunch weight, berry weight, berries per bunch or pruning weight and yield. A highly significant multilinear regression ($p<0.001$) indicted that 95% of yield variation could be accounted for by inclusion of bunches, bunch weight and berry weight in the model. The negative correlation between bunch number and bunch weight indicates that vines carrying more bunches produced smaller bunches. The Ravaz index was more impacted by the strong negative association with pruning weight than the positive association with yield.

Table 2.1.3.2. Correlation matrix, describing linear relationships between yield component variables, pruning weight and the Ravaz index of Shiraz across the full data set including block, season and rootstock (n=131). Values in bold indicate significant correlations determined using the stringent Bonferroni analysis ($p<0.05$).

	Yield	***Bunches***	***Bunch weight***	***Berry weight***	***Berries per bunch***	***Pruning weight***
Bunches	**0.84**					
Bunch weight	0.07	**-0.44**				
Berry weight	0.03	-0.21	**0.31**			
Berries/ bunch	0.21	-0.25	**0.89**	0.08		
Pruning weight	0.23	-0.11	**0.54**	**0.41**	**0.41**	
Ravaz index	**0.42**	**0.64**	**-0.41**	**-0.31**	-0.22	**-0.69**

Correlation analyses were also conducted using only the rootstock means to explore relationships between yield determinants and growth characteristics across the rootstock genotypes. Both bunch number and berry weight were significant contributors to yield variability across the rootstock genotypes. The association between yield and pruning weight was not significant. A highly significant multilinear regression ($p<0.001$) indicated that 99.5% of yield variation across the rootstock genotypes could be accounted for by inclusion of bunches ($p<0.001$), bunch weight ($p<0.001$) and berry weight ($p=0.025$) in the model. Bunch weight was positively correlated with berries per bunch and negatively correlated with berry weight. The very strong negative association between pruning weight and the Ravaz index indicates that that pruning weight (2.3-fold differences) had a stronger influence on the ratio than yield (1.5-fold differences).

Table 2.1.3.3. Correlation matrix across rootstock means (n=9) describing relationships between rootstock growth characteristics and fruit composition of Shiraz with environmental variables removed. Values in bold indicate significant correlations, $p<0.1$.

	Yield	***Bunches***	***Bunch weight***	***Berry weight***	***Berries per bunch***	***Pruning weight***
Bunches	**0.95**					
Bunch weight	-0.37	**-0.57**				
Berry weight	**0.61**	**0.62**	**-0.62**			
Berries/ bunch	-0.39	**-0.62**	**0.94**	**-0.67**		
Pruning weight	0.41	0.30	-0.43	**0.67**	-0.30	
Ravaz index	0.27	0.36	-0.18	-0.37	0.07	**-0.73**

B. Fruit composition

Berry juice composition data collected over five seasons (2013-17) for Shiraz grafted on 'near to release' selections and standard rootstocks is presented in Table 2.1.3.4. GLM, ANOVA analysis of the data showed significant effects of Block, Rootstock and Year for most variables. The results indicate that there were significant differences in maturity when samples were collected, ranging from a mean of 23.3 ^{o}Brix in 2016 to 26.7 ^{o}Brix in 2016. To test the effect associated with the differences in maturity, TSS was included as a covariate in the ANOVA analysis. There were significant effects of TSS as a covariate on pH, TA and tartrate. Hence, adjusted means are presented where applicable (Table 2.1.3.4). There were no significant rootstock and year interactions, hence, only the means of rootstocks and seasons are presented.

There were significant effects of season on all berry juice variables (Table 2.1.3.4). These included juice pH (4.29 to 4.47 in 2013 and 2016 respectively), TA (3.04 to 3.34 g/L in 2014 and 2013 respectively), malate (2.95 to 3.99 g/L in 2017 and 2014 respectively), tartrate (4.43 to 5.98 g/L in 2013 and 2014 respectively), YAN (187 to 246 g/L in 2013 and 2016 respectively), anthocyanin (0.69 to 0.90 mg/g in both 2014 and 2017 and 2016 respectively) and phenolics (1.06 to 1.68 a.u. in 2014 and 2015 respectively).

Overall, the effects of rootstock on TSS were relatively small (range 24.0 - 26.0 ^{o}Brix) with 1103 Paulsen and C112 having the highest and C20 the lowest values. Rootstocks had significant effects on all juice compositional variables, except tartrate and phenolic substances (Table 2.1.3.4). 1103 Paulsen and C112 had the highest pH while C114 and C20 had the lowest pH. Rootstock effects on TA, although significant were relatively small (2.89 - 3.70 g/L) with C114 and 140 Ruggeri having the highest and lowest levels. There were significant effects of rootstock on malate (3.08 - 4.0 g/L) with selection C112 having the highest and 140 Ruggeri the lowest levels. YAN values ranged from 164 - 226 g/L with the standard rootstocks, C112, C113 and C114 having the highest levels and C20 the lowest level. The effect of rootstock on berry anthocyanin was small with C113 having the highest and C114 the lowest values.

Table 2.1.3.4. Mean juice TSS and adjusted means based on covariate analysis of pH, TA, Malate, Tartrate, YAN, anthocyanin and phenolic substances of Shiraz grafted on four CSIRO rootstock selections and three standard rootstocks over five seasons (2013-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***TSS (°Brix)***	***pH***	***TA (g/L)***	***Mal. (g/L)***	***Tart. (g/L)***	***YAN (mg/L)***	***Anth. (mg/g)***	***Phen. (a.u.)***
Ramsey	24.6bc	4.37b	3.30b	3.86ab	4.96	231a	0.77b	1.15
1103P	26.0a	4.44a	3.11b	3.79ab	5.01	239a	0.82b	1.20
140R	24.8b	4.39ab	3.06bc	3.34bc	5.01	221a	0.76bc	1.21
C112	26.0a	4.44ab	3.20b	4.00a	5.20	231a	0.73bc	1.03
C113	25.8ab	4.43ab	3.11b	3.83ab	5.23	222a	0.95a	1.28
C114	25.1ab	4.30c	3.70a	3.84ab	5.20	241a	0.82b	1.15
C20	24.4c	4.31c	3.30b	3.54ab	4.77	192b	0.87ab	1.27
Year								
2013	23.8cd	4.29c	3.34a	3.80a	4.43d	187b	-	-
2014	23.3d	4.39b	3.04b	3.99a	5.98a	201b	0.69	1.06c
2015	28.6a	4.42ab	3.31a	3.90a	4.48d	240a	0.89	1.35a
2016	26.7b	4.47a	3.21ab	3.66a	4.98c	246a	0.90	1.22b
2017	24.2c	4.32c	3.18ab	2.95b	5.32b	241a	0.69	1.07c
Sig.								
Block[a]	*	ns	***	**	ns	***	*	ns
Rootstock.	*	***	***	**	ns	*	*	ns
Year	***	***	*	***	***	**	***	***
R x Y[b]	ns	ns	ns	ns	ns	ns	ns	ns
TSS covar.		***	*	ns	*	ns	ns	ns

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively.

[b] RxY indicates the rootstock x season interaction.

C. **Relationships between growth characteristics and fruit composition**

Correlation analyses were conducted using only the rootstock means to explore relationships between vine growth characteristics and berry juice composition across the rootstock genotypes with variation association with block and season removed (Table 2.1.3.5). Across the rootstock genotypes, yield and bunch number were negatively correlated with TA, malate but positively correlated with phenolics. Berry weight correlated negatively with pH and malate. There was significant impact of conferred vigour (pruning weight) on juice composition as shown by the negative correlations with TSS, pH, malate, tartrate and YAN. There was no significant association between pruning

weight and anthocyanin or phenolics. The Ravaz index was positively linked to TSS, pH and tartrate.

Table 2.1.3.5. Correlation matrix, describing linear relationships between growth characteristics (yield component variables, pruning weight and the Ravaz index) and berry juice composition of Shiraz across rootstock (n=8). Values in bold indicate significant correlations ($p<0.10$).

	Yield	*Bunches*	*Bunch weight*	*Berry weight*	*Berries per bunch*	*Pruning weight*	*Ravaz index*
TSS	0.10	0.24	-0.30	-0.31	-0.07	**-0.72**	**0.81**
pH	-0.16	-0.02	-0.13	**-0.61**	0.08	**-0.90**	**0.85**
TA	**-0.84**	**-0.86**	0.37	-0.54	0.47	-0.11	-0.44
Malate	**-0.69**	**-0.63**	0.25	**-0.87**	0.48	**-0.76**	0.39
Tartrate	-0.22	-0.09	-0.22	-0.37	0.04	**-0.79**	**0.64**
YAN	-0.41	-0.41	0.31	-0.52	0.43	**-0.74**	0.44
anthocyanin	0.40	0.44	-0.26	0.01	-0.23	0.25	0.11
phenolics	**0.63**	**0.64**	-0.25	0.28	-0.31	0.40	0.06

Multilinear regression techniques were used to identify the best combinations of growth traits with strong predictive value for Shiraz juice and berry composition measured across the rootstock genotypes. The results show that a significant proportion of the variability in berry composition across the rootstock genotypes could be accounted for by variation in growth characteristics based on adjusted R^2 values including:

- 44% (p=0.044) of TSS by pruning weight (-ve)
- 78% (p=0.002) of pH by pruning weight (-ve)
- 69% (p=0.006) of TA by bunch number (-ve)
- 71% (p=0.005) of malate by berry weight (-ve)
- 57% (p=0.019) of tartrate by pruning weight (-ve)
- 47% (p=0.034) of YAN by pruning weight (-ve)

E. Small-scale winemaking

Single ferment Shiraz wines were produced from selected rootstocks in 2014 with the intent to identify potential fermentation issues, assess whether pH adjustment from tartaric acid addition led to unbalanced wines; describe the impacts of rootstocks on spectral properties and to provide wine samples for tasting by industry. Formal sensory assessments were not conducted although preferences were recorded during internal assessments.

The fruit for winemaking was harvested on 13/02/2014. The must data show some minor differences in fruit maturity, which in all cases were below the desired level of around 24 ^{o}Brix. (Table 2.1.3.6). Ramsey had the highest must TSS and C114 the lowest value. Ramsey had the highest and C20 the lowest pH. C114 had the highest and Ramsey the lowest TA levels. Tartaric acid additions of 2.5-3.0 g/L were used for pH adjustment of the rootstock musts prior to fermentation with further, minor additions made during racking procedures. At bottling, C20 wine had a very low pH of 3.3 and a high TA of 8.6 g/L suggesting overcorrection of pH by acid addition leading to an unbalanced wine. pH values for the other wines were below or close to the desired maximum level of pH 3.6 with acceptable TA levels. There were only minor differences between the wines in free sulfur (30 - 38 ppm), total sulfur (60-92 ppm) and volatile acidity (0.07-14%). Ramsey wine, described as having fruit characters with soft tannins with a fresh fruity character were preferred. The C114 wine was described as having herbaceous, fruit character with soft tannins. The C20 was described as having fruity, berry flavoured characteristics. Wine spectral properties tended to reflect the different maturity levels with Ramsey and C20 having the highest colour density and highest levels of ionised anthocyanins and phenolic substances. Differences in hue indicate that C20 and C114 produced the brightest and dullest wines, respectively. C114 wines had a lower total anthocyanin than the other wines, which is not surprising given its high yield (Table 2.1.3.1). C20 wine had the highest level of anthocyanin.

Table 2.1.3.6. Must, wine data, spectral properties (colour density, hue, anthocyanin and phenolic substances) and preference rankings for Shiraz wines produced from two standard rootstocks (Ramsey and 140 Ruggeri) and three CSIRO selections, season 2014.

	Must data			***Bottling data***			***Spectral properties***					***Pref.***
	TSS (°Brix)	**pH**	**TA** (g/L)	**pH**	**TA** (g/L)	**Alc.** (%)	CD	Hue	A	IA	Phen.	
Ram.	22.8	4.17	3.92	3.62	6.54	13.7	4.46	0.65	208	35.1	23.8	****
C114	21.9	4.10	4.26	3.59	7.03	11.8	2.18	0.82	187	12.8	17.8	***
C20	22.4	4.05	4.17	3.33	8.64	12.5	4.23	0.53	230	34.9	24.1	***

F. Discussion re Shiraz and performance of CSIRO 'near to release' rootstocks

The results over five seasons show significant effects of rootstock genotype on the vine performance and fruit composition of Shiraz, associated with conferred vine vigour. In the high vigour category, the CSIRO selections C112, C113 and C114 may provide alternative rootstocks to the standard rootstocks Ramsey and 1103 Paulsen for Shiraz production in hot climates as their productivity and fruit composition were similar. C112, C113 and C114 produced riper fruit than Ramsey. In the moderate vigour rootstock category, selection C20 was able to adequately mature the crop, despite its high Ravaz index, and may be a useful alternative to 140 Ruggeri for enhanced water use efficiency, for establishment in high density plantings to improve vineyard productivity and sustainability and also for use in sites with high vigour potential.

The main driver of yield across the rootstock genotypes was bunch number (r = 0.95) and to a lesser extent berry weight (r=0.61). While they have been grouped above based on conferred vigour, the relationships with Shiraz yield and bunch number were not significant (r =0.41 and 0.30, respectively). Such results indicate that the effects of rootstock genotype on Shiraz fruitfulness warrant further investigation. Conferred vigour was however associated with the development of larger berries (r = 0.67).
Further study of the underpinning physiology leading to the relationships with inherent vine vigour and fruit composition (TSS, pH, malate tartrate, YAN) and the effects on yield, bunch number and berry weight are required. In this Shiraz study, high conferred vigour was not only negatively correlated with TSS but also pH, malate, tartrate and YAN.

It is also important to note that the effects of rootstock on inherent vigour, crop development of Shiraz were consistent across seasons, as shown by the lack of significant

rootstock x season interactions. However, there were significant rootstock x year interactions for all variables assessed for fruit composition which require further elucidation. Further study is also required to monitor whether the large increase in yield in 2017 (50%) following conversion to mechanical hedging can be maintained in future seasons by all rootstocks and to assess the longer term impacts on both growth and fruit composition. Furthermore, the underpinning causes for the significant block effects on both growth characteristics and fruit composition warrant investigation.

2.1.4. Scion x rootstock interactions

The experimental design of the trial was established to enable assessment of scion x rootstock interactions, hence the adaptability of rootstocks to different varieties. The CSIRO selection C29 was excluded from the analysis because of its poor performance with both Chardonnay and Cabernet Sauvignon and graft failure with Shiraz.

A. Yield and its components, pruning weight and Ravaz Index

The growth characteristics results (i.e. yield and its components, pruning weight and Ravaz Index) of the combined analysis across varieties and rootstocks are presented in Tables 2.1.4.1-7. Despite the fact that the trial was confined to a limited area (i.e. five rows of 60 vines) with blocks allocated along the rows, there were significant effects of block on yield (19.0-24.9.kg/vine), bunch number (211-266) and pruning weight (3.57-5.13 kg/vine) but not on bunch weight, berry weight, berries per bunch and Ravaz index. Significant scion x rootstock interactions were determined for yield, bunch number, berry weight, pruning weight and Ravaz index. The effect of rootstock on bunch weight and berries per bunch was consistent across scions.

The yield results presented in Table 2.1.4.1 show that Chardonnay and Shiraz had similar yields whereas the yield of Cabernet Sauvignon was 40% lower, despite the consistent management across the varieties. Over all scions, there was a 25% difference in yield attributed to rootstock with Ramsey, C113 and C114 producing the highest yield and 140 Ruggeri, C112 and C20 the lowest yield. The main effects of the highly significant scion x rootstock interaction were associated with the low yield of Cabernet Sauvignon grafted on 1103 Paulsen, 140 Ruggeri and C112 particularly when compared to their performance when grafted with Chardonnay and the high yield of C114 when grafted with Cabernet Sauvignon and Shiraz, compared to their yield when grafted with Chardonnay.

Chardonnay and Shiraz had similar bunch numbers whereas Cabernet Sauvignon had 20% fewer bunches (Table 2.1.4.2). Over all scions there was a 26% difference in bunch number attributed to rootstock with Ramsey, C113 and C114 having the most bunches and 1103 Paulsen, 140 Ruggeri, C112 and C20 the lowest number of bunches. The main effects of the highly significant scion x rootstock interaction were associated with the low bunch number of Cabernet Sauvignon grafted on 1103 Paulsen, 140 Ruggeri and C112 particularly when compared to Chardonnay.

Table 2.1.4.1. Mean yield per vine of three scion varieties (Chardonnay, Cabernet Sauvignon and Shiraz) grafted on four CSIRO rootstock selections and three standard rootstocks over five seasons (2013-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values GLM-ANOVA analysis is also presented. The **highest** and *lowest* mean values are shown in bold and italics respectively for each scion rootstock combination and overall rootstock response to facilitate interpretation of the scion x rootstock interactions.

Rootstock	***Chardonnay***	***Cabernet S.***	***Shiraz***	***mean***
Ramsey	**29.3**	**18.0**	**29.9**	**25.8**a
1103 Paulsen	**29.5**	*12.9*	*22.4*	21.6b
140 Ruggeri	25.0	*11.8*	25.8	*20.8*b
C112	22.9	*10.3*	25.3	*19.5*b
C113	**28.4**	**18.7**	*23.4*	23.5ab
C114	25.7	**18.4**	**33.5**	**25.9**a
C20	*19.0*	17.7	24.2	20.3b
mean	**25.2b**	**15.9c**	**27.4a**	
Summary	Block	Scion	Rootstock	Interaction
significance[a]	**	***	***	***
LSD (p = 0.05)		1.9	3.1	5.4

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively.

Table 2.1.4.2. Mean bunch number per vine of three scion varieties (Chardonnay, Cabernet Sauvignon and Shiraz) grafted on four CSIRO rootstock selections and three standard rootstocks over five seasons (2013-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented. The **highest** and *lowest* mean values are shown in bold and italics respectively for each scion rootstock combination and overall rootstock response to facilitate interpretation of the scion x rootstock interactions.

Rootstock	***Chardonnay***	***Cabernet S.***	***Shiraz***	***mean***
Ramsey	**309**	**235**	277	**273**a
1103 Paulsen	285	*175*	*208*	*222*bc
140 Ruggeri	254	*176*	264	*231*b
C112	245	*158*	242	*215*bc
C113	**302**	**246**	240	**263**ab
C114	251	231	**313**	**265**ab
C20	*179*	221	*207*	*202*c
mean	**255a**	**209b**	**264a**	
Summary	**Block**	**Scion**	**Rootstock**	**Interaction**
significance[a]	***	***	***	***
LSD (p = 0.05)	20		32	55

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively.

Chardonnay and Shiraz had similar bunch weights whereas Cabernet Sauvignon had 20% smaller bunches (Table 2.1.4.3). Over all scions there was an 18% difference in bunch weight attributed to rootstock with C20 producing the largest bunches and 140 Ruggeri and C112 the smallest bunches. The scion x rootstock interaction was not significant.

Table 2.1.4.3. Mean bunch weight of three scion varieties (Chardonnay, Cabernet Sauvignon and Shiraz) grafted on four CSIRO rootstock selections and three standard rootstocks over five seasons (2013-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***Chardonnay***	***Cabernet***	***Shiraz***	***mean***
Ramsey	97.3	74.0	110.2	**93.9ab**
1103 Paulsen	108.9	68.8	109.8	**95.8 ab**
140 Ruggeri	102.0	64.3	102.1	**89.5b**
C112	96.4	59.7	107.3	**87.8b**
C113	97.1	75.6	102.0	**91.6ab**
C114	103.6	80.4	110.5	**98.2ab**
C20	117.6	81.3	122.2	**107.1a**
mean	**103.5a**	**73.3b**	**108.3a**	
Summary	**Block**	**Scion**	**Rootstock**	**Interaction**
significance[a]	ns	***	*	ns
LSD (p = 0.05)		5.9	9.6	

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively

Shiraz had the largest berries and Cabernet Sauvignon the smallest berries (Table 2.1.4.4). Overall, there was no significant effect of rootstock on berry weight, range 1.18 - 2.25 g. The weak, but significant scion x rootstock interaction can be attributed to the effect on berry weight of Ramsey (small with Chardonnay and large with Shiraz) and C20 (large with Chardonnay and Cabernet Sauvignon and small with Shiraz).

Table 2.1.4.4. Mean berry weight of three scion varieties (Chardonnay, Cabernet Sauvignon and Shiraz) grafted on four CSIRO rootstock selections and three standard rootstocks over five seasons (2013-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented. The **highest** and *lowest* mean values are shown in bold and italics respectively for each scion rootstock combination to facilitate interpretation of the scion x rootstock interactions.

Rootstock	***Chardonnay***	***Cabernet***	***Shiraz***	***mean***
Ramsey	*1.18*	1.04	**1.49**	**1.24**
1103 Paulsen	1.20	1.02	1.32	**1.18**
140 Ruggeri	1.20	1.02	1.30	**1.17**
C112	1.22	*0.97*	1.42	**1.21**
C113	*1.19*	1.03	1.37	**1.20**
C114	1.23	**1.06**	**1.46**	**1.25**
C20	**1.29**	**1.05**	*1.20*	**1.18**
mean	**1.21b**	**1.03c**	**1.37a**	
Summary	**Block**	**Scion**	**Rootstock**	**Interaction**
significance[a]	Ns	*******	ns	*****
LSD (p = 0.05)		0.42		0.12

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively

Chardonnay and Shiraz had a similar number of berries per bunch whereas Cabernet Sauvignon had 20% fewer berries per bunch (Table 2.1.4.5). Over all scions there was an 18% difference in berries per bunch attributed to rootstock with C20 having the most berries and C112 the least berries per bunch. The scion x rootstock interaction was not significant.

Table 2.1.4.5. Mean berries per bunch of three scion varieties (Chardonnay, Cabernet Sauvignon and Shiraz) grafted on four CSIRO rootstock selections and three standard rootstocks over five seasons (2013-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***Chardonnay***	***Cabernet***	***Shiraz***	***mean***
Ramsey	82.5	71.2	84.8	**79.5ab**
1103 Paulsen	90.5	67.5	85.9	**81.3ab**
140 Ruggeri	85.0	63.5	80.7	**76.4ab**
C112	79.0	60.4	79.7	**73.0b**
C113	81.4	73.5	79.7	**78.1ab**
C114	84.7	76.4	81.9	**81.0ab**
C20	90.1	77.7	96.7	**88.2a**
Mean	**86.6a**	**71.3b**	**83.3a**	
Summary	**Block**	**Scion**	**Rootstock**	**Interaction**
significance[a]	ns	***	*	ns
LSD (p = 0.05)		5.4	8.9	

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively

There was a three-fold difference in inherent vigour between the scions, with Cabernet Sauvignon vines having highest pruning weight and Chardonnay lowest pruning weight (Table 2.1.4.6). Over all scions there was a two-fold difference in pruning weight attributed to rootstock with Ramsey, C112, C113 and C114 having the highest conferred vigour and 140 Ruggeri and C20 the lowest. The main effects of the highly significant scion x rootstock interaction were associated with the low pruning weight of Cabernet Sauvignon and Shiraz grafted on 140 Ruggeri and the relatively lower pruning weight of Shiraz grafted on C113.

Table 2.1.4.6. Mean pruning weight (kg/vine) of three scion varieties (Chardonnay, Cabernet Sauvignon and Shiraz) grafted on four CSIRO rootstock selections and three standard rootstocks over five seasons (2013-17). Means followed by the same letter are not significantly different (p = 0.05. A summary of the significance values, generated from the GLM-ANOVA analysis is also presented. The **highest** and *lowest* mean values are shown in bold and italics respectively for each scion rootstock combination and overall rootstock response to facilitate interpretation of the scion x rootstock interactions.

Rootstock	***Chardonnay***	***Cabernet***	***Shiraz***	***mean***
Ramsey	**2.57**	**8.42**	5.44	**5.49**ab
1103 Paulsen	2.53	7.08	4.58	4.73b
140 Ruggeri	2.21	*5.30*	*3.63*	*3.73*c
C112	2.32	**7.94**	**6.08**	**5.44**ab
C113	**2.88**	**7.63**	4.99	**5.17**ab
C114	2.77	7.12	**8.48**	**6.13**a
C20	*1.48*	*4.35*	*3.87*	*3.24*c
mean	**2.27c**	**6.57a**	**5.12b**	
Summary	**Block**	**Scion**	**Rootstock**	**Interaction**
significance[a]	***	***	***	**
LSD (p = 0.05)		0.40	0.64	1.12

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively

There was a five-fold difference in Ravaz index between the scions with Chardonnay having the highest and Cabernet Sauvignon the lowest value (Table 2.1.4.7). These results indicate that Cabernet Sauvignon, as managed in this study has low carbon assimilation efficiency and low water use efficiency due to the combined effects of low yield and high vigour. In contrast, Chardonnay with a high Ravaz index has high carbon assimilation efficiency and low water use efficiency due to the combined effects of its high yield and low vigour. The Ravaz index value of Shiraz fell between Cabernet Sauvignon and Chardonnay, indicating moderate carbon assimilation and water use efficiency due to a combination of high yield and high vigour. Over all scions there was a two-fold difference in Ravaz index attributed to rootstock with C20 having the highest values and C112, C113, and C114 the lowest values. The main effects of the weak but significant scion x rootstock interaction were associated with the low value of Cabernet Sauvignon and to a lesser extent Shiraz, grafted on 1103 Paulsen and C112 compared to Chardonnay. The Ravaz index results also show potential to select rootstocks with enhanced carbon assimilation and water use efficiency.

Table 2.1.4.7. Mean Ravaz index (yield/pruning weight) of three scion varieties (Chardonnay, Cabernet Sauvignon and Shiraz) grafted on four CSIRO rootstock selections and three standard rootstocks over five seasons (2013-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented. The **highest** and *lowest* mean values are shown in bold and italics respectively for each scion rootstock combination and overall rootstock response to facilitate interpretation of the scion x rootstock interactions.

Rootstock	***Chardonnay***	***Cabernet***	***Shiraz***	***mean***
Ramsey	11.81	2.44	5.69	6.65cd
1103 Paulsen	12.36	*1.95*	5.20	6.50cd
140 Ruggeri	12.16	3.22	**8.31**	7.89b
C112	11.15	*1.47*	*4.61*	*5.75d*
C113	*9.85*	2.51	*5.02*	*5.80d*
C114	*9.45*	2.95	*4.20*	*5.54d*
C20	**13.91**	**4.61**	**7.38**	**8.63**ab
mean	**12.11a**	**3.03c**	**6.29b**	
Summary	**Block**	**Scion**	**Rootstock**	**Interaction**
significance[a]	**ns**	*******	*******	*****
LSD (p = 0.05)		0.70	1.12	1.97

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively

In summary, across the five seasons and three varieties it can be concluded that:

- there was a significant effect of rootstock genotype on conferred vigour. The rootstock genotypes could be classified into four categories based on conferred vigour with C114 having very high vigour; Ramsey, C112 and C113 having high vigour; 1103 Paulsen having moderate vigour; and 140 Ruggeri and C20 having low vigour. However, care should be taken in using this general classification because of the significant scion x rootstock interaction described above. The relative poor performance of 1103 Paulsen and 140 Ruggeri in the replant situation could be due to their sensitivity to root knot nematodes in the replant situation.
- there was a significant effect of rootstock genotype on yield with Ramsey, C114 and C113 producing the highest yields compared to the lower yielding 1103 Paulsen, 140 Ruggeri, C112 and C20. However, care should be taken in using this general classification because of the significant scion x rootstock interaction described above.

- there was a significant effect of rootstock genotype on bunch number with Ramsey, C113and C114 producing high bunch numbers compared to 140 Ruggeri, 1103 Paulsen, C112 and C20.
- there was a significant effect of rootstock genotype on vine balance measured as the Ravaz index which provides an indicator of carbon and water use efficiency. C20 and 140 Ruggeri had the highest Ravaz index while Ramsey, 1103 Paulsen, C112, C113 and C114 had the lowest values. However, care should be taken in using this general classification because of the highly significant scion x rootstock interaction described above.
- there was no effect of rootstock genotype on berry weight while the effects of rootstock genotype on bunch weight and berries per bunch were small ($p<0.05$).

B. Fruit composition

The fruit compositional data from the combined analysis across varieties and rootstocks are presented in Tables 2.1.4.10 - 19. There were significant effects of block on TA, malate, YAN, and phenolics. Significant scion x rootstock interactions were only found for pH ($p<0.05$) and YAN ($p<0.001$).

The results for TSS show that all varieties were on average, harvested at a similar maturity, around 25 ^{o}Brix (Table 2.1.4.10). This result indicates that maturity differences across the varieties can be eliminated as source of variability in assessing the effects of rootstock and the scion x rootstock interactions below. The significant rootstock response shows that on average 140 Ruggeri had the highest TSS, followed by C113 and Ramsey with C20, C112, C114 and 1103 Paulsen having lowest TSS.

Chardonnay juice had a lower pH, than Cabernet Sauvignon or Shiraz at harvest, despite there being no difference in maturity (Table 2.1.4.11). The significant rootstock effect showed that 140 Ruggeri, C112, C1134 had the highest pH followed by 1103 Paulsen, C20 and Ramsey with C114 having the lowest pH. The weak scion x rootstock interaction can be attributed to the high pH of C112 and C113 and low pH of C20 with Chardonnay; the high and low pH of C20 and Ramsey respectively with Cabernet Sauvignon and the high and low pH of Shiraz with C114.

Table 2.1.4.10. Mean TSS (°Brix) of three scion varieties (Chardonnay, Cabernet Sauvignon and Shiraz) grafted on four CSIRO rootstock selections and three standard rootstocks over five seasons (2013-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***Chardonnay***	***Cabernet***	***Shiraz***	***mean***
Ramsey	25.1	25.5	25.5	**25.1ab**
1103 Paulsen	24.2	23.9	24.6	**24.2b**
140 Ruggeri	25.6	25.3	26.3	**25.6a**
C112	24.7	24.2	24.6	**24.7b**
C113	25.2	25.7	25.1	**25.2ab**
C114	24.7	25.0	24.4	**24.7b**
C20	24.8	25.1	25.3	**24.8b**
mean	**24.7**	**25.0**	**25.2**	
Summary	Block	Scion	Rootstock	Interaction
significance[a]	ns	ns	*	ns
LSD (p = 0.05)			0.9	

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively

Table 2.1.4.11. Mean pH of three scion varieties (Chardonnay, Cabernet Sauvignon and Shiraz) grafted on four CSIRO rootstock selections and three standard rootstocks over five seasons (2013-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented. The **highest** and *lowest* mean values are shown in bold and italics respectively for each scion rootstock combination and overall rootstock response to facilitate interpretation of the scion x rootstock interactions.

Rootstock	***Chardonnay***	***Cabernet***	***Shiraz***	***mean***
Ramsey	4.04	*4.24*	4.34	4.21cd
1103 Paulsen	4.02	4.35	4.33	4.23bcd
140 Ruggeri	4.04	**4.42**	**4.48**	**4.31**a
C112	**4.13**	4.38	4.36	4.29abc
C113	**4.10**	4.33	4.33	4.25abcd
C114	3.99	4.33	*4.21*	*4.17*d
C20	*3.91*	**4.43**	4.43	4.22cd
mean	**4.02**b	**4.34**a	**4.37**a	
Summary	Block	Scion	Rootstock	Interaction
significance[a]	ns	*******	******	*****
LSD (p = 0.05)		.04	.07	0.11

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively

Chardonnay juice had highest and Shiraz the lowest juice TA at harvest (Table 2.1.4.12). Across the varieties there was no effect of rootstock on the mean TA (range 3.54 - 4.00). The scion x rootstock interaction was also not significant.

Table 2.1.4.12. Mean TA (g/L) of three scion varieties (Chardonnay, Cabernet Sauvignon and Shiraz) grafted on four CSIRO rootstock selections and three standard rootstocks over five seasons (2013-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***Chardonnay***	***Cabernet***	***Shiraz***	***mean***
Ramsey	4.83	3.47	2.98	**3.76**
1103 Paulsen	4.74	3.89	3.38	**4.00**
140 Ruggeri	4.50	3.82	3.07	**3.80**
C112	4.62	3.77	3.20	**3.86**
C113	4.71	3.73	3.28	**3.91**
C114	4.75	3.57	3.09	**3.80**
C20	4.96	3.71	3.30	**3.99**
mean	**4.65a**	**3.69b**	**3.16c**	
Summary	Block	Scion	Rootstock	Interaction
significance[a]	*	***	ns	ns
LSD (p = 0.05)		0.19		

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively

Cabernet Sauvignon juice had the highest and Shiraz the lowest juice malate levels (Table 2.1.4.13). The significant rootstock effect shows that 140 Ruggeri, 1103 Paulsen and C20 had the highest malate levels while C114 and Ramsey had the lowest levels. The scion x rootstock interaction was not significant.

Cabernet Sauvignon juice had higher tartrate levels than Chardonnay or Shiraz (Table 2.1.4.14). Across the varieties, there was no effect of rootstock on mean tartrate levels (range 4.98 - 5.44). The scion x rootstock interaction was not significant.

Table 2.1.4.13. Mean malate (g/L) of three scion varieties (Chardonnay, Cabernet Sauvignon and Shiraz) grafted on four CSIRO rootstock selections and three standard rootstocks over five seasons (2013-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***Chardonnay***	***Cabernet***	***Shiraz***	***mean***
Ramsey	4.55	4.18	3.13	**3.95c**
1103 Paulsen	4.58	4.91	3.72	**4.40ab**
140 Ruggeri	4.40	5.01	3.96	**4.45a**
C112	4.68	4.68	3.57	**4.01b**
C113	4.63	4.60	3.32	**4.18ab**
C114	4.30	4.55	2.82	**3.89c**
C20	4.51	4.56	4.04	**4.38ab**
mean	**4.40b**	**4.64a**	**3.48c**	
Summary	Block	Scion	Rootstock	Interaction
significance[a]	*	***	**	ns
LSD (p = 0.05)		0.24	0.39	

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively

Table 2.1.4.14. Mean tartrate of three scion varieties (Chardonnay, Cabernet Sauvignon and Shiraz) grafted on four CSIRO rootstock selections and three standard rootstocks over five seasons (2013-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***Chardonnay***	***Cabernet***	***Shiraz***	***mean***
Ramsey	4.84	5.41	5.13	**5.13**
1103 Paulsen	5.00	5.60	4.77	**5.12**
140 Ruggeri	5.09	5.82	5.41	**5.44**
C112	5.22	5.34	4.84	**5.14**
C113	5.00	5.69	4.96	**5.22**
C114	5.02	5.45	4.46	**4.98**
C20	4.92	5.55	5.04	**5.17**
mean	**4.97b**	**5.55a**	**4.95b**	
Summary	Block	Scion	Rootstock	Interaction
significance[a]	ns	***	ns	ns
LSD (p = 0.05)		0.23		

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively

Chardonnay juice had highest and Cabernet Sauvignon the lowest levels of YAN (Table 2.1.4.15). The significant rootstock effect shows that 1103 Paulsen had the highest levels of YAN, followed by 140 Ruggeri, C113, C20 and C112 while C114 had the lowest YAN level. The highly significant scion x rootstock interaction can be attributed to the high juice YAN of Ramsey and C113 grafted with Chardonnay, the low YAN of Cabernet Sauvignon on Ramsey and the high YAN of Shiraz on C20.

Table 2.1.4.15. Mean YAN (mg/L) of three scion varieties (Chardonnay, Cabernet Sauvignon and Shiraz) grafted on four CSIRO rootstock selections and three standard rootstocks over five seasons (2013-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented. The **highest** and *lowest* mean values are shown in bold and italics respectively for each scion rootstock combination and overall rootstock response to facilitate interpretation of the scion x rootstock interactions.

Rootstock	***Chardonnay***	***Cabernet***	***Shiraz***	***mean***
Ramsey	**279**	*156*	225	220b
1103 Paulsen	**280**	**233**	230	**247**a
140 Ruggeri	270	210	219	233ab
C112	247	199	235	227ab
C113	**295**	212	189	232ab
C114	233	187	*151*	*190*c
C20	221	213	**251**	228ab
mean	**253a**	**198c**	**215b**	
Summary	Block	Scion	Rootstock	Interaction
significance[a]	*	***	**	***
LSD (p = 0.05)		16	25	44

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively

Cabernet Sauvignon had significantly higher levels of anthocyanin than Shiraz (Table 2.1.4.18). Across the varieties, there was no effect of rootstock on mean anthocyanin (range 0.76 - 1.01). The scion x rootstock interaction was not significant.

Cabernet Sauvignon had significantly higher levels of phenolic substances than Shiraz (Table 2.1.4.18). Across the varieties, there was no effect of rootstock on phenolic substances (range 1.23 -1.44). The scion x rootstock interaction was not significant.

Table 2.1.4.18. Mean anthocyanin (mg/g) of two scion varieties (Chardonnay, Cabernet Sauvignon and Shiraz) grafted on four CSIRO rootstock selections and three standard rootstocks over five seasons (2013-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***Chardonnay***	***Cabernet***	***Shiraz***	***mean***
Ramsey	-	1.05	0.73	**0.89**
1103 Paulsen	-	1.01	0.77	**0.89**
140 Ruggeri	-	1.01	0.88	**0.94**
C112	-	0.86	0.65	**0.76**
C113	-	1.08	0.87	**0.98**
C114	-	1.06	0.97	**1.01**
C20	-	0.97	0.77	**0.87**
mean	**-**	**1.00a**	**0.81b**	
Summary	Block	Scion	Rootstock	Interaction
significance[a]	ns	*******	ns	ns
LSD (p = 0.05)		0.06		

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively

Table 2.1.4.19. Mean phenolic substances of three scion varieties (Chardonnay, Cabernet Sauvignon and Shiraz) grafted on four CSIRO rootstock selections and three standard rootstocks over five seasons (2013-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***Chardonnay***	***Cabernet***	***Shiraz***	***mean***
Ramsey	-	1.50	1.19	**1.35**
1103 Paulsen	-	1.49	1.15	**1.32**
140 Ruggeri	-	148	1.20	**1.34**
C112	-	1.36	1.09	**1.23**
C113	-	1.61	1.28	**1.44**
C114	-	1.51	1.36	**1.43**
C20	-	1.42	1.17	**1.30**
mean	**-**	**1.48a**	**1.21b**	
Summary	Block	Scion	Rootstock	Interaction
significance[a]	*****	*******	ns	ns
LSD (p = 0.05)		0.05		

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively

In summary, across the five seasons and three varieties it can be concluded that:

- there was no effect of rootstock genotype on juice titratable acidity, tartrate, berry anthocyanin or phenolic substances.
- there were significant, but consistent responses of rootstock genotype across the varieties for juice TSS and malate. 140 Ruggeri had the highest TSS, followed by C113 and Ramsey. C20, C112, C114 and 1103 Paulsen had the lowest TSS. 140 Ruggeri, 1103 Paulsen and C20 had the highest malate. C114 and Ramsey had the lowest malate.
- there was a significant and reasonably consistent response of rootstock genotype on pH as the scion x rootstock interaction was just significant ($p<0.05$). 140 Ruggeri, Ruggeri, C112, C113 had the highest pH followed by 1103 Paulsen, C20 and Ramsey with C114 having the lowest pH.
- there were significant effects of rootstock genotype on YAN, however highly significant scion x rootstock interactions indicate that the effects were not consistent across the scions. 1103 Paulsen had the highest YAN, followed by 140 Ruggeri, C113, C20 and C112. C114 had the lowest levels of YAN, although above the minimum level of 150 mg/L required for fermentation.

2.1.5. 'Near to release' selections: Shiraz Padthaway

The Shiraz trial was established in a commercial vineyard in Padthaway in 2010 with 26 rootstock selections and four commercial rootstocks (Ramsey, 140 Ruggeri, 1103 Paulsen and 101-14). The 101-14 rootstock was included as it is considered by industry to be an important rootstock for the region. A further selection involving *Vitis rotundifolia*, M920, was also included as it had good performance in a high boron situation in table grape studies conducted in Carnarvon in Western Australia and was thought to have potential for salt tolerance.

The replicated trial was designed to:

- assess the performance of Shiraz on the 27 selections compared to standard commercial rootstocks when grown under moderately saline conditions in Padthaway
- test the efficacy of early screening procedures conducted in CSP 99/2.

The original 26 genotypes were selected based on screens conducted with ungrafted vines for potassium uptake, vigour, response to short term drought, root architecture, rooting and grafting. Six of the selections, covering a range in conferred vigour were identified with potential for release in the previous project CSP 0503 ('near to release' selections) having root knot nematode and phylloxera resistance, good propagation and performance characteristics (Clingeleffer and Smith 2011). Their performance has been monitored in this study, seasons 2014-2017. Soil type was a sandy loam over clay changing from a red to yellow mottle at around 45 cm depth. Soil samples were taken at harvest in 2016 at three depths, 0-30 cm (Top), 31-60 cm (Middle) and 61-90 cm (Bottom) with a hand-held auger from three sampling positions located across the trial. A 1:5 soil:water extract was prepared from each sample and electrical conductivity (EC), soluble ion (Cl^-, Ca^{2+}, K^+, Mg^{2+} and Na^+) concentrations, sodium adsorption ratio (SAR) (Richards 1954) and cation exchange capacity were determined by CSIRO Land and Water Analytical Services.

Mean total soluble salts in the irrigation water in at harvest in 2016 was 1732 $\pm$ 47 mg/L, equivalent to 2.71 dS/m. Electrical conductivity of the 1:5 soil: water extract increased

with depth, from 0.53 dS/m in the top layer to 1.0 dS/m in the bottom layer (Table 2.1.5.1). Soluble Na^+ concentrations (range 46.7 - 124.7 mg/L) increased with depth. Soluble Cl^- were highest in the middle layer (197 mg//L) and lowest in the top layer (55 mg/L). Soluble Cl^- exceeded Na^+ concentrations at all depths. Soluble S concentrations were lowest in the upper layer (4.3 mg/L). Soluble calcium (Ca^{2+}) concentrations (range 13.7- 24.3 mg/L) exceeded soluble magnesium (Mg^{2+}) concentrations (mean 4.4 mg/L) range by 2.9 to 6.4 fold, the ratio increasing with depth. Soluble potassium (K^+ mg/L) concentrations were highest in the middle layer (8.0 mg/L) and lowest in the bottom layer (5.33 mg/l). Sodium adsorption ratios were lowest in the top layer (2.13) compared to the other layers (4.4 - 4.5). Values above 3 are indicative of a sodic soil (Cass 2002). Cation exchange capacity, i.e. the capacity of the soil to hold exchangeable cations, was lower in the surface layer 12.0 ($cmol_c/kg$) compared to deeper layers (25-27 $cmol_c/kg$) (Table 2.1.5.1).

Table 2.1.5.1. Soluble concentrations of mineral elements (Ca, K, Mg, Na, S), Cl^-, cation exchange (CEC), sodium adsorption ration (SAR) and electrical conductivity (EC) of a 5:1 soil solution (n=3). ANOVA was conducted to assess the effects of depth through the profile.

		Ca *(mg/L)*	***K*** *(mg/L)*	***Mg*** *(mg/L)*	***Na*** *(mg/L)*	***S*** *(mg/L)*	***Cl*** *(mg/L)*	***CEC*** *($cmol_c/kg$)*	***SAR***	***EC*** *(ds/m)*
Depth	Top	13.7	5.67	4.66	46.7	4.3	55	12.0	2.13	0.53
	Middle	24.3	8.00	5..33	124.7	10.3	197	27.0	4.50	0.76
	Bottom	21.3	5.33	3.33	111.7	10.0	151	25.0	4.40	1.00
Significance[a]	Depth	ns	ns	ns	**	**	*	*	*	**

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively.

Some vines in the trial produced a crop in 2012/2013 but most were not in full bearing until season 2013/14. The trial was harvested on the 26/3/14, 25/2/15, 17/3/16 and 11/4/17. The C29 rootstock was excluded from the analyses because only one vine survived the establishment phase.

A. Yield and its components, pruning weight and Ravaz index

Yield and yield component data, pruning weight and the Ravaz index, collected over four seasons (2014-17) for Shiraz grafted on 'near to release' selections and standard rootstocks is presented in Table 2.1.5.2. GLM, ANOVA analysis of the data showed

significant effects of Rootstock and Year for almost all variables. There were no significant rootstock and year interactions, except for a very weak interaction with berries per bunch. Hence, only the means of rootstocks and seasons are presented in the absence of interactions.

There were significant effects of season on yield and its components, pruning weight and the Ravaz index of Shiraz (Table 2.1.5.2). Highest yield and bunch number were recorded in 2017. Season 2014, the first crop for most vines had the lowest yield and bunch number. There was no effect of season on bunch weight. Berries were smaller in 2016 than in other seasons. The highest and lowest berries per bunch were recorded in 2016 and 2015, respectively. There was no effect of season on pruning weight. There was more than a two-fold difference in the Ravaz index, due largely to the effect of the low crop in the first season, 2014.

The significant effects of rootstock on yield and growth characteristics of Shiraz indicate that rootstocks could be classified into four groups, based on yield (Table 2.1.5.2). Ramsey produced significantly more crop than all other rootstocks, followed as a group by 1103 Paulsen, C114, 140 Ruggeri and C112. C113 and C20 produced moderate yields while 101-14 had low yield and M920 a very low yield. The highest bunch numbers were produced by Ramsey, C112, 1103 Paulsen, C114 and 140 Ruggeri while M920 had very low bunch numbers. Ramsey had significantly larger bunches than all other rootstocks, followed as a group with similar bunch weights, C114, 140 Ruggeri and 1103 Paulsen. C20, C112 and C113 produced moderate size bunches while 101-14 and M920 had the smallest bunches. Ramsey, C20, 1103 Paulsen and M920 had the largest berries while berries of 101-14 were smaller than all other rootstocks.

There was a highly significant effect of rootstock on conferred Shiraz vigour (pruning weight) with C114, 1103 Paulsen, Ramsey and C112 having the highest vigour. Shiraz grafted on 101-14 and M920 had the lowest vigour. Ravaz index values were relatively high for all rootstocks but differences were not significant.

Table 2.5.1.2. Mean yield, bunches per vine, bunch weight, berry weight, berries per bunch, pruning weight and Ravaz index of Shiraz grafted on five CSIRO rootstock selections and four standard rootstocks over four seasons (2014-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***Yield (kg/vine)***	***Bunches/ vine***	***Bunch weight (g)***	***Berry weight (g)***	***Berries/ bunch***	***Pruning weight (kg/vine)***	***Ravaz Index***
Ramsey	15.6a	142a	110.2a	1.11a	103.ab	1.49b	13.3
1103P	12.3b	135a	90.3b	1.00ab	93bc	1.66a	7.9
140R	10.9bc	120ab	92.3b	0.92b	102ab	1.18b	10.1
101-14	6.3d	103	54.8d	0.77c	73cde	0.61cd	9.2
C112	10.7bc	142a	77.5cd	0.93b	89bcd	1.34b	9.2
C113	8.9c	127a	69.5d	0.98b	74cde	0.91c	11.4
C114	11.7bc	122ab	92.6b	0.98b	115a	1.75a	8.0
C20	9.1c	108b	81.9c	1.01ab	84bcd	0.80cd	15.3
M920	3.5e	57c	54.5d	1.00ab	56e	0.48d	10.2
Year							
2014	4.4c	54c	79.2	1.06a	75bc	1.04	5.8b
2015	10.0b	126b	73.4	1.07a	69c	0.96	13.2a
2016	10.4b	126b	80.2	0.77b	114a	1.18	10.9a
2017	12.7a	144a	84.1	1.00a	84b	1.17	14.0a
Significance							
Rootstock[a]	***	***	***	***	***	***	ns
Year	***	***	ns	***	***	ns	*
R x Y[b]	ns	ns	ns	ns	*	ns	ns

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively.

[b] RxY indicates the rootstock x season interaction.

Correlation analyses were conducted using only the rootstock means to explore relationships between yield determinants and growth characteristics across the rootstock genotypes (Table 2.1.5.3). Both bunch number and bunch weight were significant contributors to yield variability across the rootstock genotypes. There was also a very strong association between yield and pruning weight. A highly significant multilinear regression ($p<0.001$) indicted that 98% of yield variation across the rootstock genotypes could be accounted for by inclusion of bunches and bunch weight in the model. Bunch weight was positively correlated with both berries per bunch, berry weight and pruning weight. There was also a strong correlation between pruning weight and berries per bunch.

Table 2.1.5.3. Correlation matrix across rootstock means (n=10) describing relationships between rootstock growth characteristics and fruit composition of Shiraz with environmental variables removed. Values included are significant ($p<0.1$). Those in bold indicate significant correlations, $p<0.05$.

	Yield	***Bunches***	***Bunch weight***	***Berry weight***	***Berries per bunch***	***Pruning weight***
Bunches	**0.88**					
Bunch weight	**0.95**	**0.72**				
Berry weight			0.59			
Berries/ bunch	**0.88**	**0.72**	**0.89**			
Pruning wt.	**0.88**	**0.77**	**0.83**		**0.90**	
Ravaz index						

In 2017 it was noted that canopy and fruit condition was severely impacted by a severe drought stress caused by a break down in the irrigation system over a heatwave period in early to mid-February. This led to significant leaf loss with some rootstock genotypes, severe apoplexy (marginal leaf burn) and sunburn damage of the fruit with berry raisining, shrivel and poor colour development of exposed berries ('pinking') (Figure 2.1.5.1). Visual assessment of the canopy condition was undertaken at harvest with both the % leaf lost and % marginal burning of retained leaves recorded. There were significant effects of rootstock genotype on both leaf loss and leaf burn (Table 2.1.5.4). Selection C114, Ramsey and 140 Ruggeri showed minimal leaf loss (i.e.<15%). Selections C113, 1103 Paulsen and M920 showed some leaf loss (20-33%). Leaf loss of selection C112 was moderately high while for 101-14 it was very high. Marginal leaf burn was very low for C114, Ramsey, 1103 Paulsen and 140 Ruggeri (<13%), moderate for C112, C113, C20 and M920 (26-40%) and very high for 101-14. Overall, these results indicate that C114, Ramsey, 140 Ruggeri have very good tolerance to short term drought during ripening while 1103 Paulsen and C113 have good tolerance. In contrast, M920, C112 and C20 appear to somewhat susceptible and 101-14 highly susceptible to short term drought stress.



Figure 2.1.5.1. The impact of temporary drought stress during a heat wave in early to mid-February on canopy condition at harvest showing a largely unaffected rootstock genotype (top left), a severely affected genotype with significant leaf loss and leaf burn (bottom left), severe apoplexy with marginal leaf burn (top right) and impact on fruit condition (bottom left).

Table 2.1.5.4. Mean % canopy loss and % leaf burn of Shiraz grafted on five CSIRO rootstock selections and four standard rootstocks at harvest in 2017. Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***Canopy loss (%)***	***Leaf burn (%)***
Ramsey	13.3c	10.0c
1103P	26.7bc	10.0c
140R	10.0c	13.3c
101-14	63.3a	65.3a
C112	43.3ab	26.7bc
C113	20.0bc	26.7bc
C114	10.0c	6.7c
C20	36.7bc	40.0b
M920	33.3bc	40.0b
Significance[a]	*	**

[a] *, ** represent significance values < 0.05, 0.01 respectively.

Correlation analyses were undertaken across the 2017 data set to explore relationships between canopy condition and vine growth characteristics across rootstock genotypes (Table 2.1.5.5). Negative correlations with both canopy loss and burn and pruning weight across the rootstock genotypes indicate that genotypes with high conferred vigour are likely to suffer less from short periods of drought stress, despite having larger canopies and higher potential transpiration. Such results suggest that high conferred vigour is associated with deeper root systems and access to water from deeper in the soil profile. The negative associations between yield and canopy condition can be explained by the strong associations between yield, conferred vigour and bunch number (Table 2.1.5.3) compounded by effects of canopy loss on bunch development (Table 2.1.5.5). The negative correlations with canopy condition indicate that canopy loss and marginal leaf burn was associated with development of smaller berries and fewer berries per bunch and hence smaller bunches, presumably due to the effects over overexposure described in Figure 2.1.5.1.

Table 2.1.5.5. Correlation matrix across rootstock means (n=10) describing relationships between rootstock growth characteristics and canopy condition (% canopy loss and % marginal leaf burn). Significant values (p<0.1) are included with those in bold (p<0.05).

	Yield	***Bunches***	***Bunch weight***	***Berry weight***	***Berries per bunch***	***Pruning weight***
Canopy loss (%)	-0.51		**-0.66**	**-0.66**	-0.53	-0.53
Canopy burn (%)	**-0.78**	**-0.58**	**-0.84**	**-0.64**	**-0.76**	**-0.86**

B. Fruit composition

Berry juice composition data collected over four seasons (2014-17) for Shiraz grafted on 'near to release' selections and standard rootstocks is presented in Table 2.1.5.6. ANOVA analysis of the data showed significant effects of Rootstock and Year for some variables. There were no significant rootstock and year interactions except a weak interaction for YAN ($p<0.05$), hence, only the means of rootstocks and seasons are presented.

There was no significant effect of season on juice TSS and berry anthocyanins and phenolics (Table 2.1.5.6). Significant seasonal effects were recorded for juice pH (3.68 to 4.34 in 2016 and 2014 respectively), TA (3.07 to 4.57 g/L in 2014, 2017 and 2016 respectively), malate (1.81 to 3.52 g/L in 2016 and 2014 respectively), tartrate (5.1 to 8.71 g/L in 2017 and 2015 respectively) and YAN (174 to 224 mg/L in 2015 and 2014 respectively).

There was no effect of rootstock on TSS, pH, tartrate, YAN or phenolics (Table 2.1.5.6). M920 had the highest juice TA levels followed by 101-14, 1103 Paulsen and C112. C113 and 140 Ruggeri had the lowest levels of TA. Significant effects of rootstock on malate levels were relatively small (2.03 - 2.78 g/L) with C113 and C20 having the highest and Ramsey the lowest malate levels. The significant effect of rootstock on berry anthocyanin (1.57 - 2.05 mg/g) showed that 101-14 and C20 had the highest levels of anthocyanin, followed by 140 Ruggeri, C112 and C113 while Ramsey and C114 had the lowest levels of berry anthocyanin.

Table 2.1.5.6. Mean juice TSS, pH, TA, Malate, Tartrate, YAN, anthocyanin and phenolic substances of Shiraz grafted on five CSIRO rootstock selections and four standard rootstocks over four seasons (2014-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***TSS (ºBrix)***	***pH***	***TA (g/L)***	***Mal. (g/L)***	***Tart. (g/L)***	***YAN (mg/L)***	***Anth. (mg/g)***	***Phen (a.u.)***
Ramsey	23.2	3.95	3.63bc	2.21b	5.78	215	1.57c	1.52
1103P	25.1	4.01	3.82ab	2.32a	6.23	199	1.64c	1.65
140R	25.2	4.02	3.55cd	2.29a	6.15	202	1.96ab	1.90
101-14	24.7	3.94	3.93ab	2.22b	6.71	199	2.08a	1.92
C112	24.9	3.92	3.85ab	2.21b	6.52	207	1.83abc	1.73
C113	22.7	4.00	3.29d	2.73a	6.68	199	1.88ab	1.83
C114	24.8	4.01	3.74bc	2.44ab	6.16	203	1.58c	1.48
C20	24.7	4.05	3.70bc	2.78a	6.18	199	1.98ab	1.95
M920	23.9	3.91	4.12 a	2.38ab	6.10	192	1.67bc	1.81
Year								
2014	24.7	4.34a	3.07c	3.52a	5.33c	224a	.	.
2015	25.1	3.88b	4.19b	2.36b	8.17a	174c	1.73	1.70
2016	24.3	3.68c	4.57a	1.81c	6.38b	183b	1.82	1.72
2017	23.6	4.04b	3.07c	1.94 c	5.10c	221a	1.82	1.77
Sig.								
Rootstock.	ns	ns	**	**	ns	ns	*	ns
Year	ns	***	***	***	***	***	ns	ns
R x Y[b]	ns	ns	ns	ns	ns	*	ns	ns

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively.

[b] RxY indicates the rootstock x season interaction.

C. **Relationships between growth characteristics and fruit composition**

Correlation analyses were conducted using only the rootstock means to explore relationships between vine growth characteristics and berry juice composition across the rootstock genotypes with variation association with block and season removed (Table 2.1.5.7). Across the rootstock genotypes, yield was positively correlated with YAN. Bunch number was negatively correlated with TA and positively correlated with YAN. Bunch weight was negatively correlated with tartrate and positively correlated with YAN. Berry weight correlated negatively with tartrate and anthocyanin. There was a significant impact of conferred vigour on juice composition as shown by the positive correlation with YAN and the negative correlations with anthocyanin and phenolics. Furthermore in 2017, canopy loss was positively correlated with tartrate (r=0.59) and anthocyanin (r=0.57). Similarly canopy burn was positively correlated with tartrate (r=0.56), anthocyanins

(r=0.67) and phenolics (r=0.58). The results linking canopy condition to fruit composition are likely to be due to the effects of excessive exposure causing desiccation, development of smaller berries and concentration of tartrate, anthocyanin and phenolic substances.

Table 2.1.5.7. Correlation matrix, describing linear relationships between growth characteristics (yield component variables, pruning weight and the Ravaz index) and berry juice composition of Shiraz across rootstock genotypes (n=10). Values included are significant P<0.1). Values in bold indicate significant correlations (p<0.05).

	Yield	***Bunches***	***Bunch weight***	***Berry weight***	***Berries per bunch***	***Pruning weight***	***Ravaz index***
TSS							
pH					0.54		
TA		-0.58					
Malate							
Tartrate			**-0.66**	**-0.75**			
YAN	**0.82**	**0.71**	**0.74**		**0.71**	**0.65**	
anthocyanin				**-0.72**		-0.57	
phenolics						-0.59	

Multilinear regression techniques were used to identify the best combinations of growth traits with strong predictive value for Shiraz juice and berry composition measured across the rootstock genotypes. The results show that a significant proportion of the variability in berry composition across the rootstock genotypes could be accounted for by variation in growth characteristics based on adjusted R^2 values including:

- 88% (p=0.000) of tartrate by bunch number (+ve) and bunch weight (-ve)
- 52% (p=0.012) of tartrate by berry weight (-ve)
- 62% (p=0.004) of YAN by yield (+ve)
- 46% (p= 0.019) of anthocyanin by berry weight (-ve)

D. Plant mineral status

In the 2016 season, plant mineral status including the major ions and chloride, was determined for leaf petioles, leaf blades and berry juice at harvest. These assessments enabled relationships between mineral status and plant growth characteristics and fruit composition to be determined.

The results presented in Table 2.1.5.8 show that there were no significant effects of rootstock on petiole B, K, or P. In contrast, there were highly significant effects of rootstock on petiole Cl and S and significant effects of rootstock on Ca, Mg and Na concentrations. Ramsey and M920 had the highest petiole Cl concentrations followed by 1103 Paulsen, C20 and 101-14 whereas 140 Ruggeri, C112, C113 and to a lesser degree, C114 had the lowest Cl concentrations. Petiole S concentrations were highest with 140 Ruggeri and C20 and lowest with 1103 Paulsen, C112, C113 and C20. Petiole Ca concentrations were highest Ramsey, 1103 Paulsen, 140 Ruggeri and C114. Petiole Mg concentrations were highest for 1103 Paulsen, C114, and Ramsey and lowest with M920. Petiole Na concentrations were highest with 140 Ruggeri and M920 and lowest with C114 and to a lesser degree, C113.

There were no significant effects of rootstock on lamina Cl and K concentration (Table 2.1.5.8). Lamina B concentration was highest with M920, C114 and C112 and lowest for Ramsey, 1103 Paulsen, 140 Ruggeri and C113. Lamina Ca concentrations were highest for C114 and Ramsey and lowest with M920, C20 and 101-14. Lamina Mg concentrations were highest with C112, C113 and C114 and lowest with M920. Lamina Na concentrations were highest for M920 and lowest with C114. Lamina P concentrations were highest for C114, M920, Ramsey and 1103 Paulsen and lowest for 101-14, C112, C113 and C20. Lamina S concentrations were highest with M920 and lowest for 1103 Paulsen, 101-14, C112 and C20.

Except for Mg, there were no significant effects of rootstock on juice mineral element concentrations (Table 2.1.5.8). Juice concentrations for Mg were highest for 140 Ruggeri and lowest for C114, C20 and M920.

Table 2.1.5.8. Mean mineral element concentrations in juice, petiole and lamina of Shiraz grafted on five CSIRO rootstock selections and four standard rootstocks at harvest 2016. Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the ANOVA analysis is also presented.

	Rootstock	***Cl***	***B***	***Ca***	***K***	***Mg***	***Na***	***P***	***S***
Juice	Ramsey	84.0	4.67	143	1880	1011b	19.3	93.9	80.3
(mg/L)	1103P	94.4	5.91	145	2243	1177ab	25.2	95.0	78.6
	140R	81.2	4.90	144	2167	1205a	25.2	94.9	86.3
	101-14	94.8	4.52	148	1955	1064b	22.3	70.7	72.3
	C112	91.9	5.97	140	2329	1197ab	29.2	90.9	81.1
	C113	59.6	5.20	140	2120	1139b	20.4	83.4	77.4
	C114	82.5	5.60	108	1843	941c	16.1	93.7	70.2
	C20	78.6	5.42	140	2290	925c	25.5	90.2	71.8
	M920	120.7	4.52	130	1903	925c	32.6	102.3	80.7
Sig.[a]		ns	ns	ns	ns	**	ns	ns	ns
Petiole	Ramsey	1.81a	0.27	3.16a	1.89	0.88a	0.28b	0.06	0.09c
(% dm)	1103P	1.20b	0.30	3.03a	2.59	0.97a	0.33b	0.06	0.07d
	140R	0.53d	0.28	3.05a	2.56	0.78ab	0.51a	0.06	0.15a
	101-14	1.08b	0.30	2.67b	2.34	0.71ab	0.27b	0.07	0.09c
	C112	0.68d	0.31	2.45c	2.72	0.76ab	0.28b	0.06	0.08d
	C113	0.64d	0.28	2.57b	2.32	0.78ab	0.20c	0.06	0.08d
	C114	0.80c	0.32	2.99a	2.30	0.97a	0.12d	0.06	0.09c
	C20	1.10b	0.32	2.82ab	4.01	0.74ab	0.28b	0.06	0.07d
	M920	1.73a	0.28	2.42b	2.72	0.61b	0.47a	0.08	0.14b
Sig.		***	ns	*	ns	*	*	ns	***
Lamina	Ramsey	0.29	0.26b	3.44a	0.67	0.33b	0.11c	0.12a	0.16b
(% dm)	1103P	0.20	0.28b	3.25b	0.79	0.35b	0.12b	0.12a	0.14c
	140R	0.17.	0.24b	3.14b	0.80	0.33b	0.12c	0.11b	0.17b
	101-14	0.17	0.32ab	2.62c	0.71	0.25d	0.11c	0.10b	0.14c
	C112	0.16	0.33a	3.09b	0.76	0.37a	0.11c	0.10b	0.14c
	C113	0.33	0.28b	3.15b	0.67	0.39a	0.10c	0.11b	0.15c
	C114	0.15	0.36a	3.60a	0.85	0.38a	0.07d	0.14a	0.17b
	C20	0.19	0.32a	2.83c	0.96	0.32b	0.10c	0.11b	0.14c
	M920	0.32	0.38a	2.71c	0.82	0.28c	0.22a	0.13a	0.19a
Sig.		ns	*	**	ns	**	**	*	***

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively.

Correlation analyses were undertaken to explore relationships between growth characteristics and mineral element concentrations of juice, petioles and lamina using mean data (Table 2.1.5.9). The results show that across the rootstock genotypes, yield was negatively correlated with juice and lamina Na and positively correlated with lamina Ca and lamina and petiole Mg concentrations. Bunch number was negatively correlated with juice Cl, lamina B, Na and S and petiole P and positively correlated with juice B and Mg, lamina Ca and Mg and petiole Mg concentrations. Bunch weight was positively correlated with lamina and petiole Ca and petiole Mg. Berry weight was positively correlated with juice and lamina P concentrations. Berries per bunch, a function of both

flower number and fruit set, was positively correlated with lamina Ca, Mg and petiole Mg and negatively correlated with juice and lamina Na concentration. Pruning weight was positively correlated with juice B, lamina Ca and Mg and petiole Mg concentrations. The Ravaz index was not correlated with any mineral element, except negatively with lamina Mg.

Table 2.1.5.9. Correlation matrix, describing linear relationships between growth characteristics (yield component variables, pruning weight and the Ravaz index) and berry juice, lamina and petiole mineral element concentrations of Shiraz across rootstock genotypes (n=10). Values are included where significant ($p<0.1$). Those in bold indicate significant correlations ($p<0.05$).

	Yield	***Bunches***	***Bunch weight***	***Berry weight***	***Berries per bunch***	***Pruning weight***	***Ravaz index***
Juice							
Cl		-**0.64**					
B		0.59				**0.67**	
Mg		0.59					
Na	-0.58				**-0.63**		
P				0.62			
Lamina							
B		-0.57					
Ca	**0.72**	0.61	**0.72**		**0.71**	**0.79**	
Mg	0.61	**0.68**	0.56		0.58	**0.72**	
Na	**-0.64**	**-0.77**			**-0.70**	-0.56	
P				0.58			
S		**-0.63**					
Petiole							
Mg	**0.81**	**0.69**	**0.75**		**0.83**	**0.90**	-0.55
P		**-0.79**					

Correlation analyses were undertaken to explore relationships between fruit composition and mineral element concentrations of juice, petioles and lamina using mean data (Table 2.1.5.10). The results show that across the rootstock genotypes, TSS was negatively correlated with lamina Cl and positively correlated with petiole B concentrations. pH was negatively correlated with juice Cl and positively correlated with lamina K. Titratable acidity was positively correlated with juice Cl and Na, lamina B, Na and petiole P and negatively correlated with lamina Mg concentrations. Malate was positively correlated with lamina and petiole K. Tartrate was negatively correlated with juice P, lamina P and petiole Cl concentrations. YAN was positively correlated with petiole Mg concentration. Berry anthocyanin was negatively correlated with juice P, lamina Ca and P concentrations.

Berry phenolic substances were positively correlated with petiole K and negatively correlated with lamina Ca and P and petiole Ca concentrations.

Table 2.1.5.10. Correlation matrix, describing linear relationships between fruit composition variables and berry juice, lamina and petiole mineral element concentrations of Shiraz across rootstock genotypes (n=10). Values are included where significant ($p<0.1$). Values in bold indicate significant correlations ($p<0.05$).

	TSS	***pH***	***TA***	***Mal.***	***Tart.***	***YAN***	***Antho.***	***Phen.***
Juice								
Cl		-0.61	**0.95**					
K								
Na			0.58					
P					**-0.68**		-0.57	
Lamina								
Cl	**-0.85**							
B			**0.65**					
Ca							**-0.67**	**-0.84**
K		0.57		0.55				
Mg			-0.62					
Na			0.60					
P					**-0.70**		**-0.81**	-0.59
Petiole								
Cl					-0.56			
B	**0.66**							
Ca								-0.56
K				0.58				0.58
Mg						**0.67**		
P			**0.73**					

Correlation analyses were undertaken to explore relationships between mineral element concentrations in juice, lamina and petioles using mean data across the rootstock genotypes (Table 2.1.5.11). Juice Cl was positively correlated with juice Na, lamina B, Na and S and petiole P and negatively correlated with lamina Mg. Juice B was positively correlated with juice K, lamina and petiole Mg and negatively correlated with petiole S. Juice Ca was negatively correlated with lamina B and P. Juice K was positively correlated with juice Mg and petiole K and negatively correlated with lamina S. Juice Mg was positively correlated with juice S and negatively correlated with lamina B. Juice Na was positively correlated with lamina Na and negatively correlated with lamina and petiole Mg. Juice P was positively correlated with juice S and lamina P. Juice S was correlated negatively with petiole B.

Table 2.1.5.11. Correlation matrix, describing linear relationships between mineral elements in juice, lamina and petioles of Shiraz across rootstock genotypes (n=10). Values are included where significant ($p<0.1$). Values in bold indicate significant correlations ($p<0.05$).

	Juice							
	Cl	**B**	**Ca**	**K**	**Mg**	**Na**	**P**	**S**
Juice								
K		**0.69**						
Mg				0.61				
Na	**0.66**							
S					0.62		0.55	
Lamina								
B	0.54		**-0.63**		-0.61			
Ca						-0.58		
Mg	**-0.61**	**0.75**						
Na	**0.79**					**0.80**		
P			**-0.77**				**0.67**	
S	0.55			**-0.66**				
Petiole								
B								**-0.70**
K				0.61				
Mg		**0.64**				**-0.64**		
P	**0.86**							
S		**-0.65**						

Multilinear regression techniques were used to identify the best combinations of mineral element traits with strong predictive value for Shiraz juice and berry composition measured across the rootstock genotypes. The results show that a significant proportion of the variability in berry composition across the rootstock genotypes could be accounted for by variation in either lamina, petiole and juice mineral element concentrations based on adjusted R^2 values including:-

Lamina concentrations

- 86% ($p<0.001$) of TSS by CL (-ve) and Na (+ve)
- 60% ($p=0.017$) of pH by B (-ve) and K (+ve)
- 63% ($p=0.012$) of TA by B (+ve) and Mg (-ve)
- 75% ($p= 0.009$) of malate by Cl and K(both +ve) and Na (-ve)
- 42% ($p=0.026$) of tartrate by P (-ve)
- 61% ($p= 0.005$) of anthocyanin by P (-ve)
- 86% ($p=0.002$) of phenolics by B and Ca (both -ve) and Mg (+ve)

Petiole concentrations

- 82% (p=0.004) of TSS by B, Mg and Na (all +ve)
- 59% (p=0.018) of pH by K and Mg (both +ve)
- 78% (p=0.002) of TA by B and P (both +ve)
- 76% (p= 0.009) of malate by K (+ve) and B and Na (both -ve)
- 37% (p=0.036) of YAN by Mg (+ve)
- 42% (p=0.064) of anthocyanin by Cl and Mg (both -ve)

Juice concentrations

- 47% (p=0.045) of pH by K (+ve) and Na (-ve)
- 95% (p=0.001) of TA by Cl (+ve) and S (-ve)
- 78% (p=0.017) of tartrate by Mg and Na (both +ve) and Ca and P (both -ve)
- 94% (p=0.001) of anthocyanin by K (+ve) and B, Ca and P (all -ve)
- 76% (p=0.008) of phenolics by K (+ve) and B and Ca (both -ve)

E. Small-scale winemaking

Single ferment Shiraz wines were produced from selected rootstocks in 2016 with the intent to identify potential fermentation issues, assess whether pH adjustment from tartaric acid addition led to unbalanced wines, assess impacts of rootstocks on spectral properties and to provide wine samples for tasting by industry. The fruit for winemaking was harvested on the 17/03/2016. Note, fruit from own rooted vines was obtained from a different trial established in the same rows.

The must data show some differences in fruit maturity (Table 2.1.5.12). Own roots and Ramsey had the lowest must TSS while 1103 Paulsen and 140 Ruggeri had the highest levels. Must of own roots and to a lesser extent Ramsey, had the lowest pH values. Own roots, Ramsey and C20 had the highest levels of TA. Total tartaric acid additions for pH adjustment ranged from 0.8 g/L for own roots to 4.5 g/l for 1103 Paulsen, 140 Ruggeri and C20. Acid addition requirements were correlated with must TSS ($r=0.81$, $P<0.05$). At bottling, the pH of all wines was above the desired level of 3.6. The reasonably low titratable acidity levels in the wine, maximum 5.7 g/L, indicate that further acid adjustment of the pH could have been undertaken without leading to excessive acidity and unbalanced wines. Alcohol levels in the wine tended to reflect must TSS (r=0.92,

P<0.01), consequently they were highest for 1103 Paulsen and 140 Ruggeri and lowest for own roots, Ramsey and C113. Wine Cl concentrations were highest for own roots (273 mg/L) compared to all rootstocks with 140 Ruggeri, C113 and C114 having the lowest concentrations of Cl i.e. being the best chloride excluders.

Most wine spectral properties tended to reflect the different maturity levels, potentially masking rootstock effects. There was a significant correlation (r=0.82, p<0.05) between wine colour density and TSS with 1103 Paulsen and 140 Ruggeri wines have the highest and own roots, Ramsey and C114 having the lowest values. Wines of C114 and to a lesser degree Ramsey and C20 had higher hue values, an indication that the wines were slightly duller than the other wines. There was a significant correlation between total anthocyanin and must TSS (r=0.78, p<0.1). Wines from 140 Ruggeri and to a lesser degree 1103 Paulsen had the highest total anthocyanin while Ramsey, C113 and C114 had the lowest levels of total anthocyanin. There was a significant correlation between ionised anthocyanin and must TSS (r=0.85, p<0.05). Wines from 1103 Paulsen and 140 Ruggeri and C20 had the highest levels of ionised anthocyanin while own roots, Ramsey and C114 had the lowest levels. There was a significant correlation between wine phenolics and must TSS (r=0.78, p<0.1). Wines from 140 Ruggeri had the highest levels of phenolics while Ramsey, C113 and C114 had the lowest levels.

Table 2.1.5.12. Must, wine bottling data including total acid additions and Cl and spectral properties for Shiraz wines produced from own roots, three standard rootstocks and three CSIRO selections, season 2016.

Table	***Must data***			***Bottling data***					***Spectral properties***				
	TSS	pH	TA	Add.	pH	TA	Alc.	Cl	CD	Hue	A	IA	Phen
	°Brix		g/L	g/L		g/L	%	mg/L	a.u.		mg/L	mg/L	.a.u.
Own	22.8	3.72	3.6	0.8	3.71	5.2	12.8	273	4.88	0.64	709	19.9	19.9
Ram.	22.8	3.88	3.5	2.0	3.76	4.9	12.5	114	5.08	0.67	658	19.5	18.6
1103P	26.3	4.02	3.4	4.3	3.66	5.7	14.0	111	8.20	0.63	802	29.4	23.7
140R	25.7	4.13	3.1	4.5	3.63	5.4	14.3	89	7.16	0.64	988	25.5	30.2
C113	23.1	4.01	3.2	3.0	3.74	5.0	12.3	90	6.76	0.64	691	23.4	18.2
C114	23.6	3.98	3.2	3.0	3.68	4.9	13.1	90	4.82	0.72	671	18.8	18.8
C20	24.2	4.06	3.5	4.5	3.67	5.5	12.9	102	6.84	0.67	724	25.9	19.3

F. Discussion re Shiraz and performance of CSIRO 'near to release' rootstocks

Based on the electrical conductivity and SAR data presented in Table 2.1.5.1, the trial was planted in a soil with a moderate sodicity hazard (Lanyon 2011) and irrigated with water of moderately high salt content. The results over four seasons show significant effects of rootstock genotype on the vine performance and fruit composition of Shiraz, largely associated with conferred vine vigour. Rootstocks with the highest pruning weights were C114 and 1103 Paulsen followed by Ramsey, 140 Ruggeri and C112. Conferred vigour of C113 was moderate while for 101-14 and C20 it was low and for M920 very low.

The results from this site indicate that the CSIRO selection C114, with high conferred vigour may provide an alternative rootstock to the standard rootstocks Ramsey and 1103 Paulsen for Shiraz grown in the Padthaway region as its productivity, fruit composition and wine attributes were similar. Furthermore, C114 has shown a high degree of salt tolerance, with very low Na and Cl concentrations in juice, lamina and petioles and low levels of Cl in wine. Under short term water stress during ripening in 2017, C114 was the most drought tolerant rootstock found in the trial. Similarly, C112 which had high to moderate vigour may provide an alternative rootstock to 140 Ruggeri as its productivity and fruit composition and salt tolerance were similar. However, compared to 140 Ruggeri, C112 appeared to be more sensitive to short term drought. The moderate vigour selections C113 and C20 may offer alternatives to 101-14, which performed poorly in this study, as they had slightly higher yields, similar fruit composition and displayed good salt tolerance. They were also much more tolerant of the short term drought than 101-14 which was the most drought sensitive rootstock in the study. The low vigour, low yielding selections M920 and C29 (not included in the statistical analyses) show little promise for Shiraz grown in the Padthaway region.

The results clearly show that the main effects of rootstock on Shiraz performance were on conferred vigour (pruning weight) which was strongly linked positively to yield, bunch number, bunch weight and berries per bunch (the main drivers of yield), short term drought tolerance and YAN but negatively with berry anthocyanin and phenolic substances. It is interesting to note that yield, bunch number, bunch weight and berries per bunch were positively linked to YAN, suggesting differences between rootstocks in N uptake and movement into the berry, a result which warrants further investigation. Furthermore, negative correlations suggest that rootstocks producing larger bunches and

larger berries are likely to have less tartrate, and in the case of berry weight significantly less anthocyanin. Linkages, across the rootstock genotypes between vine growth characteristics, fruit composition and plant mineral element status (juice, petioles and lamina) warrant further study, not only from uptake perspective but also in relation to impacts on fruit composition and wine quality attributes.

2.1.6. Padthaway trials involving the near to release C7 rootstock.

2.1.6.1. Pinot Gris Padthaway site 2

The Pinot Gris trial was established in a commercial vineyard in Padthaway in 2012/13 with own root or grafted on the CSIRO salt tolerant selection C7 and commercial rootstocks, Ramsey and 140 Ruggeri. While some vines produced a crop in 2015, data were only analysed for 2016 and 2017. The trial was harvested on the 17/2/2016 and 14/3/2017.

Soil at the Padthaway site was a uniform shallow brownish gravelly calcareous mottled clay. Soil samples were taken at harvest in 2016 and 2017 at three depths, 0-30 cm (Top), 31-60 cm (Middle) and 61-90 cm (Bottom) with a hand-held auger from four sampling positions located along the trial rows. A 1:5 soil:water extract was prepared from each sample and electrical conductivity (EC), soluble ion (Cl^-, Ca^{2+}, K^+, Mg^{2+} and Na^+) concentrations, sodium adsorption ratio (SAR) (Richards 1954) and cation exchange capacity were determined by CSIRO Land and Water Analytical Services.

Mean total soluble salts in the irrigation water at harvest in 2016 was 1800 mg/L, equivalent to 2.81 dS/m. Electrical conductivity of the 1:5 soil: water extract was highest in the middle layer 0.39 dS/m compared to the top or bottom layers 0.32 dS/m (Table 2.1.6.1.1). Such values for EC are close to the threshold of below 0.3 indicative of a sodicity hazard (Lanyon 2011). Soluble Na^+ concentrations did not change with season or depth (range 43.3 - 48.3 mg/L). Soluble Cl^- appeared to increase with depth but the differences were not significant (48.4 - 63.0 mg/L). Soluble Cl^- exceeded Na^+ concentrations at all depths. Soluble S concentrations varied significantly between seasons but were similar in all soil layers. Soluble calcium (Ca^{2+}) concentrations (range 15.5 - 17.1 mg/L) exceeded soluble magnesium (Mg^{2+}) concentrations (range 2.88 - 5.25 mg/L) by 3-5.4 fold. Soluble potassium (K^+) concentrations were highest in the top layer (5.0 mg/L) compared to the other layers (1.75 mg/l). There was no effect of depth on sodium adsorption ratios 1.88 - 2.23. The SAR values are well below the threshold value of 3, which is indicative of a sodic soil (Cass 2002). Cation exchange capacity, i.e. the capacity of the soil to hold exchangeable cations, was low but similar at all depths (18.6 - 20.2 $cmol_c/kg$).

Table 2.1.6.1.1. Soluble concentrations of mineral elements (Ca, K, Mg, Na, S), Cl^-, cation exchange (CEC), sodium absorption ration (SAR) and electrical conductivity (EC) of a 5:1 soil solution (n=3). ANOVA was conducted to assess the effects block, season and of depth through the profile.

		Ca *(mg/L)*	***K*** *(mg/L)*	***Mg*** *(mg/L)*	***Na*** *(mg/L)*	***S*** *(mg/L)*	***Cl*** *(mg/L)*	***CEC*** *($cmol_c/kg$)*	***SAR***	***EC***[b] *(ds/m)*
Depth	Top	16.1	5.00a	5.25a	43.3	4.50	48.4	19.3	1.88	0.32b
	Middle	17.1	1.75b	3.13b	48.3	5.00	59.9	20.2	2.14	0.39a
	Bottom	15.5	1.75b	2.88b	48.3	5.25	63.0	18.6	2.23	0.33b
Year	2016	17.0	3.42	3.92	50.1	6.25a	55.3	20.0	2.20	-
	2017	15.5	2.25	3.58	43.1	3.58b	58.8	18.8	1.96	-
Significance[a]	Block	ns	ns	ns	**	*	*	ns	**	ns
	Depth	ns	**	***	ns	ns	ns	ns	ns	**
	Year	ns	ns	ns	ns	***	ns	ns	ns	-
	YxD	ns	ns	ns	ns	ns	ns	ns	ns	-

[a] *, **, *** represent significance values, p< 0.05, 0.01, 0.001 respectively.
[b] 2017 only

A. Yield and its components, pruning weight and Ravaz index

Yield and yield component data, collected over two seasons (2016-17) for Pinot Gris growing on own roots or grafted on Ramsey, 140 Ruggeri and the CSIRO selection, C7 are shown in Table 2.1.6.1.2. GLM, ANOVA analysis of the data showed significant effects of Block, Rootstock and Year for most variables. The significant rootstock and year interactions found for yield, bunch number, berry weight and Ravaz index were associated with variation in the magnitude of the rootstock response, not differences between rootstocks in the two seasons, hence they are not presented in detail.

The results show significant effects of season on yield and its components with the exception of berries per bunch, pruning weight and the Ravaz index (Table 2.1.6.1.2). Highest yield and bunch number were recorded in 2017, as the vines reached full productivity. Compared to season 2016 the higher yields in 2017 were associated with higher bunch numbers, larger bunches and larger berries. There was a two-fold increase in pruning weight from 2016 to 2017. The high Ravaz index values in both seasons indicate that Pinot Gris is a very efficient variety with regard to both carbon and water use efficiency.

The significant effects of rootstock on yield and growth characteristics of Pinot Gris show that own rooted vines produced the highest yield, followed by C7 while Ramsey and 140 Ruggeri had the lowest yield (Table 2.1.6.1.2). Bunch numbers, bunch weight and berries per bunch followed similar trends to yield across the rootstocks. Own rooted vines produced significantly smaller berries than those produced by the grafted vines on rootstock. Own rooted vines also had significantly higher pruning weight than vines on rootstocks. Ramsey vines had higher pruning weight (conferred vigour) than 140 Ruggeri or C7. Vine balance, based on the Ravaz index, was highest for the C7 rootstock suggesting enhanced carbon and water use efficiency compared to the other rootstocks and own roots.

Table 2.1.6.1.2. Mean yield, bunches per vine, bunch weight, berry weight, berries per bunch, pruning weight and Ravaz index of Pinot Gris growing on own roots or grafted on Ramsey, 140 Ruggeri or the CSIRO selection, C7 over two seasons (2016-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***Yield (kg/vine)***	***Bunches/ vine***	***Bunch weight (g)***	***Berry weight (g)***	***Berries/ bunch***	***Pruning weight (kg/vine)***	***Ravaz Index***
Own roots	6.95a	77.8a	86.6a	0.84b	104.6a	0.25a	28.5b
Ramsey	3.49c	47.6c	69.1c	0.88a	79.1c	0.20b	25.2b
140 Ruggeri	3.20c	44.8c	67.6c	0.86a	79.4c	0.17c	28.5b
C7	5.85b	66.2b	81.4b	0.87a	94.2b	0.17c	37.1a
Year							
2016	3.22b	45.7b	66.3b	0.75b	89.5	0.13b	33.7a
2017	6.52a	72.4a	86.0a	0.98a	89.1	0.27a	26.0b
Significance							
Block[a]	***	***	ns	ns	ns	***	*
Rootstock	***	***	***	*	***	***	***
Year	***	***	***	***	ns	***	***
R x Y[b]	***	***	ns	*	ns	ns	***

[a] *, **, *** represent significance values, p< 0.05, 0.01, 0.001 respectively.

[b] RxY indicates the rootstock x season interaction.

Because the number of rootstocks included in this study was low (n=4), valid correlation analyses could not be conducted to determine rootstock-only effects on relationships between growth characteristics. Hence the analysis was conducted for the season x rootstock means (n=8). The results of the analyses show that bunch number and bunch

weight were the main drivers of yield (Table 2.1.6.1.3). There was no significant association between berry weight or berries per bunch and yield. There was also a strong association between yield and pruning weight. However, because node number, budburst and shoot fruitfulness were not recorded in this study the underlying causes for this relationship cannot be elucidated. A highly significant multilinear regression ($p<0.001$) indicated that 99.8% of yield variation across the rootstocks and seasons could be accounted for by inclusion of bunches and bunch weight in the model. The positive correlations between bunch number and bunch weight indicate that vines carrying more bunches also had larger bunches. Both bunch weight and berry weight were positively correlated with pruning weight. Correlations between Ravaz index and growth characteristics were not significant.

Table 2.1.6.1.3. Correlation matrix, describing linear relationships between yield component variables, pruning weight and the Ravaz index for Pinot Gris across season and rootstock means (n=8). Retained r values are significant, $p<0.1$. Those highlighted in bold are significant $p<0.05$.

	Yield	***Bunches***	***Bunch weight***	***Berry weight***	***Berries per bunch***	***Pruning weight***
Bunches	**0.99**					
Bunch weight	**0.90**	**0.84**				
Berry weight			**0.70**			
Berries/ bunch			0.62			
Pruning weight	**0.72**	0.68	**0.79**	**0.81**		
Ravaz index						

B. Vine nutrient status

Lamina and petiole mineral element concentrations were assessed for leaves collected at harvest in season 2016, to determine the effects of rootstocks on plant mineral status. The results show that Block effects were limited to Mg concentrations in lamina and Na, P and Cl in petioles (Table 2.1.6.1.4). There was no effect of rootstock on K concentration in either tissue. Own roots and Ramsey had lower concentrations of B than 140 Ruggeri or C7 in both tissues, although the difference between Ramsey and C7 was not significant in petioles. Ca concentrations were highest with own roots and C7 in both tissues. Mg concentrations were highest for own rooted vines and lowest with Ramsey in both tissues. Na concentrations were lowest for own rooted vines in both tissues and C7 in petioles. Own rooted vines had the highest P concentrations in both tissues. S concentration was

highest in 140 Ruggeri laminae and 140 Ruggeri and own root petioles. Own rooted vines had significantly higher Cl in both tissues than all rootstocks, which were not significantly different in their Cl concentration except that Ramsey had higher Cl than 140 Ruggeri or C7 in the petiole.

Table 2.1.6.1.4. Mean mineral element concentration of both lamina and petioles of Pinot Gris growing on own roots or grafted on Ramsey, 140 Ruggeri or the CSIRO selection, C7 at harvest in the 2016. Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***B***	***Ca***	***K***	***Mg***	***Na***	***P***	***S***	***Cl***
	mg/kg				**%dry wt**			
Lamina								
Own roots	25.4b	3.32a	0.96	1.30a	0.27b	0.10a	0.12ab	0.70a
Ramsey	25.8b	2.95b	1.07	0.82c	0.30ab	0.07b	0.10b	0.35b
140 Ruggeri	27.3a	2.80b	0.92	1.0b	0.32a	0.05b	0.14a	0.30b
C7	27.2a	3.31a	1.07	1.01b	0.33a	0.06b	0.12ab	0.34b
Significance								
Block[a]	ns	ns	ns	*	ns	ns	ns	ns
Rootstock	**	***	ns	***	***	***	***	***
Petiole								
Own roots	26.3c	3.39a	0.64	0.45a	0.020c	0.14a	0.21a	2.13a
Ramsey	28.2b	2.89b	0.68	0.28c	0.024b	0.11b	0.20b	1.34b
140 Ruggeri	31.7a	2.81b	0.62	0.33b	0.027a	0.11b	0.22a	1.06c
C7	29.4b	3.34a	0.63	0.34b	0.022c	0.12b	0.19b	1.18c
Significance								
Block[a]	ns	ns	ns	ns	*	**	ns	*
Rootstock	***	***	ns	***	***	***	**	***

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively.

C. Fruit composition

Berry juice composition data, collected over two seasons (2016 and 2017) for Pinot Gris growing on own roots or grafted on Ramsey, 140 Ruggeri and the CSIRO selection, C7 are presented in Table 2.1.6.1.5. GLM, ANOVA analysis of the data no effect of Block

on fruit composition. There was a significant effect of season on all fruit composition variables except TSS and tartrate. Significant rootstock effects were found for TSS, pH, YAN, and Cl. The significant rootstock and year interactions found for TSS, pH and YAN were associated with variation in the magnitude of the rootstock response, not differences between rootstocks in the different seasons, hence they are not presented in detail.

The significant effects of season on fruit composition of Piot Gris, show higher pH, TA, malate, YAN and Cl in the 2017 season compared to the lower yielding, earlier ripening 2016 season (Table 2.1.6.1.5). The significant effects of rootstock on TSS show that fruit from Ramsey was the ripest (22.9 ^{o}Brix) while fruit from own roots was the least mature (21.0 ^{o}Brix). Own roots produced significantly lower juice pH (3.51) than the three rootstocks, which had similar pH values (3.56 - 3.59). YAN values ranged from 125-153 mg/L, with C7 producing the highest levels and 140 Ruggeri the lowest levels. These low YAN values are below the minimum threshold for fermentation, indicating that supplementary DAP addition would be required for successful fermentation. Juice from own rooted vines had 60% higher Cl (48.7 mg/L) than from any of the three rootstocks (29.2 - 33.9 mg/L).

Table 2.1.6.1.5. Mean juice TSS, pH, TA, Malate, Tartrate and YAN of Pinot Gris growing on own roots or grafted on Ramsey, 140 Ruggeri or the CSIRO selection, C7 over two seasons (2016-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***TSS (ºBrix)***	***pH***	***TA (g/L)***	***Malate (g/L)***	***Tartrate (g/L)***	***YAN (mg/L)***	***Chloride (mg/L)***
Own roots	21.0c	3.51b	3.81	1.19	7.57	141b	48.7a
Ramsey	22.9a	3.57a	3.89	1.34	4.75	137b	30.5b
140 Ruggeri	21.9b	3.56a	3.77	1.34	9.58	125c	29.2b
C7	21.6bc	3.59a	3.66	1.30	4.83	153a	33.9b
Year							
2016	22.0	3.49b	3.61b	0.98b	4.46	117b	27.0b
2017	21.7	3.63a	3.95a	1.60a	8.90	161a	44.2a
Significance							
Block[a]	ns	ns	ns	ns	ns	ns	Ns
Rootstock	**	*	ns	ns	ns	***	***
Year	ns	***	***	***	ns	***	***
R x Y[b]	***	***	ns	ns	ns	*	ns

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively.

[b] RxY indicates the rootstock x season interaction.

D. Relationships between growth characteristics and fruit composition

Correlation analyses were undertaken to explore relationships between vine growth characteristics and berry juice composition across the mean season x rootstock data (Table 2.1.6.1.6). There was a negative correlation between both yield and bunch number and TSS, indicative of a delay in maturity, although there were no significant correlations with either yield or bunch number and pH, TA, malate or tartrate. The positive correlations between both yield and bunch number and Cl can be attributed to the high yield and high Cl of own rooted vines. Yield was also correlated positively with YAN. Bunch weight was positively correlated with malate, YAN and Cl, the latter due to the large bunches and high juice Cl of own rooted vines. Berry weight was positively correlated with pH, TA, malate, YAN and Cl. Pruning weight was positively associated with pH, TA, malate, YAN and Cl. Vine balance as measured by the Ravaz index, was negatively associated with TSS, pH and TA.

Table 2.1.6.1.6. Correlation matrix, describing linear relationships between fruit composition and growth characteristics of Pinot Gris across season and rootstock means (n=8). Retained r values are significant, p<0.1. Those highlighted in bold are significant p <0.05.

	Yield	***Bunches***	***Bunch weight***	***Berry weight***	***Berries per bunch***	***Pruning weight***	***Ravaz index***
TSS	-0.66	**-0.72**					-0.67
pH				**0.88**		0.64	-0.62
TA				**0.86**		**0.87**	**-0.83**
Malate			0.64	**0.98**		**0.74**	
Tartrate							
YAN	0.65		**0.82**	**0.87**		**0.80**	
Chloride	**0.93**	**0.91**	**0.91**	0.62		**0.87**	

Multilinear regression techniques were used to identify the best combinations of growth traits with strong predictive value for Pinot Gris juice composition measured across the season x rootstock genotype data. They showed that a significant proportion of the variability in berry composition could be accounted for based on adjusted R^2 values including:-

- 75% (p=0.014) of TSS by bunch number (-ve) and pruning weight (+ve)
- 74% (p=0.004) of pH by berry weight (+ve)
- 98% (p<0.001) of TA by bunch number (-ve), berry weight and pruning weight (both +ve)
- 95% (p<0.001) of malate by berry weight (+ve)
- 63% (p=0.012) of YAN by bunch weight (+ve)
- 81% (p=0.002) of juice Cl by bunch number (+ve)
- 93% (p<0.001) of juice Cl by yield and pruning weight (both +ve)

E. Relationships between fruit composition variables

Correlation analyses were undertaken to explore relationships between berry juice composition variables across the means of the season x rootstock genotype data (Table 2.1.6.1.7). Correlations between TSS and other compositional variables were not significant. In contrast, pH was positively with TA, malate and YAN. Berry juice malate was positively correlated with tartrate and YAN. There was also a positive association between YAN and Cl.

Table 2.1.6.1.7. Correlation matrix, describing linear relationships between fruit composition variables of Pinot Gris across season and rootstock means (n=8). The r values retained are significant, p<0.1. Those highlighted in bold are significant p <0.05.

	TSS	***pH***	***TA***	***Malate***	***Tartrate***	***YAN***
pH						
TA		**0.77**				
Malate		**0.90**	**0.82**			
Tartrate				0.67		
YAN		**0.89**	**0.73**	**0.83**		
Chloride						0.67

F. Small-scale winemaking

Duplicate ferment Pinot Gris wines were produced for all rootstocks in 2016 and 2017 with the intent to identify potential fermentation issues, assess whether pH adjustment from tartaric acid addition led to unbalanced wines and to provide wine samples for analysis and for tasting by industry. Formal sensory assessments have not been conducted at the time of report writing. The fruit for winemaking was harvested on 17 February and

14 March in 2016 and 2017 respectively.

The must data show some differences in fruit maturity in both seasons (Table 2.1.6.8). In 2016, must of own roots and Ramsey had the highest TSS (21.6 - 21.8 ^{o}Brix) and pH (3.64 and 3.53) and lowest titratable acidity (5.4 and 5.5 g/L). Conversely in that season, 140 Ruggeri and C7 had the lowest TSS (19.8 and 18.5 ^{o}Brix, respectively) and pH (3.60 and 3.61) and highest TA (5.8 and 5.6 g/L). In 2017, must of Ramsey and 140 Ruggeri had the highest TSS (23.6 and 23.8 ^{o}Brix), pH (3.54 and 3.60) and lowest TA (4.2 and 4.3 g/L). In contrast, own roots and C7 had the lowest TSS (18.8 and 19.4 ^{o}Brix) and pH (3.3 and 3.37) and highest TA (4.7 and 4.8 g/L). In 2016 YAN levels were above the minimum acceptable standard level (150 mg/L) for winemaking, ranging from 171 - 205 mg/L, hence DAP was not added during fermentation. In 2017, YAN levels were below the acceptable standard, ranging from 77-108 mg/L. Hence 375, 450, and 500 mg/L of DAP were added to Ramsey, 140 Ruggeri, own roots and C7, respectively. In 2016, 2.0 g/L of tartaric acid was added for pH adjustment across all treatments whereas in 2017, pH adjustment was not required for own roots with a minimal addition (0.25 g/L) added to C7. In 2017, higher levels of pH adjustment were made during fermentation to 140 Ruggeri (1.75 g/L) and Ramsey (2.5 g/L).

At bottling, there were only small differences in wine pH across the rootstocks, ranging from 3.1 - 3.39 in 2016 and 3.08 - 3.23 in 2017. In 2016, wine TA levels were similar across the rootstocks, ranging from 4.9 - 5.3 g/L. In 2017, wine TA levels were relatively high, ranging from 6.15 - 7.15 g/L, with Ramsey wine having the highest level. Wine alcohol levels did not always reflect must TSS in 2016, being highest for own root wines but lowest for Ramsey and 140 Ruggeri in 2016. In 2017, wine alcohols reflected must TSS, being highest for Ramsey and 140 Ruggeri and lowest for own roots and C7. Residual sugar was not detected in 2016 wines. However, in 2017, residual sugars were detected in all wines, particularly those with high TSS in the must (i.e. Ramsey and 140 Ruggeri). It is likely that the low levels of YAN in 2017 may have impacted on the fermentation process despite the routine addition of DAP during fermentation, leading to issues with residual sugar. In both years, wine chloride concentrations were 50 - 60% higher in wines produced from own roots compared to those from rootstocks. The higher Cl concentrations in wines produced from own roots (80.5 and 69.9 mg/L) were much lower than those identified to give ‘salty taste’ to Chardonnay wine (i.e. 400 mg/L,

Walker et al. 2015) and much lower than the 607 mg/L limit allowable in Australian wine.

Table 2.1.6.1.8. Must and wine data for Pinot Gris wines produced from own roots or vines grafted on Ramsey, 140 Ruggeri and the CSIRO C7 selection in seasons 2016 and 2017. Values are means of 2 ferments.

	Must data				***Bottling data***				***chloride***
	TSS (ºBrix)	**pH**	**TA** (g/L)	**TA Added** (g/L)	**pH**	**TA** (g/L)	**Alc.** (%)	**Resid. sugar** (%)	mg/L
2016									
Own roots	21.8	3.64	5.5	2.00	3.36	5.0	13.0	-	80.5
Ramsey	21.6	3.65	5.4	2.00	3.31	4.9	10.9	-	71.4
140 Ruggeri	19.8	3.60	5.8	2.00	3.28	5.3	10.9	-	59.5
C7	18.5	3.61	5.6	2.00	3.39	4.9	11.7	-	52.9
2017									
Own roots	18.8	3.30	4.8	-	3.22	6.2	11.2	0.45	69.9
Ramsey	23.8	3.60	4.3	2.25	3.08	7.1	14.6	2.05	44.7
140 Ruggeri	23.6	3.54	4.2	1.75	3.14	6.9	14.5	1.65	43.5
C7	19.4	3.37	4.7	0.25	3.23	6.2	11.7	0.35	46.0

G. Discussion of Pinot Gris results

The trial was designed to assess the performance of the salt tolerant rootstock, C7 compared to own roots, Ramsey and 140 Ruggeri rootstocks under moderately saline conditions in Padthaway. While the trial requires ongoing evaluation for a number of seasons it is showing some interesting and unexpected results. Firstly, it is clear that Pinot Gris is a very low vigour variety but is able adequately mature the crop despite the development of very small canopies and high Ravaz index values. Secondly, to date, own rooted vines have been more productive than all rootstocks particularly Ramsey and 140 Ruggeri, suggesting semi-incompatibility issues with these rootstocks which also had fewer and smaller bunches. In contrast, the yield, bunch number and bunch weight of the C7 rootstock were much closer to own roots over the two seasons. Thirdly, lamina and petiole analyses undertaken to provide an assessment of plant mineral status at harvest in 2017 showed that the three rootstocks were good excluders of Cl compared to own roots. In contrast, Na concentrations tended to be slightly higher than for own rooted vines. Wines produced from own rooted vines had up to 52% more Cl than wines produced from rootstocks.

In summary, while the results are preliminary it can be concluded that C7 shows considerable promise as a rootstock for Pinot Gris due to its enhanced performance

compared to Ramsey and 140 Ruggeri and high salt excluding ability compared to own roots.

2.1.6.2 Shiraz Padthaway site 2

The trial was designed to assess the performance of Shiraz grafted on the salt tolerant C7 rootstock selection compared to own roots or grafted on 140 Ruggeri rootstock when grown in a commercial vineyard in Padthaway using moderately saline irrigation water. While some vines produced fruit in 2013, only data for 2014 - 2017 is included in the analyses. Harvest dates were 03/04/2014, 11/03/2015, 08/03/2016 and 27/4/2017 when harvest across the region was significantly delayed.

Soil at Padthaway was a uniform shallow brownish gravelly calcareous mottled clay. Soil samples were taken at harvest in 2016 and 2017 at three depths, 0-30 cm (Top), 31-60 cm (Middle) and 61-90 cm (Bottom) with a hand-held auger from four sampling positions located along the trial rows. A 1:5 soil:water extract was prepared from each sample and electrical conductivity (EC), soluble ion (Cl^-, Ca^{2+}, K^+, Mg^{2+} and Na^+) concentrations, sodium adsorption ratio (SAR) (Richards 1954) and cation exchange capacity were determined by CSIRO Land and Water Analytical Services.

Mean total soluble salts in the irrigation water at harvest in 2016 was 1800 mg/L, equivalent to 2.81 dS/m. Electrical conductivity of the 1:5 soil: water extract was highest in the lower layers 0.43 dS/m compared to the top or bottom layer 0.34 dS/m (Table 2.1.6.2.1). Such values for EC are close to the threshold of below 0.3 indicative of a sodicity hazard (Lanyon 2011). There were small differences between seasons in SAR, Ca, Na, S, Cl, CEC and SAR. Soluble Na^+ and Cl concentrations did not change with depth. Soluble Cl^- exceeded Na^+ concentrations at all depths. There was no effect of soil depth on Ca, Na, CEC or SAR. Soluble calcium concentrations (range 13.1-15.1 mg/L) exceeded soluble magnesium concentrations (range 2.88 - 5.38 mg/L) by 2.4 to 5.2 fold. Soluble potassium concentrations were highest in the top layer (5.5 mg/L) compared to the other layers (1.5 - 1.63 mg/l). There was no effect of depth on sodium adsorption ratios 2.71 - 2.79. The SAR values are below the threshold value of 3 that is indicative of a sodic soil (Cass 2002). Cation exchange capacity i.e. the capacity of the soil to hold exchangeable cations, was low but similar at all depths (15.6-20.6 $cmol_c/kg$).

Table 2.1.6.2.1. Soluble concentrations of mineral elements (Ca, K, Mg, Na, S), Cl^-, cation exchange (CEC), sodium adsorption ration (SAR) and electrical conductivity (EC) of a 5:1 soil solution (n=3). ANOVA was conducted to assess the effects block, season and depth through the profile.

		Ca *(mg/L)*	***K*** *(mg/L)*	***Mg*** *(mg/L)*	***Na*** *(mg/L)*	***S*** *(mg/L)*	***Cl*** *(mg/L)*	***CEC*** *($cmol_c/kg$)*	***SAR***	***EC[b]*** *(ds/m)*
Depth	Top	13.1	5.50a	5.38a	59.0	6.75	79.4	15.6	2.71	0.34
	Middle	15.1	1.50b	2.88c	59.5	5.75	82.9	20.6	2.75	0.43
	Bottom	14.4	1.63b	3.50b	59.6	6.00	89.6	18.4	2.79	0.43
Year	2016	15.9a	2.33	4.00	70.2a	6.92a	100.1a	15.1b	3.08a	-
	2017	12.5b	3.42	3.83	48.6b	4.50b	67.8b	21.3a	2.42b	-
Significance	Block	ns	ns	ns	*	ns	*	ns	*	**
	Depth	ns	***	***	ns	ns	ns	ns	ns	**
	Year	*	ns	ns	*	*	*	**	*	-
	YxD	ns	ns	*	ns	ns	ns	ns	ns	-

[a] *, **, *** represent significance values, $p< 0.05, 0.01, 0.001$ respectively.

[b] 2017 only

A. Yield and its components, pruning weight and Ravaz index

Yield and yield component data, collected over 4 seasons (2014-17) for Shiraz growing on own roots or grafted on 140 Ruggeri and the CSIRO selection, C7 (Table 2.1.6.2.2). GLM, ANOVA analysis of the data showed that there were no significant effects of Block, except for berries per bunch but significant effects of Rootstock and Year for most variables. The significant rootstock and year interactions found for yield, bunch weight, berry weight and berries per bunch were associated with variation in the magnitude of the rootstock response, not differences between rootstocks in the four seasons, hence they are not presented in detail.

The results show significant effects of season on yield and its components with the exception of berries per bunch (Table 2.1.6.2.2). The lowest yield and bunch number were recorded in 2014 before the vines had reached full maturity. The highest yield and bunch number were recorded in 2017. The smallest and largest bunches and berries were produced in 2016 and 2017, respectively. There was no significant effect of season on berries per bunch. Pruning weights were not recorded in 2014. There was a small but

significant effect of season on pruning weight which was highest in 2017. The higher Ravaz index in 2017 can be attributed to the high yield in that season rather than to pruning weight.

The significant effects of rootstock on yield and growth characteristics of Shiraz show that 140 Ruggeri rootstock produced the highest yield and had the highest number of bunches, while yields and bunch number of own roots and C7 were similar (Table 2.1.6.2.2). Bunch weight and berry weight were also highest for 140 Ruggeri. C7 and own roots vines had the highest and lowest number of berries per bunch, respectively. Rootstock effects on pruning weight and Ravaz index were not significant, possibly due to high vine to vine variability found for these traits. The results suggest vigour conferred by 140 Ruggeri was higher than that conferred by C7 or the inherent vigour of own rooted Shiraz.

Table 2.1.6.2.2. Mean yield, bunches per vine, bunch weight, berry weight, berries per bunch, pruning weight and Ravaz index of Shiraz growing on own roots or grafted on 140 Ruggeri or the CSIRO selection, C7 over four seasons (2014-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***Yield (kg/vine)***	***Bunches /vine***	***Bunch weight (g)***	***Berry weight (g)***	***Berries/ bunch***	***Pruning weight (kg/vine)***	***Ravaz Index***
Own roots	6.03b	68.9b	77.8c	1.10b	81.7b	1.03	10.1
140R	12.06a	93.0a	126.9a	1.52a	89.9ab	1.69	8.7
C7	6.00b	65.1c	91.3b	1.05b	97.7a	0.61	11.3
Year							
2014	3.34c	33.0d	92.8bc	1.07b	85.5	.	.
2015	7.18b	65.1c	102.5b	1.37a	78.1	1.09b	7.4c
2016	7.02b	82.4b	80.4c	1.04b	111.0	1.04b	9.2b
2017	14.46a	122.3a	118.8a	1.40a	84.5	1.19a	13.4a
Significance[a]							
Block	ns	ns	ns	ns	*	ns	ns
Rootstock	***	***	***	***	**	ns	ns
Year	***	***	**	***	ns	***	***
R x Y	**	ns	**	***	***	ns	ns

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively.

R x Y rootstock x year interaction

Because the number of rootstocks included in this study was low (n=3), valid correlation analyses could not be conducted to determine rootstock-only effects on relationships

between growth characteristics. Hence, the analysis was conducted for season x rootstock means (n=12 or 9 in the case of pruning weight and Ravaz index). The results of the analyses show that both bunch number and bunch weight were the main drivers of yield (Table 2.1.6.2.3).

A highly significant multilinear regression ($p<0.001$) indicted that 96.5% of yield variation across the rootstocks and seasons could be accounted for by inclusion of bunches and bunch weight in the model. There was also a strong association between yield and pruning weight. However, because node number, budburst and shoot fruitfulness were not recorded in this study the underlying causes for this relationship cannot be elucidated. The positive correlations between bunch number and bunch weight or berry weight indicate that vines carrying more bunches also had larger bunches with larger berries. Bunch number was also positively correlated with pruning weight and the Ravaz index. Both bunch weight and berry weight were positively correlated with pruning weight. The negative correlation between berry weight and berries per bunch indicates that bunches which had more berries had smaller berry weight.

Table 2.1.6.2.3. Correlation matrix across season x rootstock means (n=12 except for pruning weight and Ravaz index where n=9) describing relationships between rootstock growth characteristics. Retained r values are significant, $p<0.1$. Those highlighted in bold are significant $p<0.05$.

	Yield	***Bunches***	***Bunch weight***	***Berry weight***	***Berries per bunch***	***Pruning weight***
Bunches	**0.93**					
Bunch weight	**0.78**	0.55				
Berry weight	**0.64**	0.48	**0.74**			
Berries/ bunch				**-0.63**		
Pruning weight	**0.71**	0.53	**0.76**	**0.76**		
Ravaz index		**0.57**				

B. Fruit composition

Berry juice composition data, collected over four seasons (2014-17) for Shiraz growing on own roots or grafted on 140 Ruggeri and the CSIRO selection, C7 is presented in Table 2.1.6.2.4. GLM, ANOVA analysis of the data show no effect of Block on fruit composition except malate and anthocyanin. There was a significant effect of season on all fruit composition variables. Significant rootstock effects were found for all variables except TA and anthocyanin. The significant rootstock and year interactions, found for pH, tartrate, YAN and anthocyanin, were associated largely with variation in the magnitude of the rootstock response, not differences between rootstocks across the seasons, hence they are not presented in detail. TSS was also shown to be a significant covariate for a number of the other variables, hence adjusted rootstock means are also presented, where appropriate, in Table 2.1.6.2.4.

The significant effect of season on TSS (Table 2.1.6.4) shows that the highest and lowest levels of maturity were achieved in 2016 and 2017, respectively despite the fact that harvest occurred on 8 March in 2016 but was delayed until 27 April in 2017. Juice pH was highest in 2014 and lowest in 2017. TA was highest in 2016 and lowest in 2017, when maturation was delayed. Malate levels were highest in 2014 and lowest in 2016 whereas tartrate levels were also highest in 2014 but lowest in 2017. YAN was highest in 2014 and lowest in 2016. Anthocyanin and phenolic substances were lowest in 2017.

The significant effects of rootstock on TSS show that fruit from own roots was the ripest (22.8 ^{o}Brix) compared to the grafted vines on 140 Ruggeri and C7 (21.5 and -21.9 ^{o}Brix). Own roots produced significantly lower juice pH and malate levels while 140 Ruggeri had the highest pH and malate levels. C7 had significantly lower pH and malate levels than 140 Ruggeri. There was no effect of rootstock on TA. Tartrate levels were highest with C7 although significantly not different to own roots, but lower with 140 Ruggeri. C7 had the lowest YAN levels. These low YAN levels across all rootstocks are below the minimum threshold for fermentation, indicating that supplementary DAP addition would be required for successful fermentation. While there was no effect of rootstock on anthocyanins, own roots had the highest concentration of phenolic substances. Juice Cl concentrations were 60% higher with own rooted vines (63.3 mg/L) compared to both

rootstocks (39.4-44.3 mg/L). The effect of rootstock on Cl was even greater when the rootstock means were adjusted for the significant TSS covariate (i.e. a 2.4-fold difference between own roots and C7).

It should also be noted that leaf lamina and petiole Cl was also assessed for samples collected at harvest in 2014 and 2016. The significant ($p<0.001$) results showed that own rooted vines had the highest concentration of lamina Cl (1.35 % dry weight) and petiole Cl (2.74%), compared to 140 Ruggeri concentrations of 0.24% and 0.60 % and C7 concentrations of 0.37% and 1.36% for lamina and petioles, respectively.

Table 2.1.6.2.4. Mean juice TSS, pH, TA, Malate, Tartrate, YAN, anthocyanin, phenolic substances and Cl of Shiraz grafted own roots or grafted on 140 Ruggeri or the CSIRO selection, C7 over four seasons (2014-17). Means followed by the same letter are not significantly different ($p = 0.05$). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***TSS (ºBrix)***	***pH***	***TA (g/L)***	***Malate (g/L)***	***Tartrate (g/L)***	***YAN (mg/L)***	***Anth. (mg/g)***	***Phen. (a.u.)***	***Juice Cl (mg/L)***
Own roots	22.8a	3.58a	5.00	2.63c	6.36a	132a	1.02	1.43a	63.3a
140R	21.9b	3.68c	5.02	3.34a	6.06b	135a	0.95	1.17b	44.3b
C7	21.5b	3.62b	5.01	2.94b	6.47a	121b	0.89	1.18b	39.4b
Own roots adj.	-	3.55b	5.02	-	6.25ab	127b	0.93	1.34a	74.1a
140R adj.	-	3.68a	5.01	-	6.09b	136a	0.93	1.15b	37.0b
C7 adj.	-	3.64a	4.97	-	6.54a	124b	0.90	1.18b	30.9b
Year									
2014	23.6b	3.87a	5.00b	3.73a	8.28a	160a	.	.	-
2015	21.1c	3.56c	5.05b	2.82b	6.81b	81d	1.12a	1.27b	-
2016	25.0a	3.44d	5.65a	2.32c	6.57b	127c	1.00a	1.62a	-
2017	18.6d	3.63b	4.32c	3.00b	3.52c	149b	0.75b	0.89c	-
Significance.[a]									
Block	ns	ns	ns	**	ns	ns	*	ns	ns
Rootstock	***	***	ns	***	*	*	ns	***	**
Year	***	***	***	***	***	***	***	***	-
RxY [b]	ns	*	ns	ns	**	*	***	ns	-
TSS covariate	-	***	*	ns	**	***	*	***	**

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively.

[b] RxY indicates the rootstock x season interaction.

C. Relationships between growth characteristics and fruit composition

Correlation analyses were undertaken to explore relationships between Shiraz vine growth characteristics and berry juice composition across the mean season x rootstock data (Table 2.1.6.2.5). Cl could not be included in the analysis as data were only determined in one season (2016). There was a negative association between both yield and bunch number and TSS, indicative of a delay in maturity, although there were no significant correlations with either yield or bunch number and pH. Yield was also negatively correlated with TA, while both yield and bunch number were negatively correlated with tartrate, anthocyanin and phenolics. Bunch weight was negatively correlated with TSS, TA and phenolics but positively correlated with malate. There were no significant correlations between berry weight or pruning weight and any measure of fruit composition. Berries per bunch was positively correlated with TA. The Ravaz index, largely driven by effects of yield on berry composition rather than pruning weight was negatively correlated with TSS, TA, tartrate and anthocyanin and positively correlated with YAN.

Table 2.1.6.2.5. Correlation matrix, describing linear relationships between fruit composition and growth characteristics of Shiraz across season and rootstock means (n=12 or 9). Retained r values are significant, $p<0.1$. Those highlighted in bold are significant $p<0.05$.

	Yield	***Bunches***	***Bunch weight***	***Berry weight***	***Berries per bunch***	***Pruning weight***	***Ravaz index***
TSS	**-0.67**	**-0.57**	**-0.57**				-0.49
pH							
TA	-0.48		-0.48		0.49		-0.48
Malate			0.50				
Tartrate	**-0.83**	**-0.92**					**-0.70**
YAN							0.53
anthocyanin	-0.48	**-0.64**					**-0.90**
phenolics	**-0.66**	**-0.58**	**-0.64**				

Multilinear regression techniques were used to identify the best combinations of growth traits with strong predictive value for Shiraz juice composition measured across the season x rootstock genotype data. They showed that a significant proportion of the variability in berry composition could be accounted for based on adjusted R^2 values including:

- 76% (p=0.014) of TSS by bunch weight (-ve) and pruning weight (+ve)
- 82% (p=0.003) of malate by bunch weight (+ve) and pruning weight (-ve)

- 84% (p=0.000) of tartrate by bunch number (-ve)
- 83% (p=0.005) of tartrate by yield (-ve) and pruning weight (+ve)
- 75% (p=0.006) of anthocyanin by bunch number (-ve) and pruning weight (+ve)
- 68% (p=0.033) of phenolic substances by yield (-ve), berries per bunch and pruning weight (both +ve)

D. Relationships between fruit composition variables

Correlation analyses were undertaken to explore relationships between berry juice composition variables across the means of the season x rootstock genotype data (Table 2.1.6.2.6). TSS was positively correlated with TA, tartrate anthocyanin and phenolic substances. pH was positively correlated with malate and YAN and negatively correlated with phenolic substances. TA was positively correlated with tartrate and phenolics. Malate was positively correlated with YAN and negatively associated with phenolics. Tartrate was positively correlated with anthocyanins and phenolics. YAN was negatively associated with anthocyanin. Interestingly, the expected link between anthocyanin and phenolic substances was not significant (r=0.43) across the season x rootstock means.

Table 2.1.6.2.6. Correlation matrix, describing linear relationships between fruit composition variables of Shiraz across season and rootstock means (n=12 or 9). Retained r values are significant, p<0.1. Those highlighted in bold are significant p <0.05.

	TSS	***pH***	***TA***	***Malate***	***Tartrate***	***YAN***	***Antho.***
pH							
TA	**0.81**						
malate		**0.93**					
tartrate	**0.74**		**0.59**				
YAN		0.56		0.48			
anthocyanin	0.48				**0.64**	-0.49	
phenolics	**0.91**	**-0.90**	**0.88**	**-0.81**	**0.79**		

E. Wine

Duplicate ferment Shiraz wines were produced for own roots and the two rootstocks in 2016 and 2017 with the intent to identify potential fermentation issues, assess whetherpH adjustment from tartaric acid addition led to unbalanced wines and to provide wine samples for analysis and for tasting by industry. Formal sensory assessments had not been conducted at the time of report writing. The fruit for winemaking was harvested on 8 March and 27 April in 2016 and 2017 respectively.

The must data show that a desirable level of TSS (i.e. 24.0 ^{o}Brix) was achieved in 2016 but not in 2017 (mean 20 ^{o}Brix), despite the seven week delay in harvest date (Table 2.1.6.2.7). In both seasons, must of own roots had the highest TSS (24.8 and 20.9 ^{o}Brix) and the lowest pH compared to the rootstocks. Must of 140 Ruggeri was slightly riper than C7 in 2016 but not in 2017. Differences in TA between own roots and the rootstocks were small in both seasons. YAN levels were below the minimum acceptable standard level (150 mg/L) for winemaking, in both seasons ranging from 85-114 mg/L, hence blanket treatments of 200 mg/L DAP were added during fermentation to all treatments.

At bottling, there were only small differences in wine pH across the rootstocks, ranging from 3.61 - 3.67 in 2016 and 3.43 - 3.52 in 2017. Wine TA levels were similar across the rootstocks in both years, ranging from 4.9 - 5.4 g/L. Wine alcohol levels reflected must TSS in both years, being highest for own root wines. In both years, wine chloride concentrations were 3 - 4 times higher in wines produced from own roots compared to 140 Ruggeri or C7. The higher Cl concentrations in wines produced from own roots (314 and 257 mg/L) were approaching levels identified to give ‘salty taste’ to Shiraz wine (i.e. 380 mg/L, Walker et al. 2015) but still lower than the 607 mg/L limit allowable in Australian wine. Cl levels were lowest in Shiraz wine produced from 140 Ruggeri in 2016, whereas in 2017 both 140 Ruggeri and C7 had low levels.

There were large differences in wine spectral properties between the seasons. Compared to 2017, wines in 2016 had higher colour density, hue values and total anthocyanin and lower levels of phenolic substances. Compared to own roots or C7, wines produced from 140 Ruggeri had the lowest colour density, total and ionised anthocyanin and phenolic substances. Wines produced from C7 rootstock had the highest colour density, total and

ionised anthocyanin in 2016. In 2017, own rooted wines had the highest colour density, total and ionised anthocyanin and phenolic substances.

Table 2.1.6.2.7. Must, wine data, spectral properties (colour density, hue, total anthocyanin, ionised anthocyanin and phenolic substances) of Shiraz wines produced from own roots, 140 Ruggeri and C7 rootstocks in 2016 and 2017.

	Must data			***Bottling data***				***Spectral properties***					***Cl***
	TSS (°Brix)	**pH**	**TA** g/L	**TA Added** g/L	**pH**	**TA** (g/L)	**Alc.** (%)	CD a.u.	Hue	A mg/L	IA mg/L	Phen. a.u.	mg/L
2016													
Own	24.8	3.37	5.2	0.25	3.67	4.9	15.2	4.33	0.75	561	16.8	12.8	314
140R	24.3	3.53	5.1	2.75	3.61	5.1	14.7	3.43	0.88	526	15.2	9.9	67
C7	23.9	3.64	4.8	1.25	3.64	5.0	14.4	4.46	0.86	634	19.8	11.5	105
2017													
Own	20.9	3.56	3.8	1.00	3.49	5.2	12.6	2.38	0.61	342	19.3	23.3	257
140R	19.3	3.77	3.6	3.50	3.43	5.4	11.5	1.58	0.68	246	12.4	16.9	69
C7	19.6	3.70	3.6	2.25	3.52	5.0	11.6	1.81	0.69	299	13.6	21.8	67

F. Discussion of Shiraz results

The trial was designed to assess the performance of Shiraz grafted on the salt tolerant C7 rootstock selection compared to own roots or grafted on 140 Ruggeri rootstock grown in a commercial vineyard in Padthaway using saline irrigation water. Under the commercial management regime, yields of C7 were equivalent to own rooted vines at 10-12 t/ha. In contrast, yields of 140 Ruggeri were very high and beyond expectations for the region at around 22 t/ha due to the retention of high bunch numbers and development of large bunches with large berries, compared to own roots and C7. Compared to own roots, both 140 Ruggeri and C7 proved to be good Cl excluders, with low Cl in juice and wine. Compared to 140 Ruggeri, C7 displayed enhanced juice composition with lower pH, lower levels of malate and higher levels of tartrate. Furthermore, compared to 140 Ruggeri less tartaric acid was required for pH adjustment of C7 must and the C7 wines had enhanced spectral properties.

In summary, it can be concluded that C7 shows considerable promise as a rootstock for Shiraz in Padthaway as a moderate vigour, salt excluding rootstock with similar growth characteristics to own roots and fruit composition and wine quality attributes more comparable to own roots than 140 Ruggeri which had excessive vigour and yield.

2.1.7. Summary of the performance of 'near to release' selections

Promising rootstock selections identified in previous projects have been evaluated in various trials located in a hot irrigated region at Irymple in Victoria and in the milder Padthaway region, with supplementary irrigation with moderately saline water. In undertaking these evaluations, significant interactions with region and scion and rootstock performance as grafted vines have been established. These interactions indicate that a suite of new rootstocks is required to meet industry requirements for new rootstocks suited to different varieties and regions.

From the trial conducted in a replant situation in a hot climate across five seasons with three varieties, it can be concluded that:

- there was a significant effect of rootstock genotype on conferred vigour which could be classified into four categories with C114 having very high vigour; Ramsey, C112 and C113 having high vigour; 1103 Paulsen having moderate vigour; and 140 Ruggeri and C20 having low vigour. The highly significant scion x rootstock interaction was associated with the low pruning weight of Cabernet Sauvignon and Shiraz grafted on 140 Ruggeri and the relatively lower pruning weight of Shiraz grafted on C113.
- there was a significant effect of rootstock genotype on yield with Ramsey, C114 and C113 producing the highest yields compared to the lower yielding 1103 Paulsen, 140 Ruggeri, C112 and C20. The highly significant scion x rootstock interaction was associated with the low yield of Cabernet Sauvignon grafted on 1103 Paulsen, 140 Ruggeri and C112 compared to their performance when grafted with Chardonnay and the high yield of C114 when grafted with Cabernet Sauvignon and Shiraz compared to its yield when grafted with Chardonnay.
- there was a significant effect of rootstock genotype on bunch number with Ramsey, C113 and C114 producing high bunch numbers compared to 140 Ruggeri, 1103 Paulsen, C112 and C20. The highly significant scion x rootstock interaction was associated with the low bunch number of Cabernet Sauvignon grafted on 1103 Paulsen, 140 Ruggeri and C112 particularly when compared to Chardonnay.
- there was no effect of rootstock genotype on berry weight while the effects of rootstock genotype on bunch weight and berries per bunch were small.

- there was no effect of rootstock genotype on juice titratable acidity, tartrate, berry anthocyanin or phenolics.
- there was a significant effect of rootstock genotype on juice TSS. 140 Ruggeri had the highest TSS, followed by C113 and Ramsey. C20, C112, C114 and 1103 Paulsen had the lowest TSS.
- there was a significant and reasonably consistent response of rootstock genotype on pH as the significant scion x rootstock interaction was small. 140 Ruggeri, C112, C113 had the highest pH followed by 1103 Paulsen, C20 and Ramsey with C114 having the lowest pH.
- there was a significant effect of rootstock genotype on malate. 140 Ruggeri, 1103 Paulsen and C20 had the highest malate. C114 and Ramsey had the lowest malate.
- there were significant effects of rootstock genotype on YAN, however a highly significant scion x rootstock interaction indicated that the effects were not consistent across the scions. 1103 Paulsen had the highest YAN, followed by 140 Ruggeri, C113, C20 and C112. C114 had the lowest level of YAN. The highly significant scion x rootstock interaction can be attributed to the high juice YAN of Ramsey and C113 grafted with Chardonnay, the low YAN of Cabernet Sauvignon on Ramsey and the high YAN of Shiraz on C20.
- further research is required to monitor whether the large increases in yield in 2017 following conversion to mechanical hedging at the Irymple site can be maintained in future seasons and to assess the longer term impacts on both growth and fruit composition.

From the Padthaway Shiraz trial involving the suite of 'near to release' selections assessed above it can be concluded that:

- there was a highly significant effect of rootstock on conferred Shiraz vigour with C114, 1103 Paulsen, Ramsey and C112 having the highest vigour. Shiraz grafted on 101-14 and M920 had the lowest vigour.
- there was a highly significant effect of rootstock on yield. Ramsey produced significantly more crop than all other rootstocks, followed as a group by 1103 Paulsen, C114, 140 Ruggeri and C112. C113 and C20 produced moderate yields while 101-14 produced low yield and M920 a very low yield.

- there was a highly significant effect of rootstock on bunch number. Highest bunch numbers were produced by Ramsey, C112, 1103 Paulsen, C114 and 140 Ruggeri while M920 had very low bunch numbers.
- there was a significant effect of rootstock on bunch weight. Ramsey had significantly larger bunches than all other rootstocks, followed as a group with similar bunch weights, C114, 140 Ruggeri and 1103 Paulsen. C20, C112 and C113 produced moderate size bunches while 101-14 and M920 had the smallest bunches.
- there were significant effects of rootstock on berry weight. Ramsey, C20, 1103 Paulsen and M920 had the largest berries while berries of 101-14 were smaller than all other rootstocks.
- there were no significant effects of rootstock on berry TSS, pH, tartrate, YAN or phenolics.
- there were significant effects of rootstock on TA. M920 had the highest juice TA followed by 101-14, 1103 Paulsen and C112. C113 and 140 Ruggeri had the lowest TA.
- there were significant effects of rootstock on malate with C113 and C20 having the highest and Ramsey the lowest malate levels.
- there were significant effects of rootstock on berry anthocyanin with 101-14 and C20 having the highest levels of anthocyanin, followed by 140 Ruggeri, C112 and C113 while Ramsey and C114 had the lowest levels.

From the Padthaway Shiraz trial involving the salt tolerant C7 selection it can be concluded that C7 shows considerable promise for Shiraz in Padthaway as a moderate vigour, salt excluding rootstock with similar growth characteristics to own roots and fruit composition and wine quality attributes more comparable to own roots than 140 Ruggeri, which had excessive vigour and yield.

From the preliminary results for the Padthaway Pinot Gris trial involving the salt tolerant C7 selection it can be concluded that that C7 shows considerable promise as a rootstock for Pinot Gris, a very low vigour variety, due to its enhanced performance compared to Ramsey and 140 Ruggeri and high salt excluding ability compared to own roots.

Based on the results of these studies conducted over five seasons, a number of selections have been identified for release to industry. A summary of the recommendations for each of the 'near to release' selections evaluated is provided below.

Selection C 114

It is recommended that the C114 selection be released for adoption by industry as a very high vigour, high yielding, salt tolerant alternative to Ramsey (which is not resistant to phylloxera), particularly for white wine production. It has shown resistance to selected root knot nematode and phylloxera isolates. Across varieties and sites C114 had the highest conferred vigour, yield and bunch number. At Padthaway it was the most tolerant rootstock to short term drought. In general, C114 fruit composition was similar to Ramsey with low TSS, pH, malate, YAN, berry anthocyanin and colour density and ionised anthocyanin in wine. Chardonnay wine produced from C114 was preferred over other rootstocks. C114 may be useful for red wine production as a risk management tool against drought and salinity, where water for irrigation is limited and because of delayed ripening, to extend the season. C114 has also performed well in dried grape trials when grafted with Carina, Sultana and Sunmuscat including maintaining high productivity with deficit irrigation. It is likely to be widely adopted across both the dried grape and table grape industries. Significant mother vine plantings of C114 have been established by licensed nurseries.

Selection C113

It is recommended that the C113 selection be released for adoption by industry as a high vigour and high yielding alternative to Ramsey in hot climates and as a moderate vigour, salt tolerant alternative to the salt and drought sensitive 101-14 in Padthaway. It has shown resistance to selected root knot nematode and phylloxera isolates. Wines produced from C113 at Padthaway had higher colour density, total and ionised anthocyanins than own rooted vines. In the replant situation at Irymple it offered distinct advantages over the lower yielding 1103 Paulsen and 140 Ruggeri, which may have been impacted by nematode infestation. C113 has also performed well in dried grape trials when grafted with Carina, Sultana and Sunmuscat including maintaining high productivity with deficit irrigation. Mothervine plantings of C113 have been established by licensed nurseries. It is also likely to be adopted across both the dried grape and table grape industries.

Selection C112

The high vigour C112 selection offered no significant advantages over C113 in the hot irrigated region trials, having lower yield, bunch number and lower TSS, high pH, malate and YAN. At Padthaway, C112 had higher vigour, yield and K in juice and leaves than C113. It is recommended that the performance of C112 continue to be monitored at both sites but that it not be released at this point of time as small-scale wine lots have not been produced and it is not included in dried grape trials. Feedback from regional trials established as part of this project will be taken into account before a final decision is made regarding this selection.

Selection C20

It is recommended that the moderate vigour, C20 selection be released as an alternative to 140 Ruggeri and 1103 Paulsen which have performed poorly in replant situations in the hot irrigated region. For the Padthaway region it is recommended as a moderate vigour, salt tolerant alternative to the salt and drought tolerant 101-14 which has been widely planted. In the hot region C20 had good yields, particularly with Cabernet Sauvignon due to high bunch numbers, most likely due to increased fruitfulness associated with an improved microclimate. It had the highest Ravaz index of all the rootstocks across all varieties in both regions due to its moderate conferred vigour and good yields, an indicator of good vine balance as it was able to adequately mature the fruit and high water use and carbon efficiency. It has shown resistance to selected root knot nematode and phylloxera isolates. Wines produced from C20 in Padthaway have high colour density and ionised anthocyanins.

Selection C7

The Padthaway Shiraz and Pinot Gris trials indicate that C7 shows considerable promise for Shiraz in Padthaway as a moderate vigour, salt excluding rootstock with similar growth characteristics to own roots and fruit composition and wine quality attributes more comparable to own roots than 140 Ruggeri or Ramsey (in the case of Pinot Gris). It should also be noted that C7 has a higher level of resistance to root knot nematodes than 140 Ruggeri and is also resistant to selected strains of phylloxera. Because the results of the Padthaway trials are preliminary, particularly for Pinot Gris, it is recommended that a decision to name and release be deferred until more data are available and sensory

analysis of the wines is completed. However, it is recommended that industry be encouraged to develop further regional semi-commercial evaluation sites where saline irrigation water is an issue including Coonawarra, Langhorne Creek and Padthaway covering a diverse range of scion varieties. Sufficient material from mother vine plantings should be available to facilitate this process. It should be noted that C7 has been included in recent dried grape trials with Carina, Sultana and Sunmuscat which include management under deficit irrigation which will produce the first significant crop in 2018.

Selection C29

C29 was established in trials located at Irymple and in Padthaway. It has performed very poorly with poor establishment and very low conferred vigour. It is recommended that C29 be removed from the CSIRO rootstock development program.

Selection C5

C5 was included for its salt tolerance in a Shiraz trial planted at site 2 in Padthaway which was established. In the trial C5 performed very poorly with low establishment and very low conferred vigour. It was not included in statistical analyses and most vines have been removed. It is recommended that C5 be removed from the CSIRO rootstock development program.

Selection M920

M 920 (a rootstock selection involving *V. rotundifolia* and *V. Vinifera*) was included in the 'near to release' Shiraz Padthaway trial at site 1 because it had showed excellent tolerance to high B when used for table grape production in Carnarvon, WA and was considered as a potential salt tolerant type. In the trial, M920 performed very poorly, having the lowest conferred vigour, yield, bunch number, bunch weight and berries per bunch of all the rootstocks. It also had low TSS and high Na in juice, petioles and lamina. It is recommended that C5 be removed from the CSIRO rootstock development program.

2.2. Next generation rootstocks

2.2.1. Shiraz CSIRO Irymple

The Shiraz trial, planted in 2010, was designed to evaluate the field performance of 55 of the most promising drought tolerant selections, under nursery conditions, from 310 genotypes in the previous project (CSP 0503). It included own roots and five commercial rootstocks (Ramsey, 140 Ruggeri, 1103 Paulsen, Merbein 5489Ω and Schwarzman). The trial incorporated two irrigation treatments, a full irrigation (Control, 5.5 ML/ha) and a deficit irrigation treatment (2.5 ML/ha), which were applied to individual blocks. Data recorded over five seasons (2013 to 2017) are included in this analysis.

Volumetric soil moisture, monitored over for two seasons, 2015-16 and 2016-17 is shown in Figure 2.2.1.1. The refill point when irrigation was applied during the growing season was when the volumetric water content at 30 cm of the control treatment was between 24.5 and 25.0 % moisture. Consequently, the average water content of the Control treatment during the growing season at 30 cm was 22.0 - 22.5% whereas at 60 cm it was between 20.0 - 20.5%. In contrast, the average water content over the growing season of the deficit treatment at 30 cm was around 15% and at 60 cm, 16.0 - 16.5%.

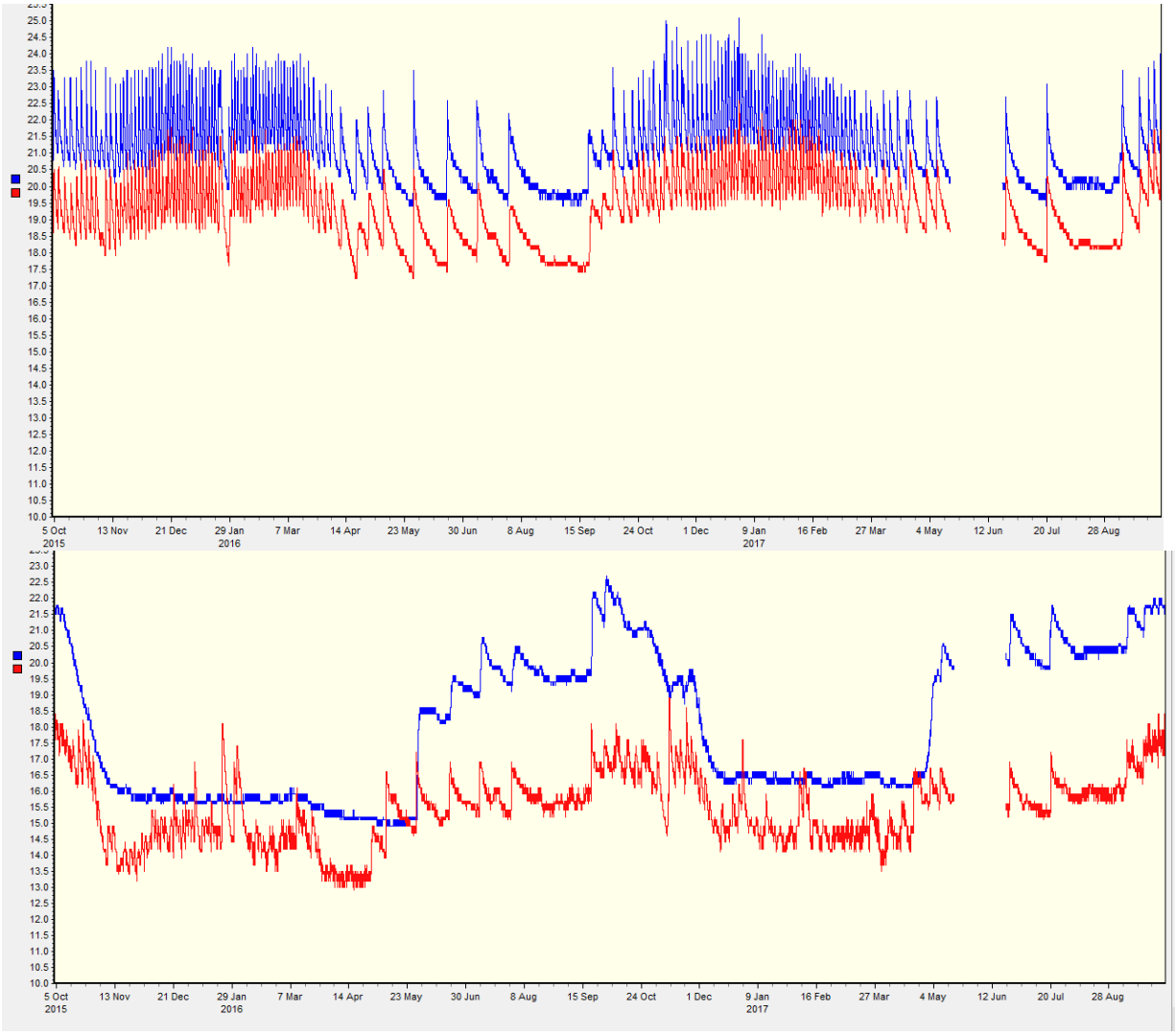


Figure 2.2.1.1. Soil moisture (% v/v) recorded in the Control irrigation (Top) and deficit irrigation treatment (Bottom) at 30 cm and 60 cm. In the Control (Top) graph, blue and red lines are for 30 cm and 60 cm measurements, respectively. In the deficit graph (Bottom), blue and red lines are for 60 cm and 30 cm, respectively.

2.2.1.1. Analyses including both irrigation treatments

A. Yield and its components, pruning weight and Ravaz index

Yield and yield component data, pruning weight and the Ravaz index, collected over five seasons (2013-17), for Shiraz grafted on '55 genotypes' and five standard rootstocks and own rooted vines (Table 2.2.1.1). GLM, ANOVA analysis of the data showed significant effects of Rootstock, Irrigation and Year for almost all variables. However, there were no significant rootstock and year interactions. Hence, only the means of rootstocks and

seasons are presented in the absence of interactions. The experimental design, where irrigation treatments were embedded in blocks did not allow assessment of the rootstock x irrigation interaction.

There were significant effects of season on yield and its components, pruning weight and the Ravaz index of Shiraz (Table 2.2.1.1.). Highest yield and bunch number were recorded in 2017, post conversion to simulated mechanical hedging in winter 2016. Season 2014 had the lowest yield. Bunch numbers were lowest in 2013. Largest bunches were produced in 2013. Largest berries were recorded in 2013 and 2015. The highest and lowest berries per bunch were recorded in 2013 and 2015, respectively. The highest and lowest pruning weights were recorded in 2015 and 2017, respectively. There was more than a three-fold difference in the Ravaz index, an indicator of vine balance and a surrogate for carbon efficiency and water use efficiency, across the seasons being highest in in 2017 post conversion to mechanical hedging.

There were significant effects of irrigation on yield and its components, pruning weight and the Ravaz index of Shiraz, except berries per bunch (Table 2.2.1.1). On average across the rootstock genotypes and seasons the deficit treatment reduced yield, bunch weight, berry weight, and pruning weight by 7%, 13%, 9% and 23% respectively. The 9% increase in bunches per vine with the deficit treatment, which partly offset the yield reductions listed above, may have been due to improved shoot fruitfulness associated with a reduction in conferred vigour and improved light interception. The deficit treatment also produced a small (14%) but significant increase in the Ravaz index.

Rootstock genotype had highly significant effects on all growth characteristics of Shiraz except berries per bunch (Table 2.2.1.1.). Differences between the 60 rootstocks genotypes in yield (three-fold), bunch number (2.4-fold), bunch weight (71 %), berry weight (27%), pruning weight (eight-fold) and Ravaz index (9.3-fold) were very large. The distribution of the growth characteristics across the 60 rootstock genotypes is shown in Figure 2.2.1.2. With the exception of berry weight, most variables appeared to be normally distributed across the rootstock genotypes. Berry weight displayed a skewed distribution towards the larger berry categories across the rootstock genotypes.
Of all the rootstock genotypes in the trial, Ramsey which had the highest bunch numbers produced the highest yield. Merbein 5489 had the lowest bunch number and yield. Bunch weight, berry weight, berries per bunch and pruning weight of own roots and Merbein

5489 were lower than the other standard rootstocks. Schwarzman had the highest pruning weight of all the rootstocks. Vine balance, as determined by the Ravaz index, of own roots and Merbein 5489 was higher than the other standard rootstocks, approaching optimum levels suggested for Cabernet Sauvignon by Dokoozlian 2011.

Table 2.2.1.1. Mean yield, bunches per vine, bunch weight, berry weight, berries per bunch, pruning weight and Ravaz index of Shiraz grafted on 55 CSIRO rootstock selections, five standard rootstocks or grown as own roots over five seasons (2013-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***Yield (kg/vine)***	***Bunches /vine***	***Bunch weight (g)***	***Berry weight (g)***	***Berries/ bunch***	***Pruning weight (kg/vine)***	***Ravaz Index***
Own roots	8.45	111.5	81.9	1.19	68.7	1.49	6.59
Ramsey	13.17	125.6	109.6	1.31	84.0	3.72	4.18
Schwarzman	9.57	88.5	108.1	1.35	81.2	5.28	3.27
1103P	10.00	98.5	100.0	1.28	79.1	2.76	4.75
140R	9.76	98.7	100.2	1.33	75.0	2.85	4.91
Merbein 5489	6.63	85.8	72.8	1.06	66.6	1.53	6.48
minimum	4.61	52.7	70.7	1.06	57.0	0.67	1.66
maximum	13.17	125.6	120.7	1.35	123.1	5.28	15.42
Irrigation							
Control	8.45a	81.9b	103.1a	1.30a	79.1	2.77a	5.20b
Deficit	7.90b	89.2a	90.0b	1.18b	77.1	2.12b	5.91a
Year							
2013	5.79cd	47.5d	119.0a	1.31a	91.1a	2.21c	3.84c
2014	5.54d	59.1cd	91.4b	1.18b	76.5b	2.81b	3.38c
2015	6.38c	68.8c	90.1b	1.31a	68.9c	3.02a	3.36c
2016	8.51b	94.1b	90.1b	1.21b	74.6bc	2.23c	6.78a
2017	14.67a	158.3a	90.0b	1.22b	77.1b	1.98cd	10.42a
Significance[a]							
Rootstock	***	***	***	***	ns	***	***
Irrigation	**	**	***	***	ns	***	*
Year	***	***	**	***	***	***	***

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively.

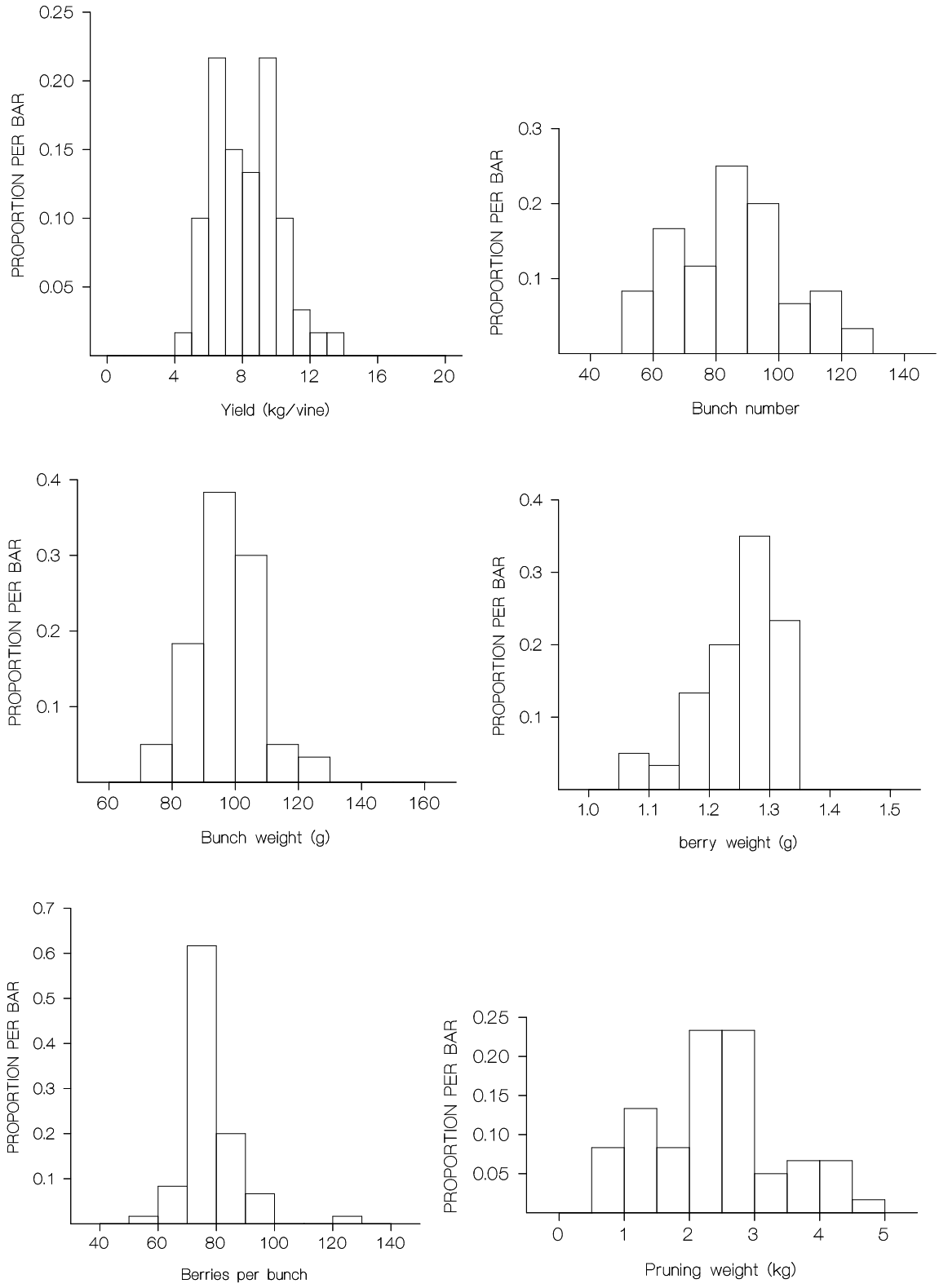


Figure 2.2.1.2. Distribution of mean values of yield and its components and pruning weight of 60 rootstock genotypes over two irrigation treatments (Control and Deficit) and five seasons (2013-15).

Correlation analyses were conducted using only the rootstock means to explore relationships between yield determinants and growth characteristics across the rootstock genotypes (Table 2.2.1.2). Bunch number and to a lesser extent bunch weight were significant contributors to yield variability across the rootstock genotypes. A highly significant multilinear regression ($p<0.001$) indicted that 94.6% of yield variation across the rootstock genotypes could be accounted for by inclusion of bunches and bunch weight in the model. Bunch weight was positively correlated with both berry weight and berries per bunch and pruning weight. There was a strong positive correlation between berry weight and pruning weight. Interestingly the relationship between yield and pruning weight while significant, was low.

Table 2.2.1.2. Correlation matrix across rootstock means (n=60) describing relationships between rootstock growth characteristics across rootstock genotypes. Values of r shown are significant $p<0.05$. Highly significant correlations ($p<0.001$) are in bold.

	Yield	***Bunches***	***Bunch weight***	***Berry weight***	***Berries per bunch***	***Pruning weight***
Bunches	**0.86**					
Bunch weight	**0.59**					
Berry weight	0.31		**0.61**			
Berries/ bunch	**0.50**		**0.72**			
Pruning weight	0.24		**0.46**	**0.70**	0.36	
Ravaz index			-0.32	**-0.61**		**-0.77**

B. Fruit composition

Berry juice composition data collected over five seasons (2013-17) for Shiraz across the 60 rootstock genotypes is presented in Table 2.2.1.3. GLM, ANOVA analysis of the data showed significant effects of Rootstock, Irrigation and Year for most variables. The results indicate that there were significant differences in maturity when samples were collected, ranging from a mean of 22.4 ^{o}Brix to 26.6 ^{o}Brix. To test the effect associated with the differences in maturity, TSS was included as a covariate in the ANOVA analysis. There were significant effects of TSS as a covariate on all variables (data not shown in detail).

The results show significant effects of season on all berry juice variables (Table 2.2.1.3). TSS was highest in 2014 and lowest in 2017, following conversion to mechanical hedging. pH was highest in 2014 and lowest in 2013. TA was highest in 2013 and lowest in 2016. Malate was highest in 2015 and lowest in 2017. Tartrate was highest in 2013 and lowest in 2017. YAN was highest in 2014 and lowest in 2017. Anthocyanin and phenolic substances were highest in 2015 and lowest in 2017. Irrigation treatments had a significant effect on all fruit composition variables. The deficit treatment, compared to the control treatment had significantly higher TSS, pH, tartrate, anthocyanin and phenolic substances and lower TA, malate and YAN.

Rootstocks had significant effects on most juice compositional variables, except tartrate, anthocyanin and phenolic substances as shown by the differences in minimum and maximum values (Table 2.2.1.3). The distribution of the berry composition variables across the 60 rootstock genotypes shown in Figure 2.2.1.3, generally followed a normal distribution. Differences in fruit composition between the standard rootstocks were generally small with Merbein 5489 (25.8 ^{o}Brix) and own roots (24.2 ^{o}Brix having the highest and lowest TSS. Ramsey had the highest pH while Merbein 5489 and own roots had the lowest pH. Schwarzman had the highest TA, malate and YAN levels while Merbein 5489 and own roots had the lowest levels of these compounds. Merbein 5489 had significantly more anthocyanin and phenolic substances than all the other standard rootstocks, including own roots.

Table 2.2.1.3. Mean juice TSS, pH, TA, Malate, Tartrate, YAN, anthocyanin and phenolic substances of Shiraz grafted on 60 CSIRO rootstock genotypes over five seasons (2013-17). Means followed by the same letter are not significantly different ($p < 0.05$). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***TSS (ºBrix)***	***pH***	***TA (g/L)***	***Malate (g/L)***	***Tartrate (g/L)***	***YAN (mg/L)***	***Anth. (mg/g)***	***Phen. (a.u.)***
Own roots	24.2	4.17	3.45	2.95	5.81	188	1.16	1.45
Ramsey	25.1	4.30	3.91	4.43	5.90	297	1.07	1.39
Schwarzman	24.9	4.22	4.51	5.01	5.83	319	1.15	1.42
1103P	25.5	4.27	3.86	3.82	5.63	237	1.20	1.43
140R	24.6	4.26	3.90	4.37	5.55	291	0.95	1.26
Merbein 5489	25.8	4.14	3.46	3.09	5.49	194	1.61	2.03
minimum	22.4	3.99	2.96	2.40	4.82	141	0.92	1.26
maximum	26.6	4.36	4.51	5.01	6.07	333	1.61	2.03
Irrigation								
Control	24.5b	4.17b	3.80a	3.74a	5.41b	226a	1.06b	1.31b
Deficit	25.3a	4.25a	3.32b	3.30b	5.67a	215b	1.20a	1.52a
Year								
2013	25.0b	4.00c	4.45a	3.26b	7.08a	232b	-	-
2014	26.1a	4.47a	3.12d	4.47a	5.78b	280a	1.02bc	1.67b
2015	25.2b	4.21b	3.86b	4.48 a	4.73c	208c	1.47a	1.74a
2016	25.8ab	4.33 b	2.91d	2.75c	5.42b	202c	1.27b	1.60b
2017	22.3c	4.05c	3.46bcd	2.64c	4.68c	181d	0.76c	1.05c
Significance[a]								
Rootstock	***	**	***	***	ns	***	ns	ns
Irrigation	***	***	***	***	***	*	***	***
Year	***	***	***	***	***	***	***	***
TSS covariate	-	***	***	***	**	***	***	***

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively.

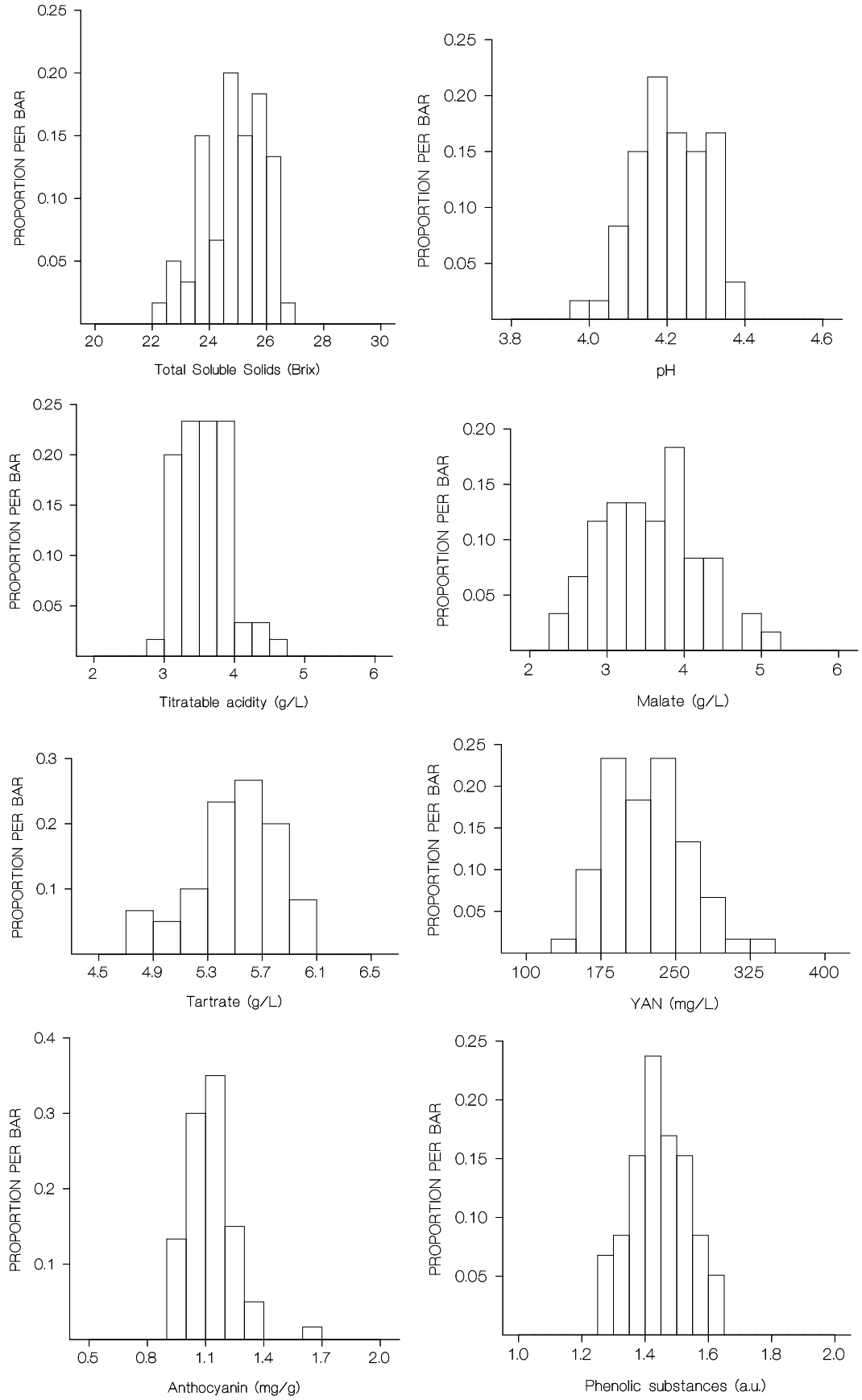


Figure 2.2.1.3. Distribution of mean values of berry composition variables of 60 rootstock genotypes over two irrigation treatments (Control and Deficit) and five seasons (2013-15).

C. Juice nutrient status

In 2016, juice samples were analysed by ICP to determine nutrient status (Table 2.2.1.4). Compared to the control irrigation, the deficit treatment produced significant increases in B and Na, but reduced the concentration of Ca, P and S in juice. Rootstocks had a significant effect on most mineral elements except Ca and P with 2.8-fold differences in B, 2.5-fold difference in K, two-fold differences in Mg, three-fold difference in P and a 2.8-fold difference in S. Across the standard rootstocks including own roots, B values were relatively high (17.2- 20.6 mg/L), Ca was highest with 1103 Paulsen and Merbein 5489 and lowest with Schwarzman and Ramsey; K was highest with 1103 Paulsen and Ramsey and lowest with own roots; there was little variation in Mg concentration; own roots and Ramsey had higher Na concentrations than the other standards rootstocks; P was highest with 1103 Paulsen and lowest with own roots while S was highest with Ramsey and lowest with own roots and Merbein 5489. The distribution of the mineral elements across the 60 rootstock genotypes is shown in Figure 2.2.1.4. Most elements followed a normal distribution. However, in the case of Na, the distribution was highly skewed toward lower Na concentrations, indicating significant Na exclusion by most rootstocks.

Table 2.2.1.4. Mean mineral element concentrations in juice of Shiraz grafted on 60 CSIRO rootstock genotypes in season 2016. Means followed by the same letter are not significantly different ($p < 0.05$). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***B (mg/L)***	***Ca (mg/L)***	***K (mg/L)***	***Mg (mg/L)***	***Na (mg/L)***	***P (mg/L)***	***S (mg/L)***
Own roots	17.2	106.5	1717	112.9	118.2	82.9	64.2
Ramsey	19.0	98.1	2450	107.5	90.1	112.1	97.8
Schwarzman	17.6	95.3	2143	112.95	24.2	120.5	75.4
1103P	20.6	121.0	2603	111.8	24.0	170.2	85.2
140R	18.6	103.9	2083	116.6	34.4	102.2	80.8
Merbein 5489	20.0	120.3	2129	119.8	23.6	125.1	69.6
minimum	8.3	77.8	1108	71.3	7.8	59.6	37.0
maximum	23.5	151.2	2785	144.5	273.0	173.8	102.4
Irrigation							
Control	14.3b	123.4a	2038	100.9	22.9b	135.8a	71.5a
Deficit	17.8a	111.5b	2049	101.4	56.7a	100.0b	65.6b
Significance[a]							
Rootstock	***	ns	***	***	***	ns	**
Irrigation	***	***	ns	ns	***	***	*
TSS covariate	***	*	***	***	ns	***	***

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively.

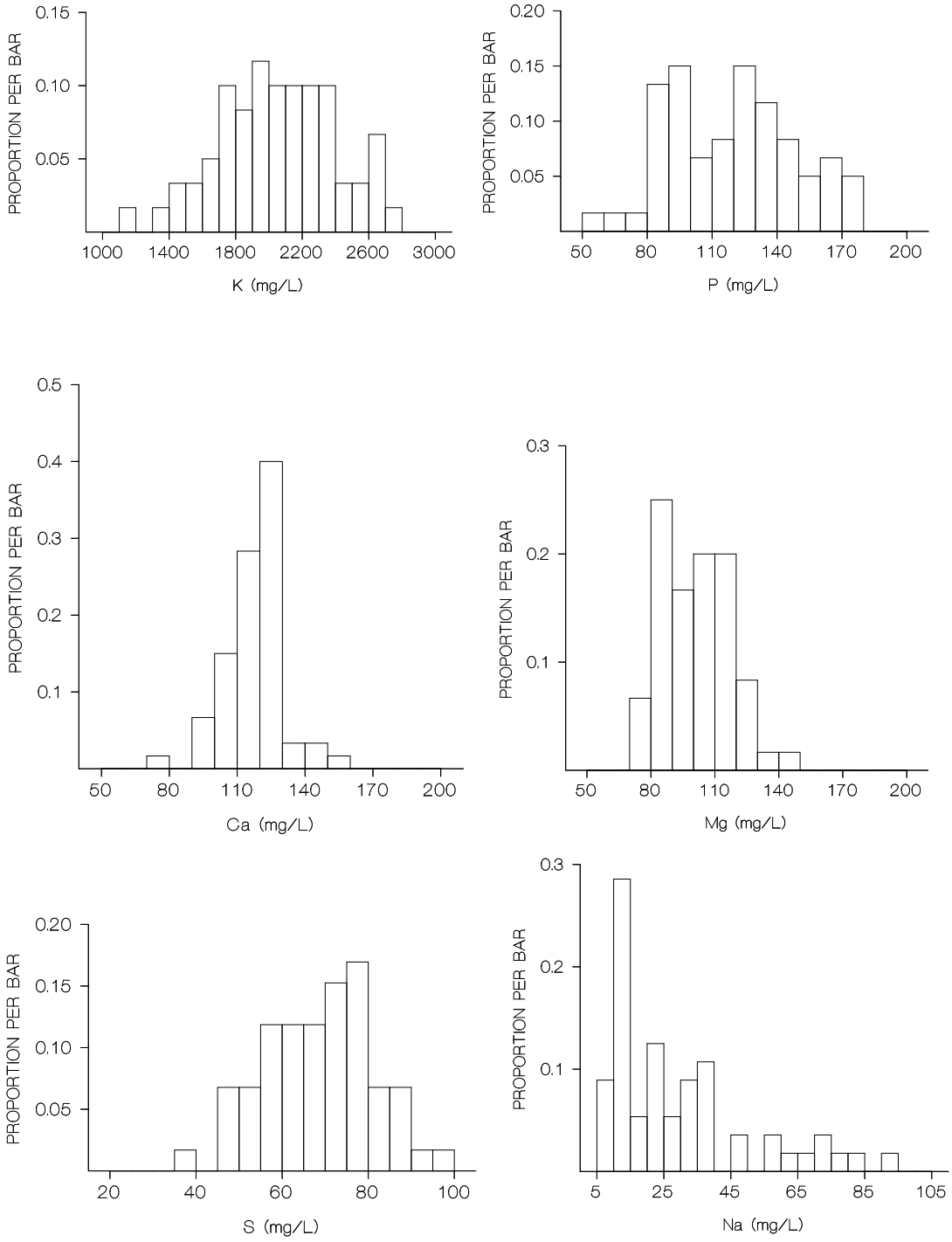


Figure 2.2.1.4. Distribution of mean values of juice mineral elements of 60 rootstock genotypes over two irrigation treatments (Control and Deficit), season 2016.

D. Relationships between growth characteristics and fruit composition

Correlation analyses were undertaken to explore relationships between Shiraz vine growth characteristics and berry juice composition using rootstock means with effects of irrigation and season removed (Table 2.2.1.5). Adjusted fruit composition means generated from the inclusion of the significant TSS were also included to removes effects of fruit maturity. The results clearly show that rootstock effects on vine growth characteristics can have a significant influence on fruit composition. The results indicate that yield differences between rootstock genotypes had little effect on fruit composition, except weak but significant negative correlations with TSS and anthocyanin and phenolic substances, which disappeared when adjusted for TSS. There were also weak negative correlations between yield and juice Ca and P and positive correlations with adjusted B and S. Rootstock bunch number was negatively correlated with TSS and pH and anthocyanin, which disappeared when adjusted for TSS. Bunch number correlated negatively with Ca and P but positively with adjusted B. Rootstock bunch weight was positively correlated with TA, malate and YAN and negatively correlated with anthocyanin and phenolic substances. These responses, except TA were retained when adjusted for TSS. Berry weight strongly influenced fruit composition with positive correlations with TSS, pH, TA, malate, tartrate, YAN and negative correlations with anthocyanin and phenolic substances. When adjusted for TSS, the effects of berry weight on pH, TA and tartrate disappeared whereas the negative correlations with anthocyanin and phenolic substances were stronger. Berry weight was also positively correlated with K, P, and S, with both the unadjusted and adjusted means. Berries per bunch had little effect on fruit composition, except a weak positive correlation with TA which disappeared when adjusted for TSS and a weak correlation with adjusted malate.

The eight-fold difference in conferred vigour across the rootstock genotypes had highly significant influences on fruit composition. Pruning weight was positively correlated with TSS, pH, TA, malate, tartrate and YAN but not anthocyanin or phenolics (Table 2.2.1.5). When adjusted using the TSS covariate, pruning weight correlations with pH and tartrate disappeared. Pruning weight could also be linked to juice mineral concentration being positively correlated with B, K, Mg, P and S, using both the unadjusted and TSS adjusted means. Pruning weight negatively correlated with juice Ca, for both the unadjusted and adjusted means. Vine balance, as determined using the Ravaz index also strongly influenced fruit composition, most probably due to the effects of pruning weight already

established and to a lesser degree yield. Consequently, it was negatively correlated with TSS, pH, TA, malate, tartrate and YAN but not anthocyanin or phenolics. When adjusted for TSS the correlations with TA and tartrate disappeared and weak, while positive correlations with anthocyanin and phenolic substances were shown. The Ravaz index was negatively correlated with juice B, K, Mg, P and S, using both the unadjusted and adjusted means. It was also positively correlated with adjusted Ca.

Table 2.2.1.5. Correlation matrix, describing linear relationships between mean growth characteristics (yield component variables, pruning weight and the Ravaz index), berry juice composition of Shiraz across rootstock genotypes (n=60). Values retained are significant ($p<0.05$). Highly significant correlations ($p<0.001$) are in bold.
Note: adjusted means using the significant TSS covariate were also included in the analysis. Mineral element data for 2016 only.

	Yield	***Bunches***	***Bunch weight***	***Berry weight***	***Berries per bunch***	***Pruning weight***	***Ravaz index***
TSS	-0.28	-0.37		0.31		**0.55**	**-0.69**
pH		-0.29		0.25		**0.45**	**-0.53**
TA			0.37	**0.57**	0.27	**0.76**	**-0.57**
Malate			0.32	**0.62**		**0.86**	**-0.74**
Tartrate				0.32		**0.47**	**-0.57**
YAN			0.27	**0.57**		**0.83**	**-0.71**
Anthocyanin	-0.34	-0.24	**-0.42**	-0.26			
Phenolics	-0.27		-0.37	-0.37			
pH adj.							
TA adj.						**0.79**	**-0.63**
Malate adj.			0.38	**0.61**	0.28	**0.80**	**-0.61**
Tartrate adj.							
YAN adj.			0.34	**0.53**		**0.74**	**-0.55**
Antho. adj.			-0.37	**-0.42**			0.29
Phen. adj.			-0.27	**-0.49**		-0.32	0.32
B						**0.49**	**-0.55**
Ca	-0.28	-0.31				-0.28	
K				**0.43**		**0.63**	**-0.65**
Mg						0.30	-0.36
P	-0.30	**-0.44**		0.38		**0.56**	**-0.51**
Na							
S				**0.45**		**0.69**	**-0.65**
B adj.	0.25	0.30				0.28	-0.31
Ca adj.						-0.39	0.29
K adj.				0.39		**0.41**	-0.37
Mg adj.							-0.24
P adj.				0.34		0.31	
Na adj.							
S adj.	0.30			0.40		**0.52**	**-0.42**

Multilinear regression techniques were used to identify the best combinations of growth traits with strong predictive value for Shiraz juice composition across the rootstock genotypes (n=60). They showed that a significant proportion of the variability in berry composition across could be accounted for based on adjusted R^2 values including:-

- 47% (p=0.000) of TSS by bunch number, bunch weight (both -ve) and pruning weight (+ve)
- 25% (p=0.000) of pH by bunch number (-ve) and pruning weight (+ve)
- 56% (p=0.000) of TA by pruning weight (+ve)
- 73% (p=0.000) of malate by pruning weight (+ve)
- 25% (p=0.000) of tartrate by berries per bunch (-ve) and pruning weight (+ve)
- 72% (p=0.000) of YAN by berries per bunch (-ve) and pruning weight (+ve)
- 37% (p=0.000) of anthocyanin by bunch weight and berry weight (both -ve) and pruning weight (+ve)
- 24% (p=0.000) of phenolic substances by berry weight (-ve) and pruning weight (+ve)
- 35% (p=0.000) of B by bunch weight (-ve) and pruning weight (+ve)
- 14% (p=0.004) of Ca by bunch number and pruning weight (both -ve)
- 43% (p=0.000) of K by bunch weight (-ve) and pruning weight (+ve)
- 20% (p=0.001) of Mg by bunch weight (-ve) and pruning weight (+ve)
- 47% (p=0.000) of P by bunch number (-ve) and pruning weight (+ve)
- 49% (p=0.000) of S by bunch weight (-ve) and pruning weight (+ve)

Relationships between pruning weight and pH and malate, and berry weight and malate and YAN across means generated over seasons and irrigation treatments for the 60 rootstock genotypes are shown in Figure 2.2.1.1.5.

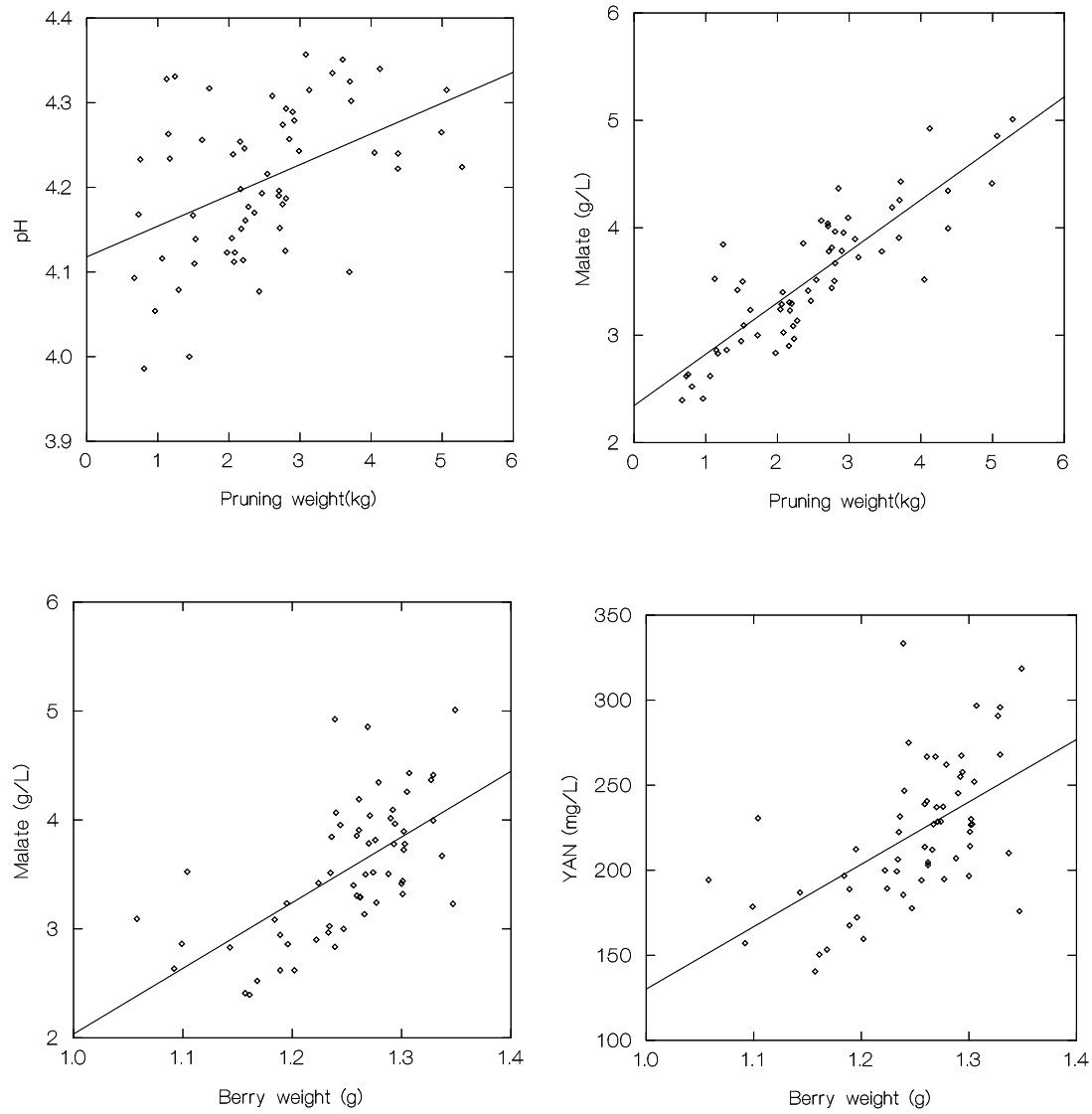


Figure 2.2.1.5. Relationships between pruning weight and pH or malate (Top) and berry weight and malate or YAN (Bottom) across 60 rootstock genotypes over two irrigation treatments (Control and Deficit) and five seasons (2013-15).

F. Relationships between fruit composition variables

Correlation analyses were undertaken to explore relationships between Shiraz berry juice composition variables using rootstock means with effects of irrigation and season removed (Table 2.2.1.6). Adjusted fruit composition means generated from the inclusion of the significant TSS covariate were also included to removes effects of fruit maturity. Across the rootstocks, there were significant effects of fruit maturity described by the significant positive correlations between TSS and pH, TA, malate, tartrate, YAN, anthocyanin, K, Mg, P, and S. Juice pH was positively correlated with malate, tartrate and YAN. However, when adjusted for TSS the correlations with tartrate disappeared and significant negative correlations with anthocyanin and phenolics were shown. Juice pH was positively correlated with K, Mg, P, and S and when adjusted for TSS, positively correlated with B, K, and S. TA was positively correlated with malate, tartrate and YAN using both the unadjusted and adjusted means. The strong correlations with malate highlight the dominance of this organic acid on TA across the rootstocks compared to tartrate. TA was positively correlated with B, K, Mg, P and S using both the adjusted and unadjusted means. Juice malate was positively correlated with tartrate and highly correlated with YAN and adjusted pH, TA, tartrate and YAN but negatively correlated with adjusted anthocyanin and phenolics. There were also positive correlations between malate and B, K, Mg, P and S using both the unadjusted and adjusted means. There were also negative correlations between malate and unadjusted or adjusted Ca. Juice tartrate was positively correlated with YAN and adjusted TA, malate and YAN and negatively correlated with adjusted anthocyanin and phenolics. Tartrate was also positively correlated with B, K, Mg, P, Na and S using both the unadjusted and adjusted means, with the exception of adjusted P. Juice YAN, was positively correlated with adjusted pH, TA, malate, tartrate, and negatively correlated with anthocyanin and phenolics. YAN was positively correlated with both the unadjusted and adjusted means for B, K, Mg, P and S and negatively correlated with both Ca means. There were strong positive correlations between anthocyanin and unadjusted and adjusted phenolics and unadjusted P.

Linear relationships between K and pH, malate and YAN and YAN and malate across 60 rootstock genotypes over two irrigation treatments (Control and Deficit) and five seasons (2013-15) are shown in Figure 2.2.1.6.

Table 2.2.1.6. Correlation matrix, describing linear relationships between Shiraz juice composition variables across rootstock (n=60). Values retained are significant ($p<0.05$). Highly significant correlations ($p<0.001$) are in bold.

	TSS	***pH***	***TA***	***Malate***	***Tartrate***	***YAN***	***Antho.***	***Phen.***
pH	**0.75**							
TA	0.24							
malate	**0.54**	**0.55**	**0.78**					
tartrate	**0.56**	**0.53**	0.36	**0.57**				
YAN	**0.56**	**0.57**	**0.71**	**0.94**	**0.56**			
anthocyanin	0.38							
phenolics							**0.91**	
pH adj.		**0.73**		0.27		0.27	-0.28	-0.33
TA adj.			**0.99**	**0.82**	**0.41**	**0.75**		
Malate adj.		0.38	**0.81**	**0.96**	**0.46**	**0.89**		-0.25
Tartrate adj.			0.27	0.32	**0.83**	0.30		
YAN adj.		0.34	**0.74**	**0.88**	**0.42**	**0.94**		-0.26
Antho. adj.		**-0.44**		-0.37	-0.32	-0.35	**0.78**	**0.80**
Phen. adj.		**-0.52**		**-0.48**	-0.38	**-0.46**	**0.61**	**0.81**
B	**0.55**	**0.56**	**0.43**	**0.67**	**0.61**	**0.65**		
Ca				-0.29		-0.34		
K	**0.73**	**0.73**	**0.44**	**0.78**	**0.62**	**0.74**		
Mg	0.39	0.32	0.34	**0.50**	**0.54**	**0.45**		
P	**0.74**	**0.58**	0.36	**0.55**	0.31	**0.54**	0.35	
Na					**0.43**			
S	**0.65**	**0.59**	**0.55**	**0.78**	**0.54**	**0.81**		
B adj.		0.26	0.36	**0.48**	**0.48**	**0.49**		
Ca adj.		-0.31	-0.25	-0.40		**-0.44**		
K adj.		0.38	0.39	**0.61**	**0.50**	**0.60**		
Mg adj.			0.31	**0.41**	**0.48**	0.37		
P adj.			0.30	0.30		0.34		
Na adj.					**0.41**			
S adj.		0.28	**0.52**	**0.63**	**0.42**	**0.70**		

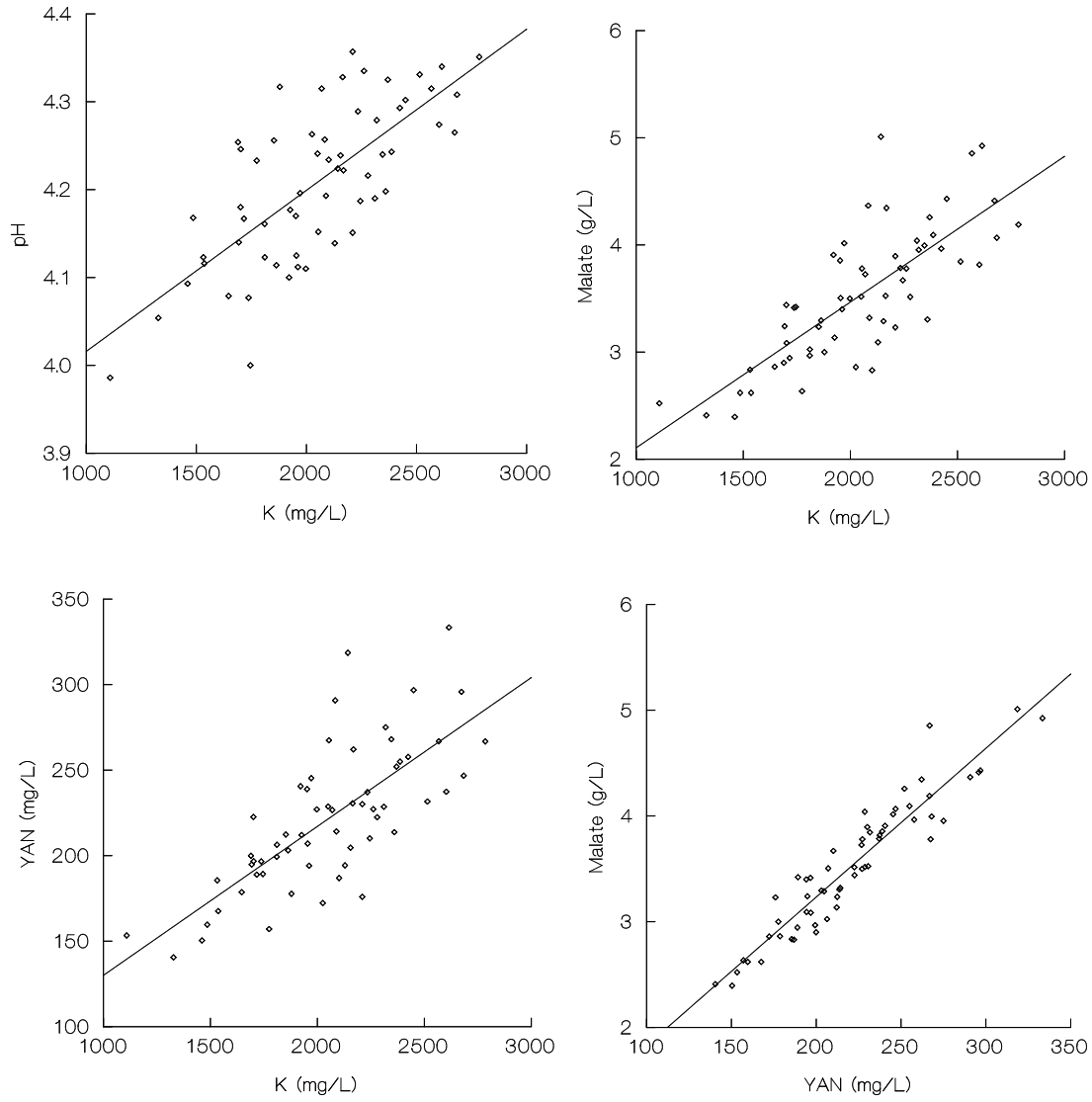


Figure 2.2.1.6. Relationships between K and pH, malate and YAN and YAN and malate across 60 rootstock genotypes over two irrigation treatments (Control and Deficit) and five seasons (2013-15).

G. Impact of irrigation treatments on relationships between growth characteristics and fruit composition

Rootstock and seasonal effects were also analysed by GLM, ANOVA for the individual irrigation treatments (data not shown or discussed in detail). With the control irrigation, well-watered treatment there were significant effects of rootstock on growth characteristics, fruit composition except tartrate, anthocyanin and phenolics. TSS as a covariate was highly significant for all variables. Under the well-watered control conditions significant rootstocks effects on juice mineral elements were only found for B, Mg and Na. TSS as a covariate was significant for K, P, and S. With the deficit treatment there were significant effects of both season and rootstock on growth characteristics except berries per bunch, fruit composition except tartrate, anthocyanin and phenolics. TSS as a covariate was highly significant for all variables except TA. There were significant rootstocks effects on B, K, Mg, Na and S. TSS as a covariate was significant with the deficit treatment for K, Mg, P, S. While not presented, the mean data for both irrigation treatments was used in correlation analyses to explore relationships across the rootstock genotypes between growth characteristics and fruit composition with the aim to identify if irrigation treatment had effects on key relationships. Adjusted fruit composition means generated from the inclusion of the significant TSS were also included to removes effects of fruit maturity.

The results clearly show that irrigation treatments significantly alter the relationships between growth characteristics and fruit composition (Table 2.2.1.7). With the well-watered control, yield was negatively correlated with TSS, pH, anthocyanin, phenolics, K, P and S. These relationships disappeared when adjusted using TSS as a covariate, an indication that the yield effects were associated with differences in maturity. With deficit irrigation, yield was also negatively correlated with anthocyanin and phenolics which largely disappeared when adjusted for TSS. There were also weak correlations with between yield and adjusted YAN and Na. In the control irrigation treatment, bunch number was negatively correlated with TSS, pH, malate, YAN, anthocyanin, phenolics, K, P and S which disappeared when adjusted for TSS, except for TA and malate. In contrast, with deficit irrigation there were only weak negative correlations between bunch number and TSS and pH, which disappeared when adjusted for TSS and, negative correlations with Ca, K, P, and a positive correlation with adjusted Na.

In the control irrigation, bunch weight had little impact on fruit composition except for a weak negative correlation with anthocyanin and a positive correlation with adjusted malate (Table 2.2.1.7). In contrast, bunch weight in the deficit irrigation treatment was associated with key aspects of fruit composition being positively correlated with unadjusted and adjusted TA, malate and YAN and negatively correlated with unadjusted and adjusted anthocyanin and phenolics. In the deficit treatment, bunch weight was also positively correlated with S and adjusted Na and negatively correlated with Mg, Na and adjusted Mg and S. In the control treatment berry weight strongly influenced fruit composition with positive correlations with TSS, pH, TA, malate, tartrate and YAN but not anthocyanins or phenolics. When adjusted for TSS, the effects of berry weight on pH disappeared while a negative relationship with phenolics was found. Berry weight was also positively correlated with unadjusted K, P, and S and adjusted B, K, and S. In the deficit treatment berry weight correlated positively with TSS and unadjusted and adjusted TA, malate and YAN and negatively correlated with unadjusted and adjusted anthocyanin and phenolics. Berry weight also correlated positively with K, P and S.

Conferred vigour (i.e. pruning weight) across the rootstock genotypes had highly significant influences on fruit composition under both irrigation treatments (Table 2.2.1.7). In the control treatment pruning weight was positively correlated with TSS, pH, TA, malate, tartrate, YAN, anthocyanin and phenolics. When adjusted using the TSS covariate the pruning weight correlations with pH, anthocyanin and phenolics disappeared. In the control treatment pruning weight was also positively correlated with B, K, Mg, P and S using both the unadjusted and adjusted means, except P and negatively correlated with juice Ca for both the unadjusted and adjusted means. In the deficit treatment pruning weight was positively correlated with TSS, pH, TA, malate, tartrate and YAN and negatively correlated with phenolics. When adjusted for TSS, positive correlations with pruning weight and TA, malate and YAN were maintained while for anthocyanin there was a negative correlation. In the deficit treatment pruning weight also correlated positively with K, Mg, P and S although these relationships disappeared when adjusted for TSS.

Table 2.2.1.7. Correlation matrix, describing linear relationships for the control irrigation and deficit irrigation treatments between mean growth characteristics (yield component variables, pruning weight and the Ravaz index), berry juice composition of Shiraz across rootstock genotypes (n=60). Values retained are significant ($p<0.05$). Highly significant correlations ($p<0.001$) are in bold. Note: adjusted means using the significant TSS covariate were also included in the analysis. Mineral element data for 2016 only. (Note: the calculated variables, berries per bunch and the Ravaz index are not included).

	Yield		***Bunches***		***Bunch weight***		***Berry weight***		***Pruning weight***	
Irrigation	**Con**	**Def**	**Con**	**Def**	**Con**	**Def**	**Con**	**Def**	**Con**	**Def**
TSS	**-0.42**		**-0.52**	-0.28			0.26	0.36	**0.57**	**0.52**
pH	-0.30		**-0.44**	-0.26			0.26		**0.44**	0.34
TA						0.29	**0.46**	**0.55**	**0.73**	**0.70**
Malate			-0.35			0.24	**0.57**	**0.54**	**0.84**	**0.78**
Tartrate							0.34		**0.57**	0.30
YAN			-0.35			0.33	**0.45**	**0.55**	**0.83**	**0.79**
anthocyanin	**-0.42**	-0.31	**-0.42**		-0.25	**-0.51**		**-0.47**	**0.44**	
phenolics	-0.31	-0.26	-0.30			**-0.42**		**-0.50**	0.24	-0.23
pH adj.										
TA adj.			-0.24			0.29	**0.48**	**0.55**	**0.79**	**0.71**
Malate adj.			-0.24		0.28	0.22	**0.57**	**0.46**	**0.78**	**0.66**
Tartrate adj.						-0.21	0.24		0.33	
YAN adj.		0.25				0.32	**0.43**	**0.47**	**0.75**	**0.67**
Antho. adj.		-0.22				**-0.50**		**-0.57**		-0.36
Phen. adj.						-0.38	-0.24	**-0.56**		**-0.42**
B							0.29		**0.55**	0.36
Ca				-0.29					-0.27	
K	-0.27		**-0.47**	-0.23			**0.45**	0.30	**0.65**	**0.43**
Mg						-0.22			0.39	0.25
P	**-0.48**		**-0.62**	-0.29			0.32	0.32	**0.53**	**0.59**
Na						-0.22				
S	-0.25		**-0.41**			0.22	0.39	0.38	**0.67**	**0.58**
B adj.							0.25		**0.43**	
Ca adj.				-0.25					-0.31	
K adj.							0.39		**0.40**	
Mg adj.						-0.24			0.28	
P adj.										
Na adj.		0.32		0.29		0.22				
S adj.						-0.22	0.35		**0.53**	

Multilinear regression techniques were used to identify the best combinations of growth traits with strong predictive value for Shiraz juice composition across the rootstock genotypes (n=60) under the well-watered control irrigation regime. For the well-watered control irrigation they showed that a significant proportion of the variability in berry composition across rootstocks could be accounted for, based on adjusted R^2 values, including:-

- 48% (p=0.000) of TSS by yield (-ve) and pruning weight(both +ve)

- 25% (p=0.000) of pH by yield (-ve) and pruning weight(both +ve)
- 52% (p=0.000) of TA by pruning weight (+ve)
- 71% (p=0.000) of malate by pruning weight (+ve)
- 31% (p=0.000) of tartrate by pruning weight (+ve)
- 68% (p=0.000) of YAN by pruning weight (+ve)
- 33% (p=0.000) of anthocyanin by bunch weight (-ve) and pruning weight (+ve)
- 10% (p=0.019) of phenolic substances by bunch weight (-ve) and pruning weight (+ve)
- 30% (p=0.000) of B by pruning weight (+ve)
- 49% (p=0.000) of K by bunch number (-ve) and pruning weight (+ve)
- 13% (p=0.002) of Mg by pruning weight (+ve)
- 47% (p=0.000) of P yield (-ve) and pruning weight (+ve)

Similarly, for the deficit irrigation treatment, the multilinear regression techniques showed that a significant proportion of the variability in berry composition across could be accounted for based on adjusted R^2 values including:-

- 37% (p=0.000) of TSS by bunch number (-ve) and pruning weight (+ve)
- 18% (p=0.000) of pH by yield (-ve) and pruning weight (+ve)
- 49% (p=0.000) of TA by pruning weight (+ve)
- 66% (p=0.000) of malate by yield (-ve) and pruning weight (+ve)
- 14% (p=0.006) of tartrate by berries per bunch (-ve) and pruning weight (+ve)
- 65% (p=0.000) of YAN by yield (-ve) and pruning weight (+ve)
- 35% (p=0.000) of anthocyanin by bunch weight and berry weight (both -ve) and pruning weight (+ve)
- 24% (p=0.000) of phenolic substances by berry weight (-ve)
- 12% (p=0.005) of B by pruning weight (+ve)
- 15% (p=0.007) of Ca by yield (+ve), bunch number and bunch weight (both -ve)
- 23% (p=0.000) of K by bunch number (-ve) and pruning weight (+ve)
- 21% (p=0.001) of Mg by yield (-ve), bunches and pruning weight and pruning weight (both +ve)
- 45% (p=0.000) of P bunch number (-ve) and pruning weight (+ve)
- 32% (p=0.000) of S by pruning weight (+ve)

H. Impact of irrigation treatments on relationships between fruit composition variables

The effect of irrigation on relationships between fruit composition variables was also assessed (Table 2.2.1.8.). Data for anthocyanin and phenolics are not included as few relationships were found with other variables in the combined analysis presented above (Table 2.2.1.6). In general, relationships between fruit composition variables were similar for both irrigation treatments. In the case of TSS, positive correlations were found for both treatments across the 60 rootstock genotypes with pH, malate, tartrate, YAN, B, K, Mg, P, and S. Positive correlations between TSS and anthocyanin and phenolics were only found with the control irrigation. Positive correlations between TSS and TA and Ca were only found with the deficit treatment. For pH, positive correlations were found with malate, tartrate, YAN and adjusted malate and YAN but not adjusted tartrate for both irrigation treatments. In both treatments pH was negatively correlated with adjusted anthocyanin and phenolics. In both treatments, pH was positively correlated with unadjusted B, K, Mg, P and S but only B and K when adjusted for TSS. pH was also positively correlated with anthocyanin in the unadjusted control treatment and with adjusted Mg in the deficit treatment. In both irrigation treatments TA was positively correlated with malate, tartrate, YAN and adjusted malate and YAN. TA was also positively correlated with B, K, Mg, P and S and adjusted B and Mg and negatively correlated with adjusted pH. Furthermore, for the control treatment TA was positively correlated with anthocyanin, which disappeared when adjusted for TSS, and adjusted TA, K and S and negatively correlated with unadjusted and adjusted Ca. For the deficit treatment only, TA was negatively correlated with adjusted anthocyanin and phenolics and positively correlated with adjusted Na.

In both irrigation treatments, malate correlated positively with unadjusted tartrate and YAN, adjusted TA, malate and YAN and negatively with adjusted phenolics (Table 2.2.1.8). In both irrigation treatments malate was positively correlated with B, K, Mg, P, S and adjusted B, K and Mg. In the control treatment, malate also correlated positively with anthocyanin, a relationship which disappeared when adjusted for TSS, adjusted tartrate and adjusted S and negatively with unadjusted and adjusted Ca. In the deficit treatment malate was negatively correlated with phenolics, adjusted anthocyanins and phenolics and positively with adjusted Na. For both irrigations, tartrate was positively

correlated with adjusted YAN, adjusted TA, B, K, Mg, Na, S and adjusted Mg and S. In the control, tartrate was also positively correlated with adjusted malate and adjusted YAN, B, K and Na. In the deficit treatment tartrate was positively correlated with P but negatively correlated with adjusted phenolics and adjusted P. In both irrigation treatments YAN was positively correlated with adjusted TA and malate and unadjusted and adjusted B, K, Mg and unadjusted P and S. In the control irrigation treatment YAN was also positively correlated with anthocyanin, a relationship which disappeared when adjusted for TSS, adjusted tartrate and S and negatively correlated with unadjusted and adjusted Ca. In the deficit treatment YAN also correlated positively with adjusted P and Na and negatively with unadjusted and adjusted anthocyanin and phenolics.

Table 2.2.1.8. Correlation matrix, describing linear relationships for the control irrigation and deficit irrigation treatments between fruit composition variables (TSS, pH, TA, malate, tartrate and YAN. Values retained are significant ($p<0.05$). Highly significant correlations ($p<0.001$) are in bold. Note: adjusted means using the significant TSS covariate were also included in the analysis. Mineral element data for 2016 only.

	TSS		***pH***		***TA***		***Malate***		***Tartate***		***YAN***	
Irrigation	**Con**	**Def**	**Con**	**Def**	**Con**	**Def**	**Con**	**Def**	**Con**	**Def**	**Con**	**Def**
pH	**0.76**	**0.67**										
TA		0.32										
malate	**0.52**	**0.55**	**0.50**	**0.56**	**0.74**	**0.76**						
tartrate	0.32	**0.72**	0.28	**0.56**	**0.55**	0.24	**0.64**	**0.45**				
YAN	**0.57**	**0.55**	**0.50**	**0.50**	**0.71**	**0.73**	**0.92**	**0.91**	**0.65**	**0.41**		
Antho.	**0.62**		0.32		0.29		0.29				0.31	-0.26
Phen.	**0.47**							-0.25				-0.34
pH adj.			**0.76**	**0.67**	-0.27	-0.34						
TA adj.					**0.99**	**1.00**	**0.79**	**0.77**	**0.58**	0.25	**0.77**	**0.73**
Malate adj.			0.33	0.32	**0.77**	**0.74**	**0.97**	**0.90**	**0.63**		**0.86**	**0.80**
Tartr. adj.					**0.49**		**0.44**		**0.91**	**0.79**	**0.42**	
YAN adj.			0.30	0.25	**0.76**	**0.70**	**0.88**	**0.79**	**0.64**		**0.95**	**0.90**
Antho. adj.			-0.26	-0.38		-0.29		**-0.45**				**-0.50**
Phen. adj.			-0.31	**-0.52**		-0.25	-0.28	**-0.48**		-0.31	-0.29	**-0.53**
B	**0.57**	**0.56**	**0.47**	**0.60**	0.39	0.40	**0.66**	**0.67**	**0.55**	**0.57**	**0.61**	**0.56**
Ca		0.35			-0.27		-0.38		-0.40	0.32	-0.34	
K	**0.71**	**0.66**	**0.65**	**0.76**	0.37	0.38	**0.76**	**0.74**	**0.53**	**0.59**	**0.69**	**0.64**
Mg	**0.41**	0.35	0.24	0.33	0.32	0.39	**0.46**	**0.54**	0.37	**0.55**	**0.43**	0.38
P	**0.74**	**0.63**	**0.57**	**0.64**	0.26	**0.51**	**0.49**	**0.74**		**0.44**	**0.52**	**0.73**
Na									0.29	0.44		
S	**0.66**	**0.55**	**0.50**	**0.60**	**0.48**	**0.57**	**0.75**	**0.78**	**0.52**	**0.52**	**0.75**	**0.80**
B adj.			0.29	0.29	0.38	0.25	**0.56**	**0.41**	**0.57**		**0.52**	0.33
Ca adj.					-0.28		**-0.42**		**-0.41**		-0.38	
K adj.			0.26	0.31	0.35		**0.58**	0.34	**0.59**		**0.51**	0.30
Mg adj.				0.22	0.30	0.35	0.36	**0.46**	0.36	**0.44**	0.35	0.30
P adj.										-0.29		0.27
Na adj.						0.31		0.34	0.29			**0.43**
S adj.					**0.49**		**0.65**		**0.57**	**0.47**	**0.67**	

G. Winemaking 2017

Growth characteristic and fruit composition data based on the Shiraz means over seasons (2013-16) were sorted to identify the most promising new CSIRO selections with potential for future release. Key criteria used in the selection were yield and vigour, ability to maintain productivity under the deficit irrigation treatment, pH, malate and berry anthocyanin. Wines were made from these selections and the six standards in season 2017.

Growth characteristics over all seasons (2013-17) results are presented in Table 2.2.1.9. Of the standard rootstocks, only Ramsey, the highest yielding genotype, maintained its yield under the deficit treatment with 1103 Paulsen, 140 Ruggeri and Merbein 5489 having significant losses. In contrast, all the selections, except 230-10 maintained their productivity over five seasons of deficit irrigation treatment. In some cases, including Ramsey, the number of bunches increased with the deficit treatment suggesting impacts on vine and shoot fruitfulness. In general, the deficit treatment produced small reductions in bunch weight compared to the control which were particularly noticeable with Merbein 5489 and 230-10. Similarly, the deficit treatment also produced small reductions in berry weight which was particularly noticeable with 1103 Paulsen, 140 Ruggeri and 230-10. The deficit treatment had little impact on pruning weight except for significant reductions with Ramsey, 140 Ruggeri and Merbein 5489. Interestingly, the values of the Ravaz index were generally lower with the deficit treatment because the effect on crop development was larger than on pruning weight, the exception being with Merbein 5489.

Table 2.2.1.9. Mean yield, bunches per vine, bunch weight, berry weight, berries per bunch, pruning weight and Ravaz index of Shiraz grafted on 12 CSIRO rootstock selections showing promise, five standard rootstocks or grown as own roots under two irrigation strategies, a well-watered control and a deficit treatment over five seasons (2013-17).

Rootstock	***Yield (kg/vine)***		***Bunches /vine***		***Bunch weight (g)***		***Berry weight (g***		***Pruning weight (kg/vine)***		***Ravaz***	
	Con	**Def**	**Con**	**Def**	**Con**	**Def**	**Con**	**Def**	**Con**	**Def**	**Con**	**Def**
Own root	9.0	7.9	117	106	81	83	1.19	1.19	1.20	1.79	8.4	4.7
Ramsey	12.9	13.4	114	137	113	106	1.34	1.28	4.64	2.80	3.0	5.4
Schwarz.	9.8	9.3	89	88	117	99	1.40	1.30	4.09	6.47	3.9	2.6
1103P	12.8	7.1	120	77	112	88	1.40	1.15	2.81	2.71	5.1	4.4
140R	11.4	6.4	106	84	111	79	1.39	1.20	3.51	1.53	4.9	4.9
M 5489	8.2	5.6	89	84	90	61	1.15	1.00	2.64	0.79	4.0	8.2
230-10	9.2	5.1	80	61	123	79	1.40	1.13	2.94	2.36	3.8	6.4
230-30	13.6	10.2	121	97	115	118	1.28	1.21	2.05	2.11	6.7	5.6
230-45	12.0	11.2	101	98	122	119	1.30	1.30	2.27	2.59	6.9	5.0
231-03	9.8	11.8	89	131	103	92	1.31	1.21	2.01	2.30	8.3	5.3
231-20	8.7	9.4	84	88	105	92	1.31	1.21	1.79	2.93	5.5	4.7
231-30	9.1	10.9	95	125	95	89	1.31	1.26	2.98	2.59	4.0	6.6
231-11	9.7	9.3	92	95	107	99	1.29	1.25	2.22	1.78	7.3	6.4
231-51	10.6	10.2	103	98	105	104	1.30	1.23	1.88	2.67	5.6	4.3
233-68	7.8	8.6	93	103	86	80	1.14	1.07	0.84	1.70	11.1	6.4
235-01	7.9	12.6	94	148	92	88	1.14	1.23	2.05	2.39	8.9	5.6
235-70	9.9	9.8	87	100	112	102	1.32	1.18	2.24	2.23	4.3	5.9
235-74	10.0	9.3	117	101	99	94	1.32	1.16	2.38	1.57	8.9	6.7

Fruit composition data for the CSIRO selections and standard rootstocks is presented in Table 2.2.1.10. With few exceptions, including Ramsey and Merbein 5489, the deficit treatment compared to the control produced higher TSS and pH. The aim of identifying selections with lower pH than standard rootstocks was achieved. This was particularly noticeable under deficit irrigation as almost all selections had lower pH than all standard rootstocks including own roots, with the exception of Merbein 5489. In general, most of the selections, except 230-10 tended to have lower malate than the standard rootstocks, except own roots. In almost all cases tartrate increased with the deficit irrigation treatment, possibly due to reductions in berry size associated with berry shrivelling. All rootstock genotypes had acceptable levels of YAN (i.e. >150 mg/L), which in a number of cases increased under the deficit treatment. Berry anthocyanins were generally increased by deficit irrigation. Merbein 5489 had the highest berry anthocyanin levels with both irrigation treatments.

Table 2.2.1.10. Mean juice TSS, pH, TA, Malate, Tartrate, YAN, anthocyanin and phenolic substances of Shiraz grafted on 12 CSIRO rootstock selections showing promise, five standard rootstocks or grown as own roots under two irrigation strategies, a well-watered control and a deficit treatment, over five seasons (2013-17).

Rootst.	***TSS (ºBrix)***		***pH***		***TA (g/L)***		***Malate (g/L)***		***Tartrate (g/L)***		***YAN (mg/L)***		***Antho. (mg/g)***	
	C	D	C	D	C	D	C	D	C	D	C	D	C	D
Own	22.2	26.2	3.99	4.35	3.9	3.0	2.6	3.3	5.6	6.1	168	209	1.08	1.23
Ram.	25.1	25.0	4.31	4.29	4.0	3.8	4.9	4.0	5.9	5.9	304	290	1.09	1.05
Schw.	23.9	26.0	4.18	4.27	4.3	4.7	4.5	5.6	5.6	6.1	248	389	1.05	1.22
1103P	25.0	26.1	4.17	4.38	3.8	3.9	3.3	4.3	5.6	5.6	194	277	1.06	1.34
140R	24.0	25.9	4.20	4.37	4.2	3.2	4.6	3.8	5.6	5.6	322	229	0.87	1.12
M5489	26.7	25.1	4.25	4.06	3.6	3.4	3.7	2.6	5.3	5.6	232	168	1.32	1.82
230-10	25.8	26.1	4.31	4.30	3.8	3.3	4.5	3.7	5.2	5.9	264	235	1.00	1.26
230-30	21.7	24.3	4.06	4.21	3.7	3.0	3.6	2.9	5.6	5.2	208	167	0.77	1.19
230-45	22.7	25.1	4.06	4.09	3.9	3.7	3.6	3.2	5.2	5.6	208	185	1.00	1.25
231-03	23.4	24.6	4.17	4.22	3.3	3.3	3.3	3.4	5.1	5.7	204	222	1.02	1.08
231-20	23.8	25.2	4.10	4.24	4.2	3.7	3.8	4.0	5.5	6.1	229	248	1.09	1.07
231-30	24.6	24.8	4.11	4.15	4.0	3.6	3.8	3.2	5.7	5.3	191	225	1.07	1.10
231-11	23.1	23.3	4.13	4.15	3.6	3.3	3.5	2.8	5.3	5.2	200	188	1.02	0.99
231-51	24.0	26.6	4.10	4.26	3.6	3.5	2.9	3.4	5.6	6.1	172	252	0.93	1.14
233-68	23.5	24.4	3.95	4.20	3.7	2.9	2.9	2.8	4.5	5.4	163	193	1.24	1.30
235-01	23.3	24.4	4.23	4.27	3.1	3.0	3.2	3.0	5.4	5.4	199	194	1.05	1.09
235-70	22.9	24.4	4.13	4.18	3.5	3.4	3.2	2.8	5.1	5.5	167	219	0.86	1.15
235-74	24.9	24.8	4.10	4.15	3.3	3.0	3.3	2.4	5.0	5.3	191	180	1.06	1.20

As indicated above, wines were produced from these selections in 2017, after conversion to mechanical hedging. Following sampling of the trial on 11 March, harvest was conducted between 11 March and 20 March with the aim to account for irrigation and rootstock effects on maturity. The actual mean data for the growth characteristics from 2017 is presented in Table 2.2.1.11. In general, yield of the promising selections was higher than own roots but less than Ramsey. The deficit treatment reduced yield of Merbein 5489, 230-10 and 231-51 due to reductions in bunch number and bunch weight. Berry weight was slightly lower for most rootstock genotypes with deficit irrigation. The pruning weight data indicates that most of the selections were moderate vigour, more closely aligned to own roots and Merbein 5489.

Table 2.2.1.11. Mean yield, bunches per vine, bunch weight, berry weight, pruning weight and Ravaz index of Shiraz grafted on 12 CSIRO rootstock selections showing promise, five standard rootstocks or grown as own roots, under two irrigation strategies, a well-watered control and a deficit treatment, season 2017.

Rootstock	***Yield (kg/v)***		***Bunches per vine***		***Bunch weight (g)***		***Berry weight (g)***		***Pruning weight (g)***	
Irrigation	**Con**	**Def**	**Con**	**Def**	**Con**	**Def**	**Con**	**Def**	**Con**	**Def**
Own root	12.7	12.8	195	219	64	58	1.14	1.04	1.43	1.60
Ramsey	22.3	19.4	212	213	106	91	1.24	1.27	4.04	2.23
Schwarz	16.7	21.4	166	179	101	118	1.35	1.19	3.34	5.20
140R	22.0	.	231	.	95	.	1.49	.	2.23	.
1103P	17.2	15.3	167	156	100	99	1.38	1.27	3.13	1.98
M5489	17.4	7.3	191	121	91	51	1.09	1.07	1.46	0.56
230-10	20.0	12.0	183	142	110	78	1.43	1.24	2.55	1.46
230-30	17.8	13.6	186	156	103	87	1.23	1.12	1.72	1.35
230-45	17.5	18.2	178	160	99	115	1.18	1.35	1.42	2.18
231-03	16.4	22.3	136	251	116	88	1.29	1.12	1.16	2.03
231-20	14.9	18.9	140	160	112	105	1.16	1.17	1.73	2.40
231-30	16.9	18.4	183	259	91	70	1.22	1.13	1.57	1.07
231-51	14.1	9.7	161	125	81	74	1.25	1.19	1.69	1.30
231-11	17.3	19.9	169	204	103	94	1.43	1.10	2.36	2.28
233-68	10.8	14.9	162	192	62	74	1.14	0.99	0.80	1.32
235-01	9.3	20.2	148	258	62	79	0.87	1.25	0.86	2.50
235-70	18.3	18.2	152	199	127	87	1.39	1.17	2.44	1.94
235-74	11.9	14.8	192	184	60.4	79	1.16	1.05	0.77	1.53
Irrigation mean	**16.3**	**16.3**	**175**	**187**	**93.6**	**85.2**	**1.25**	**1.16**	**1.93**	**1.94**

Mean data of fruit composition collected before harvest in 2017 (Table 2.2.1.12). Tartrate levels (not shown) were similar across rootstocks and irrigation (mean 4.70 g/L). At sampling, TSS of most rootstock genotypes was below 24 ^{o}Brix desired required for winemaking. Hence harvest date of each genotype was adjusted to accommodate the variability. The pH of almost all rootstock selections was below those determined for own roots and the standard rootstocks, except Merbein 5489 in both the control and deficit irrigation treatments. For most rootstock genotypes deficit irrigation reduced TA, due to reductions in malate. YAN levels for a number of the rootstock selections were lower than the desired level of 150 mg/L. Berry anthocyanins were highest with Merbein 5489 with both irrigation treatments. With some exceptions, particularly 231-11, 235-70, 231-30, the CSIRO rootstock selections had higher berry anthocyanins than the standard rootstocks, i.e. Ramsey, Schwarzman, 140 Ruggeri and 1103 Paulsen.

Table 2.2.1.12. Mean juice TSS, pH, TA, Malate, Tartrate, YAN, anthocyanin and phenolic substances of Shiraz grafted on 12 CSIRO rootstock selections showing promise, five standard rootstocks or grown as own roots, under two irrigation strategies, a well-watered control and a deficit treatment, season 2017.

Roots.	***TSS (ºBrix)***		***pH***		***TA (g/L)***		***Malate (g/L)***		***YAN***		***Anthocyanin (mg/g)***	
Irrig.	**Con**	**Def**	**Con**	**Def**	**Con**	**Def**	**Con**	**Def**	**Con**	**Def**	**Con**	**Def**
Own	21.6	24.1	3.94	4.28	3.60	2.83	1.85	2.55	173	179	0.79	0.98
Ramsey	22.2	20.8	4.22	4.15	3.77	3.38	3.90	2.90	266	191	0.62	0.80
Schwarz	24.2	21.6	4.32	4.14	3.96	4.50	4.65	4.25	290	283	0.61	0.53
140R	21.9	.	4.00	.	3.75	.	2.85	.	186	.	0.49	.
1103P	20.4	24.2	4.00	4.49	4.50	2.85	4.10	3.15	279	235	0.38	0.73
M5489	20.1	25.4	3.82	3.83	3.79	3.67	2.05	1.93	118	120	0.86	1.16
230-10	22.6	21.2	4.06	4.04	3.59	2.98	2.70	2.13	182	142	0.74	0.89
230-30	20.9	21.9	3.99	3.95	3.75	2.84	3.15	1.30	184	149	0.54	0.90
230-45	21.1	21.3	3.83	3.79	4.04	3.82	2.70	2.20	184	136	0.87	0.96
231-03	21.7	20.5	3.86	4.10	3.25	3.05	2.25	2.35	178	157	0.50	0.63
231-20	21.4	23.5	3.86	4.18	4.28	3.81	2.65	3.90	144	244	0.76	0.75
231-30	22.3	20.4	3.87	3.78	3.89	3.44	2.85	1.55	142	136	0.45	0.52
231-51	21.6	20.6	3.95	4.08	3.91	3.26	3.45	2.45	208	179	0.68	0.64
231-11	24.3	23.4	4.09	4.08	3.56	3.32	2.80	2.45	151	214	0.66	0.63
233-68	22.4	21.5	3.74	3.97	3.41	2.83	1.90	1.65	103	150	0.81	1.06
235-01	22.3	23.0	3.98	4.22	2.79	3.03	1.60	2.55	145	201	0.70	0.65
235-70	22.3	22.6	4.11	4.05	3.60	3.47	3.30	2.50	152	209	0.27	0.67
235-74	24.1	21.1	3.89	3.86	3.44	3.59	2.05	1.80	130	171	0.78	0.81
Irrig. mean	**22.1**	**22.2**	**3.97**	**4.06**	**3.71**	**3.31**	**2.82**	**2.45**	**179**	**182**	**0.64**	**0.78**

The results for the must and total tartaric acid addition to adjust must and wine pH are presented in Table 2.2.1.13. In general, TSS levels were below the desired level for winemaking of 24 ºBrix, particularly those of 140 Ruggeri, 1103 Paulsen, 230-30, 231-03, 231030 and 233-68 in the well-watered control treatment and 231-30 with the deficit treatment. Own roots and Merbein 5489 had lower pH than the other standard rootstocks. Must pH of most of the rootstock selections were lower than standard rootstocks, Ramsey, Schwarzman, 140 Ruggeri and 1103 Paulsen. The amount of acid addition required for pH adjustment varied greatly across the rootstock genotypes. Of the standard rootstocks it was lowest for own roots and Merbein 5489 and highest for Ramsey, Schwarzman and 1103 Paulsen. For most of the rootstock selections the requirement for acid addition was lower than the standard rootstocks. Selection 231-30 had a very low requirement for pH adjustment.

Table 2.2.1.13. Mean TSS, pH, TA of must and total acid addition for pH adjustment of must and wine of Shiraz grafted on 12 CSIRO rootstock selections showing promise, five standard rootstocks or grown as own roots, under two irrigation strategies, a well-watered control and a deficit treatment, season 2017.

Rootstock	***TSS (^{O}Brix)***		***pH***		***TA (g/L)***		***Tartrate Added (g/L)***	
	Con	**Def**	**Con**	**Def**	**Con**	**Def**	**Con**	**Def**
Own root	22.2	22.1	4.03	4.08	3.67	3.21	3.5	4.2
Ramsey	23.3	23.4	4.41	4.26	3.04	2.74	7.9	6.0
Schwarz	24.4	23.0	4.43	4.29	3.53	4.09	8.3	8.8
140R	21.9	.	4.19	.	3.34	.	6.7	.
1103P	21.0	23.4	4.19	4.44	3.05	2.97	5.6	7.9
M5489	22.8	23.2	4.09	3.84	2.97	3.62	3.9	2.3
230-10	22.6	22.5	4.22	4.24	3.09	2.93	5.7	5.5
230-30	19.8	22.5	4.25	4.32	2.89	2.50	5.0	5.5
230-45	22.1	22.3	4.14	4.25	2.98	2.64	4.3	5.0
231-03	21.1	22.4	4.21	4.23	2.78	2.63	4.7	5.1
231-20	22.8	21.5	4.21	4.12	2.96	3.72	5.6	6.3
231-30	21.7	21.4	3.87	3.94	3.49	3.00	2.5	2.9
231-51	22.8	23.4	4.29	4.21	3.54	2.68	7.1	5.4
231-11	22.9	22.3	4.12	4.19	3.53	3.05	4.9	6.9
233-68	20.9	22.3	3.89	4.06	3.20	2.74	3.6	3.7
235-01	22.1	23.7	4.19	4.25	2.45	3.00	5.7	5.8
235-70	22.6	23.2	4.16	4.18	2.69	2.79	5.3	4.8
235-74	23.2	23.4	3.99	4.11	2.93	2.47	3.3	4.1
Irrigation mean	**22.2**	**22.7**	**4.16**	**4.18**	**3.11**	**2.99**	**5.2**	**5.3**

Bottled wine analyses are presented in 2.2.1.14. There was no residual sugar in any of the wines. It should be noted that acid adjustment of wine to the desired level of pH 3.6 or below, was not continued if wine TA exceeded 8.0 g/L, which was the case with Schwarzman (both irrigation treatments), 1103 Paulsen (deficit) and 231-51 (control). Wine alcohol levels ranged from 11.4 % (selection 233-68, control) to 15.0 (Merbein 5489, control). Wine VA levels were highest with 1103 Paulsen and Schwarzman. A number of the selections had very low levels of VA.

Table 2.2.1.14. Wine pH, TA, alcohol and volatile acidity of Shiraz grafted on 12 CSIRO rootstock selections showing promise, five standard rootstocks or grown as own roots under two irrigation strategies, a well-watered control and a deficit treatment, season 2017.

Rootstock	***pH***		***TA (g/L)***		***Alcohol (%)***		***VA (g/L)***	
Irrigation	**Con**	**Def**	**Con**	**Def**	**Con**	**Def**	**Con**	**Def**
Own root	3.59	3.58	7.48	7.38	13.2	13.5	0.17	0.17
Ramsey	3.61	3.60	7.60	6.93	13.9	14.7	0.15	0.15
Schwarz	3.66	3.77	8.64	8.99	14.2	14.6	0.22	0.18
140R	3.65	.	7.55	.	13.0	.	0.19	.
1103P	3.65	3.65	7.66	8.01	13.2	13.6	0.20	0.22
M5489	3.57	3.49	7.21	7.32	15.0	13.9	0.12	0.14
230-10	3.59	3.53	7.71	7.49	13.5	13.7	0.13	0.14
230-30	3.53	3.54	7.17	6.92	12.8	14.6	0.14	0.04
230-45	3.50	3.55	7.33	6.53	14.2	14.2	0.14	0.12
231-03	3.62	3.58	6.77	6.90	12.7	12.3	0.16	0.15
231-20	3.59	3.63	7.63	7.41	13.5	12.3	0.20	0.14
231-30	3.57	3.58	6.60	6.08	13.8	13.5	0.18	0.14
231-51	3.65	3.59	8.21	7.50	13.0	13.9	0.22	0.14
231-11	3.62	3.59	7.67	6.96	13.8	12.8	0.17	0.13
233-68	3.53	3.57	6.37	6.50	11.4	14.7	0.16	0.05
235-01	3.54	3.59	7.02	7.75	13.6	14.3	0.07	0.16
235-70	3.61	3.58	7.37	6.92	13.4	13.8	0.12	0.13
235-74	3.57	3.59	6.96	6.83	14.1	14.0	0.16	0.13

The spectral properties for each of the Shiraz wines are reported in Table 2.2.1.15. In general wines produced from own roots, Schwarzman, Merbein 5489 and selections 235-01 and 235-74 had the highest colour density. In contrast, wines from 140 Ruggeri (control only), 1103 Paulsen and selections 230-30, 231-03, 231-20, 231-11 and 233-68 had the lowest levels. Wine colour density was enhanced by deficit irrigation with a number of selections including 230-30 and 231-30. In general, the deficit irrigation treatment produced brighter wines with lower hue values, exceptions being wines from selections 232-11 and 235-74. Own roots, Merbein 5489 and selections 230-10, 23045 and 235-01 produced the brightest wines. Wine total anthocyanin was highest in wines from Merbein 5489, 230-45 (control), 235-01 and 235-74 (control). Ionised anthocyanins in wines were highest for own roots, Schwarzman, Merbein 5489 and the selection 230-10, 230-30 (deficit), 231-30 (deficit), 235-01, and 235-74 (control). Schwarzman, Merbein 5489 and selections 231-30 (deficit) and 235-01 had the highest levels of wine phenolic substances. Wines from 140 Ruggeri (control only), 231-03 and 233-68 (control)

had low phenolic substances.

Table 2.2.1.15. Wine spectral properties of Shiraz grafted on 12 CSIRO rootstock selections showing promise, five standard rootstocks or grown as own roots under two irrigation strategies, a well-watered control and a deficit treatment, season 2017.

	Colour density (a.u.)		***hue***		***Total anthocyanin (mg/L)***		***Ionised anthocyanin (mg/l)***		***Total phenolics (a.u.)***	
	con	**def**	**con**	**def**	**con**	**def**	**con**	**def**	**con**	**def**
Own root	5.67	5.52	0.65	0.65	781	731	17.8	16.7	13.6	13.4
Ramsey	4.14	4.71	0.71	0.71	650	745	13.2	15.0	10.5	13.4
Schwarz	6.44	6.72	0.80	0.66	792	717	17.8	19.9	17.8	16.3
140 R	2.35	.	0.81	.	460	.	9.1	.	6.4	.
1103P	3.38	4.92	0.77	0.70	497	582	12.9	17.8	8.0	12.5
M5489	6.07	7.92	0.67	0.57	892	825	17.7	20.7	14.6	16.2
230-10	4.78	5.59	0.69	0.66	796	715	16.2	17.2	13.6	13.3
230-30	2.85	6.57	0.80	0.67	484	761	9.3	17.9	6.0	14.7
230-45	4.72	5.07	0.64	0.64	846	585	9.7	6.1	14.5	8.0
231-03	3.51	3.44	0.75	0.74	643	439	6.0	9.5	5.8	4.6
231-20	3.36	4.21	0.74	0.70	628	588	9.1	5.6	8.7	7.2
231-30	3.80	6.87	0.75	0.63	560	809	13.0	20.8	9.7	16.7
231-51	4.67	3.37	0.71	0.74	723	457	14.3	12.0	12.3	6.3
231-11	4.21	2.29	0.72	0.84	672	475	13.5	6.8	10.6	6.3
233-68	3.39	4.54	0.68	0.71	473	716	5.7	13.4	4.6	11.9
235-01	6.08	7.21	0.66	0.61	861	847	18.1	21.5	17.1	17.4
235-70	4.52	5.58	0.72	0.66	723	734	13.9	17.4	11.6	13.6
235-74	6.49	4.37	0.62	0.72	801	629	20.8	14.5	14.8	11.3
Irrigation mean	**4.47**	**5.22**	**0.72**	**0.68**	**682**	**667**	**13.2**	**14.5**	**11.1**	**11.9**

Correlation analyses were undertaken to explore relationships between vine growth characteristics and must and wine attributes across the rootstock genotypes and irrigation treatments (Table 2.2.1.16, n=35). There were significant positive correlations between yield and must pH, the amount of acid addition required for pH adjustment, wine pH and hue (i.e. less bright wines) and a negative correlation between yield and wine colour density (just significant). There were no correlations between bunch number and must or wine parameters. Bunch weight was positively correlated with must pH, must acid addition, wine pH, wine TA and hue and negatively correlated with wine colour density, ionised anthocyanin and total phenolics. Berry weight was positively correlated with must pH, must addition, wine pH, wine TA, volatile acidity and hue and negatively associated with wine colour density. Berries per bunch was positively correlated with must pH, must addition, wine pH and hue and negatively correlated with colour density and ionised anthocyanin. Conferred vigour (pruning weight) was positively correlated with must pH,

and TA, must addition (highly significant), wine pH (highly significant), wine TA and volatile acidity but not to any spectral properties. The Ravaz index was negatively linked to must pH, must additions, wine pH and hue.

In summary it can be concluded that rootstock growth characteristics can be linked to must and wine quality attributes. Rootstocks with good yields but low moderate conferred vigour (i.e. enhanced vine balance as determined by the Ravaz index), which produce smaller bunches with fewer, small berries are likely to have lower must pH and require less acid addition for pH adjustment, leading to wines without excessive levels of acidity and with enhanced spectral properties.

Table 2.2.1.16. Correlation matrix, describing linear relationships between growth characteristics (yield component variables, pruning weight and the Ravaz index) and must and wine attributes of Shiraz across 18 rootstock genotypes and two irrigation systems (control, deficit, n=35). Values retrained are significant correlations ($p<0.05$). Highly significant correlations ($p<0.001$) are in bold.

	Yield	***Bunches***	***Bunch weight***	***Berry weight***	***Berries per bunch***	***Pruning weight***	***Ravaz index***
Must TSS							
Must pH	0.40		**0.54**	0.35	0.50	**0.58**	**-0.52**
Must TA						0.38	
Must Additions	0.44		**0.53**	0.36	0.47	**0.75**	-0.50
Wine pH	0.47		0.47	0.41	0.37	**0.75**	-0.45
Wine TA			0.32	0.37		**0.65**	-0.46
Alcohol							
Volatile acidity				0.49		0.32	
Colour density	-0.29		-0.38	-0.28	-0.33		
Hue	0.37		0.46	0.34	0.40		0.38
Total anthocyanin							
Ionised anthocyanin			-0.43		-0.42		
Total phenolics			-0.29				

Multilinear regression techniques were used to identify the best combinations of growth traits with strong predictive value for Shiraz must and wine composition across the rootstock 18 genotypes and two irrigation treatments (control, deficit, n=35). The results show that a significant proportion of the variability in must and wine composition across the rootstock irrigation data could be accounted for, based on adjusted R^2 values, including:

- 34% (p=0.000) of must pH by pruning weight (+ve)
- 23% (p=0.005) of must TA by berries per bunch (-ve) and pruning weight (+ve)
- 62% (p=0.000) of tartaric acid addition for pH adjustment by pruning weight (+ve)
- 57% (p=0.000) of wine pH by pruning weight (+ve)
- 60% (p=0.000) of wine TA by yield (-ve), berry weight and pruning weight (both +ve)
- 21% (p=0.003) of wine volatile acidity by berry weight (+ve)
- 12% (p=0.025) of wine colour density by bunch weight (-ve)
- 28% (p=0.002) of wine ionised anthocyanins by bunch weight (-ve) and pruning weight (+ve)
- 16% (p=0.040) of wine phenolic substances by yield (-ve), bunch number and pruning weight (both +ve)

Because pruning weight was the most important growth characteristic impacting fruit and wine composition, these relationships are shown in more detail for berry composition in Figure 2.2.1.7 and wine in Figure 2.2.1.8. They highlight the negative effects of high conferred vigour on fruit composition (i.e. berry pH, malic acid, anthocyanin and phenolic substances), acid addition required for pH adjustment and final wine pH, TA and volatile acidity).

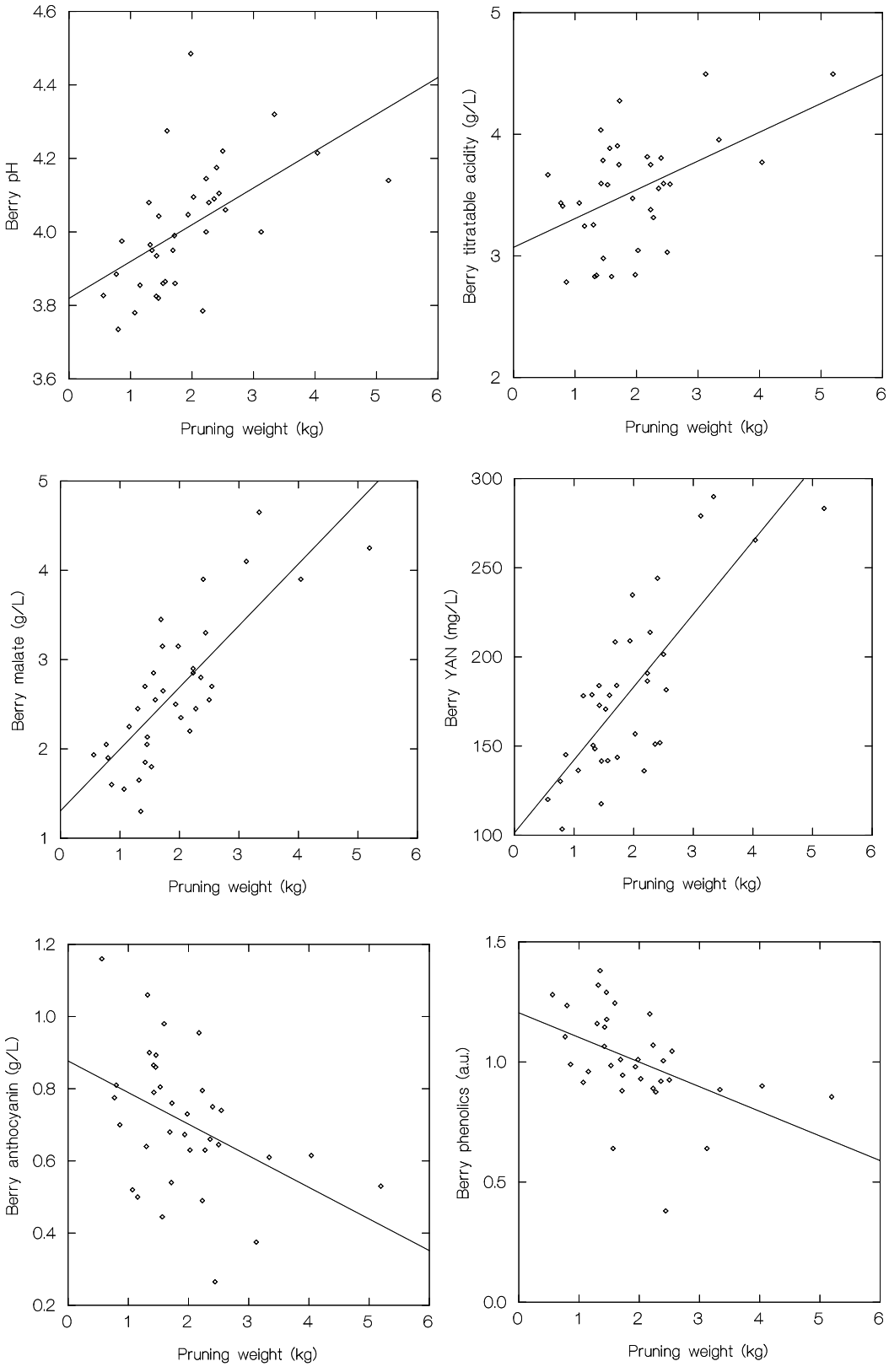


Figure 2.2.1.7. Relationships between pruning weight and berry composition (pH, TA, malate, YAN, anthocyanin and phenolic substances across 18 rootstock genotypes selected for winemaking and two irrigation treatments (well-watered control and deficit), season 2017.

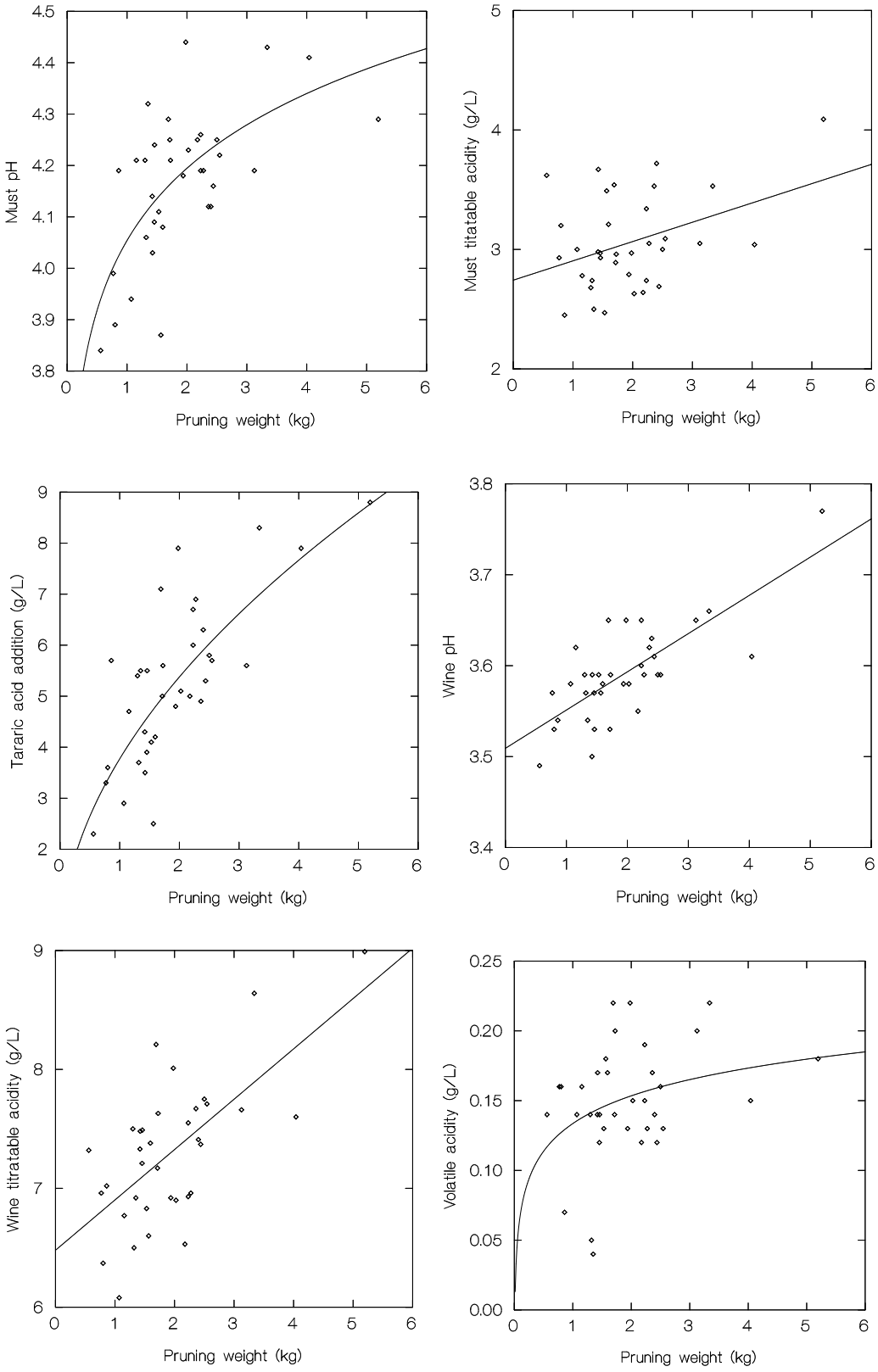


Figure 2.2.1.8. Relationships between pruning weight and must and wine composition (must pH and TA, total acid required for pH adjustment, wine pH, wine TA acidity and VA) across 18 rootstock genotypes selected for winemaking and two irrigation treatments (well-watered control and deficit), season 2017.

Correlation analyses were undertaken to explore relationships between measurements of berry composition and must and wine attributes across the rootstock genotypes and irrigation treatments (Table 2.2.1.17, n=35). Sequential harvesting was undertaken to minimise to reduce the effect of fruit maturity on wine composition. Consequently, there were few correlations between berry TSS and must or wine data except for weak positive correlations with must TSS, TA and wine colour density. In contrast, there were positive correlations between berry pH and must TSS and pH, acid addition for pH adjustment, wine pH, wine TA and volatile acidity. Berry malate levels were strongly correlated with must pH and must TA, acid additions for pH adjustment, wine pH, wine TA, volatile acidity and wine hue. There was a weak positive correlation between berry tartrate and must TA. Berry YAN was strongly correlated with must pH and TA, acid additions for pH adjustment, wine pH, TA, volatile acidity and hue. Berry anthocyanin was positively correlated with wine alcohol, colour density and total anthocyanin but negatively correlated with acid additions for pH adjustment, wine pH, volatile acidity, and hue. Berry phenolics were positively correlated with wine alcohol, colour density but negatively correlated with acid additions for pH adjustment, wine pH, volatile acidity and hue. Berry K, measured only in 2016, was positively correlated with means of must, acid addition for pH adjustment and wine pH.

Table 2.2.1.17. Correlation matrix, describing linear relationships between berry composition (TSS, pH, TA, Malate, tartrate, YAN, anthocyanin and phenolics) and must and wine attributes of Shiraz across 18 rootstock genotypes and two irrigation systems (control, deficit). Values retained are significant ($p<0.05$). Highly significant correlations ($p<0.001$) are in bold.

	TSS	***pH***	***TA***	***Mal***	***Tart***	***YAN***	***anth***	***phen***	***K***
Must TSS	0.32	0.42							
Must pH		**0.64**		0.50		**0.64**			0.40
Must TA	0.39		0.51	0.51	0.30	0.36			
Must Additions		**0.69**		**0.69**		**0.78**	-0.32	-0.28	0.42
Wine pH		0.51	0.38	**0.68**		**0.72**	**-0.53**	-0.45	0.28
Wine TA		**0.59**	0.39	**0.72**		**0.67**			
Alcohol							0.30		
Volatile acidity		0.30	0.48	**0.62**		0.44	-0.36	-0.38	
Colour density	0.29						0.39	0.33	
hue				0.42		0.37	-0.55	-0.48	
Total anthocyanin							0.35		
Ionised anthocyanin									
Total phenolics									

Multilinear regression techniques were used to identify the best combinations berry composition with strong predictive value for Shiraz must and wine composition across the rootstock 18 genotypes and two irrigation treatments (control, deficit, n=35). The results show that a significant proportion of the variability in must and wine composition across the rootstock irrigation data could be accounted for, based on adjusted R^2 values, including:

- 26% (p=0.006) of must TSS by berry pH, TA and malate (all +ve)
- 52% (p=0.000) of must pH by berry TSS and pH (both +ve)
- 47% (p=0.000) of must TA by berry TSS and TA (both +ve)
- 72% (p=0.000) of tartaric acid addition by berry pH and malate (both +ve) and tartrate (-ve)
- 56% (p=0.000) of wine pH by berry pH and TA (+ve) and tartrate (-ve)
- 63% (p=0.000) of wine titratable acidity by berry pH and TA (both +ve)
- 40% (p=0.000) of wine volatile acidity by berry pH and TA (both +ve)
- 14% (p=0.037) of wine colour density by berry TSS and phenolics (both +ve)

Correlation analyses were undertaken to explore relationships between must composition and wine attributes across the rootstock genotypes and irrigation treatments (Table 2.2.1.18, n=35). Must TSS was positively correlated with must pH, the requirement for acid addition and wine TA, alcohol, colour density, total anthocyanin, ionised anthocyanin and total phenolic substances. Must pH was positively correlated with the requirement for acid addition and wine TA and hue. Must TA was positively correlated with wine pH, wine TA and volatile acidity. Requirement for acid addition was positively correlated with wine pH, TA, volatile acidity and hue. Wine pH was positively correlated with wine TA, volatile acidity and hue. Wine TA was positively correlated with volatile acidity, ionised anthocyanin and total phenolics. Wine alcohol was positively with colour density, total anthocyanin, ionised anthocyanin and total phenolic substances and negatively correlated with hue. Colour density was negatively correlated with hue but positively correlated with total anthocyanin, ionised anthocyanin and total phenolic substances. Total anthocyanin was negatively correlated with hue but positively correlated with ionised anthocyanin and total phenolic substances.

Table 2.2.1.17. Correlation matrix, describing linear relationships between must and wine composition variables (must TSS, pH and TA); requirements for acid addition for pH adjustment; and, wine pH, TA, alcohol, volatile acidity, colour density and total anthocyanin of Shiraz across 18 rootstock genotypes and two irrigation systems (control, deficit) . Values retained are significant ($p<0.05$). Highly significant correlations ($p<0.001$) are in bold.

	Must				***Wine***					
	TSS	pH	TA	Add.	pH	TA	Alc.	VA	CD	Total Anth.
Must pH	0.34									
Must TA										
Must Additions	0.33	**0.87**								
Wine pH		0.45	0.46	**0.69**						
Wine TA	0.44	**0.56**	**0.55**	**0.72**	**0.65**					
Alcohol	**0.59**									
Volatile acidity			**0.56**	0.32	**0.53**	**0.55**				
Colour density	0.45						**0.56**			
Hue		0.35		0.40	0.35		-0.34		**-0.79**	**-0.70**
Total anthocyanin	0.40						**0.60**		**0.83**	
Ionised anthocyanin	0.49					0.30	**0.52**		**0.77**	**0.69**
Total phenolics	**0.52**					0.29	**0.67**		**0.89**	**0.91**

H. Discussion and summary

The Shiraz trial, planted in 2010, was designed to evaluate the field performance, under well-watered and deficit irrigations treatments, of 55 of the most promising drought tolerant selections under nursery conditions in the previous project (CSP 0503). It included own roots and five commercial rootstocks. A systematic approach was used to select the most promising rootstock genotypes based on their field performance and fruit composition. Key criteria used in this process were ability of rootstock genotypes to maintain acceptable productivity under deficit irrigation with low pH and malate levels and high berry anthocyanins in berries. In 2017 small-scale wines were produced from the 12 most promising selections with the aim to identify selections for further development. To the best of the author's knowledge, the study covering a high number of rootstock genotypes and their response to deficit treatments, augmented by targeted winemaking is unique. Consequently, not only has the study identified a number of promising new rootstock genotypes that warrant further development, it has also identified key

relationships relating vine growth characteristics to fruit and wine composition across the irrigations treatments.

Major findings from this study included:

- Confirmation that rootstocks provide a powerful tool to manipulate vine growth characteristics with implications for fruit and wine composition and carbon and water use efficiency. Across the 60 rootstock genotypes there were eight-fold differences in conferred vigour (pruning weight) compared to three-fold differences yield and a nine-fold differences in the Ravaz index (yield to pruning weight ratio).
- Fruit composition variables were significantly impacted by rootstock genotype except for tartrate, anthocyanin and phenolics. The results showed significant potential to select rootstocks with lower pH, malate and YAN than most standard rootstocks currently grown. This was confirmed in winemaking studies undertaken in 2017.
- Confirmation that rootstocks are a powerful tool to manipulate berry juice mineral ion content with 2-3 fold differences in B, K, Mg, P and S. This is particularly relevant for K, the dominant ion in juice, which has been linked to negative wine attributes (see below) as many of the CSIRO selections had concentrations below the most widely adopted standard rootstocks including 1103 Paulsen and Ramsey.
- Long term deficit irrigation treatment (45% of control) over all rootstock genotypes reduced yield by only 6%, due in part to an average 9% increase in fruitfulness (bunches per vine) despite significant reductions in bunch weight (13%) associated largely with reduced berry size (9%). The increased fruitfulness is likely to be a result of improved light conditions during fruit bud initiation associated with a 13% reduction in pruning weight, and by inference canopy size.
- Long term deficit irrigation, despite the small differences in yield, significantly impacted fruit composition with early ripening (0.8 ^{o}Brix), higher pH, lower TA, malate and YAN but increased tartrate, anthocyanin and phenolic substances. The increases in tartrate, anthocyanin and phenolics is consistent with effects of concentration associated with the reduction in berry size described above.
- Long term deficit irrigation significantly altered mineral ion balance in juice with a two-fold increase in juice Na and a small but significant increase B and small but significant reductions in Ca, P and S but had no effect on K or Mg.

- Demonstration that vine growth characteristics, particularly conferred vigour, have a major impact on fruit and wine composition. Consequently, rootstocks and deficit irrigation, which have significant effects on vine growth characteristics, provide growers with powerful tools to enhance wine quality attributes. Outcomes from key relationships across both irrigation treatments, in order of importance show that:
    - rootstocks with high conferred vigour are likely to have high berry TSS, pH, TA, malate, tartrate, YAN, K, P and S; high must pH and TA; high requirement for acid addition for pH adjustment; high wine pH, TA and VA.
    - rootstocks with large berries are likely to have high berry TSS, pH, TA, malate, tartrate, YAN, K, P and S but low anthocyanin and phenolics; high must pH; high requirement for acid addition for pH adjustment; high wine pH, TA, VA and hue (i.e. less bright) and reduced colour density.
    - rootstocks with large bunches are likely to have high berry TA, malate and YAN, but low anthocyanin and phenolics; high must pH; high requirement for acid addition for pH adjustment; high wine pH and hue and reduced colour density.
    - high yielding rootstocks are likely to have slightly lower berry TSS, anthocyanin, phenolics, Ca and P; high must pH; high requirement for acid addition for pH adjustment; high wine pH and hue and reduced colour density.
    - rootstocks with high bunch numbers are likely to have low berry TSS, pH, anthocyanin, Ca and P.
    - vine balance, as determined by the Ravaz index (yield/pruning weight), while impacted by rootstock genotype with nine-fold differences, added little to the understanding of rootstock effects fruit and wine composition as correlations were generally similar to or lower than with pruning weight alone, exceptions being stronger negative correlations between the Ravaz index and TSS, pH and tartrate. These results can largely be explained by the weak relationships between yield and measurements of fruit and wine composition.
- Demonstrated that not all relationships between growth characteristics and fruit across rootstock genotypes were stable across irrigation treatments. Key findings included:

- relationships with pruning weight and fruit composition were generally stable, except for negative correlations with berry anthocyanin and phenolics with the deficit treatment that were not apparent with full irrigation.
- relationships with berry weight were generally stable, except that the positive correlations with juice pH and tartrate with well-watered vines disappeared under deficit treatment. Furthermore, significant negative correlations between berry weight and berry anthocyanin and phenolics were not apparent with full irrigation.
- relationships between bunch weight and fruit composition were generally confined to the deficit treatment.
- strong negative correlations with bunch number and malate, YAN, anthocyanin and phenolics found in the well-watered treatment disappeared with deficit irrigation.
- negative correlations with yield and TSS and pH found in the well-watered treatment disappeared with deficit irrigation.

- Identified key relationships between fruit and wine composition variables across rootstock genotypes, which in some cases were impacted by irrigation treatment. These included:
  - Strong positive associations between TSS pH, TA (deficit only), malate, tartrate, YAN, anthocyanin and phenolics (control only), B, Ca (deficit only), K, Mg, P and S. Such results that TSS should be included as a covariate in ANOVA analysis of rootstock effects on fruit composition. The relationships between TSS and anthocyanin or phenolics, apparent in the control irrigations, appears to be uncoupled with deficit irrigation. The association between TSS and TA with deficit irrigation appears to be linked with a very strong correlation with tartrate ($r = 0.72$).
  - pH was positively associated across rootstock genotypes and irrigation treatments with malate, tartrate and YAN although linkages with tartrate disappeared when corrected for TSS.
  - TA was positively associated across rootstock genotypes and irrigation treatments with malate, tartrate and YAN although the linkage with tartrate disappeared with deficit irrigation when adjusted for TSS. TA was also negatively associated with pH when adjusted for TSS.

- Malate was positively associated across rootstock genotypes and irrigation treatments with tartrate and YAN (very strong, r>0.91) and TSS adjusted TA, tartrate (control only) and YAN.
- Tartrate was positively associated across rootstock genotypes and irrigation treatments with YAN and TSS adjusted TA, malate (control irrigation only) and YAN (control irrigation only).
- YAN was positively associated across rootstock genotypes and irrigation treatments with TSS adjusted TA, malate, tartrate (control only).
- Strong positive associations between K, the dominant cation and TSS, pH, TA, malate, tartrate and YAN across both irrigation treatments. When adjusted using TSS as a covariate these were generally weaker or disappeared in the case of TA and tartrate under deficit irrigation. These results indicate that two mechanisms are likely to be involved in berry K including a strong linkage with TSS during ripening but also an independent effect of rootstock genotype. The results also show that the relationships between K and TA and tartrate is uncoupled by deficit irrigation.
- Strong positive associations were also found between other elements (B, Mg, P and S) and pH, TA, malate, tartrate and YAN. The associations between Mg, P, S and pH all disappeared when adjusted for TSS. Associations with P and TA, malate, tartrate and YAN disappeared when adjusted for TSS. Furthermore, associations with S and TA, malate and YAN disappeared with deficit irrigation when adjusted for TSS. The associations between B and tartrate disappeared when adjusted for TSS with deficit irrigation.
- Ca was negatively linked to TA, malate, tartrate and YAN with the control irrigation across the rootstock genotypes.
- The results linking berry mineral accumulation to juice pH, organic acids and YAN indicate that further research is required to understand mineral element transport into berries and the impacts of the ripening process, rootstock and irrigation treatment on ion balance and the role of organic acids within the berry.
- Anthocyanin and phenolics were positively associated with TSS only with control irrigation, an indication that this process is uncoupled by deficit irrigation, possibly due to berry wilting. Furthermore, when adjusted for TSS to describe rootstock genotype responses, anthocyanin and phenolics were

negatively linked to pH, TA, malate and YAN (in particular with deficit irrigation). These results indicate that further study is required to understand the effects of deficit irrigation on anthocyanin and phenolics development and linkages with fruit composition once effects of maturity are removed.

- Adoption of a systematic screening approach, based on performance and fruit composition over four seasons, successfully identified 12 new rootstock genotypes with enhanced water use efficiency, capable of maintaining productivity under deficit irrigation treatments with enhanced fruit and wine composition compared to most standard rootstocks. Key attributes of most of the selections were their moderate conferred vigour, low must pH with reduced requirement for acid addition for pH adjustment with enhanced spectral properties. Further assessment of these selections is required to see if their potential can be maintained under the mechanical pruning regime implemented in winter 2016.

In summary it can be concluded that rootstock growth characteristics can be linked to must and wine quality attributes. Rootstocks with good yields but moderate conferred vigour which produce smaller bunches with fewer, small berries are likely to have lower must pH and require less acid addition for pH adjustment, leading to wines without excessive levels of acidity and with enhanced spectral properties. The results show significant potential to identify new rootstocks with enhanced fruit and wine composition and enhanced water use efficiency, capable of maintaining productivity with reduced water supply. Rootstock behaviour and impacts on fruit composition varied across irrigations treatments, indicating a requirement for rootstock evaluation to be conducted under both well-watered and limited water supply conditions. Further research is required to understand the effects of rootstock genotype and deficit irrigation treatments on ripening processes and their effects on relationships between fruit composition parameters including mineral element and ion balance in berries.

2.2.2. Shiraz Padthaway

The Shiraz trial, planted in 2010, was designed to evaluate the field performance of 59 rootstock genotypes from a single family tested for drought tolerance, under nursery conditions in the previous project (CSP 0503). It also included own roots and seven commercial rootstocks (Dog Ridge, 140 Ruggeri, 1103 Paulsen, 101-14, Merbein 5489Φ, Merbein 5512Φ, Merbein 6262Φ) and M920 (described in 2.1.5 above). Data recorded over five seasons, harvests 2013 to 2017 are included in this analysis. The soil information provided for this site is described in 2.1.5 above. Harvest dates were 8/03/13, 26/03/14, 25/02/15, 10/03/16 and 11/04/17.

In 2017 it was noted that canopy and fruit condition were severely impacted by a severe drought stress, caused by a breakdown in the irrigation system over a heatwave period in early to mid-February. This led to significant leaf loss with some rootstock genotypes, severe apoplexy (marginal leaf burn) and sunburn damage of the fruit with berry shrivel and poor colour development of exposed berries ('pinking') as described above (Figure 2.1.5.1). Visual assessment of the canopy condition was undertaken at harvest with both the % leaf lost and % marginal burning of retained leaves recorded.

A. Yield and its components, pruning weight and Ravaz index

Yield and yield component data, pruning weight and the Ravaz index, collected over five seasons (2013-17) for Shiraz grafted on '53 CSIRO selections', five standard rootstocks and own rooted vines (Table 2.2.2.1).

There were significant effects of season on yield and its components, pruning weight and the Ravaz index of Shiraz (Table 2.2.2.1.). Highest yield and bunch number were recorded in 2017. Season 2014 had the lowest yield. Bunch numbers were lowest in 2013. Largest bunches were produced in 2013. Largest berries were recorded in 2013. The highest and lowest berries per bunch were recorded in 2016 and 2014, respectively. The highest and lowest pruning weights were recorded in 2016 and 2015 respectively. There was more than a 2.8-fold difference in the Rravaz index, an indicator of vine balance and a surrogate for carbon efficiency and water use efficiency, across the seasons being highest in 2015 and lowest in 2014.

Rootstock genotype had highly significant effects on all growth characteristics of Shiraz, except berries per bunch (Table 2.2.2.1). There were large differences between the 59 rootstocks genotypes in yield (6.7-fold), bunch number (2.2-fold), bunch weight (2.8-fold), berry weight (1.4-fold), berries per bunch (2.5-fold), pruning weight (8.5-fold), Ravaz index (3.3-fold), canopy loss (5.8-fold) and leaf burn (3.7-fold). The distribution of the growth characteristics across the 60 rootstock genotypes is shown in Figure 2.2.2.1. With the exception of pruning weight, most variables displayed a skewed distribution with a high proportion of genotypes falling into high categories. Consequently, the highest proportion of rootstock genotypes fell in the 9-10 kg yield category, the 100-110 bunches category, the 80-90 g bunch weight category, the 0.9 - 1.0 g berry weight category and the 90-100 berries per bunch category.

1103 Paulsen produced the highest yield due largely to the development of larger bunches with more berries per bunch than the other rootstocks (Table 2.2.2.1). Yield of own roots, Dog Ridge, 140 Ruggeri, 101-14 and Merbein 5489 were all similar as were their bunch weight, berry weight and berries per bunch. While Dog Ridge and 140 Ruggeri had the highest number of bunches, their yields were lower than 1103 Paulsen because of the development of the smaller bunches. Yields of Merbein 5512 and M 920 were lower due to development of fewer, smaller bunches. Merbein 6262 had the least number of bunches and by far the lowest yield.

Own roots and Dog Ridge had the highest pruning weights, followed by 1103 Paulsen (Table 2.2.2.1). Pruning weights of 140 Ruggeri and 101-14 were lower than 1103 Paulsen but higher than the moderate vigour rootstocks, Merbein 5489 and Merbein 5512. Merbein 6262 and to a lesser degree, M 920 had the lowest pruning weight. Vine balance, as determined by the Ravaz index, of own roots, Dog Ridge and Merbein 5512 were close to the optimum values (Dokoozlian 2011), while for 1103 Paulsen, 140 Ruggeri, 101-14, and Merbein 5489 the Ravaz index values were higher (i.e. 14.8-20.2). Ravaz index values for the low vigour rootstocks M920 and Merbein 6262 were even higher.

There were significant effects of rootstock genotype on both leaf loss and leaf burn (Table 2.2.2.1). 140 Ruggeri and Dog Ridge were most the most tolerant rootstocks to short term drought stress during ripening, having the lowest canopy loss and leaf burn than the other

rootstocks. The most sensitive rootstocks to the short term drought stress were 101-14, Merbein 6262 and M920.

Table 2.2.2.1. Mean yield, bunches per vine, bunch weight, berry weight, berries per bunch, pruning weight, Ravaz index, canopy loss and leaf burn in 2017 of Shiraz grafted on 53 CSIRO rootstock selections, eight standard rootstocks or grown as own roots over five seasons (2013-17). Means followed by the same letter are not significantly different (p = 0.05). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***Yield (kg/v)***	***Buns./ vine***	***Bunch weight (g)***	***Berry weight (g)***	***Berrs./ bunch***	***Prun. Wt. (kg/v)***	***Ravaz Index***	***Can. loss (%)***	***Leaf burn (%)***
Own roots	9.76	111	88.3	0.97	93.6	1.05	10.9	29.6	41.8
Dog Ridge	9.95	117	84.0	0.93	91.8	1.08	10.0	20.0	26.7
1103 Paulsen	11.21	102	101.5	0.93	107.6	0.89	14.8	30.0	40.0
140 Ruggeri	9.58	116	81.0	0.93	88.2	0.77	16.3	26.0	27.5
101-14	9.47	105	82.2	0.90	91.9	0.70	16.4	57.5	70.0
Merbein 5489	9.11	107	79.3	0.96	86.2	0.60	20.2	44.0	58.0
Merbein 5512	6.56	114	58.8	0.79	72.6	0.68	9.7	46.7	56.7
Merbein 6262	2.20	61	44.9	0.77	57.8	0.24	26.3	67.5	85.0
M 920	7.24	95	80.1	0.87	92.4	0.44	22.3	77.5	80.0
Minimum	2.20	59.6	41.2	0.73	46.7	0.22	7.9	15.0	23.3
Maximum	14.80	130.6	116.8	1.06	117.0	1.87	26.3	86.7	86.7
Year									
2013	5.55c	48.1d	113.7a	1.14a	100.0a	-	-	-	-
2014	4.18c	62.6c	66.4d	0.92c	72.1b	0.78b	5.9c	-	-
2015	9.45b	117.9b	75.0c	1.03b	73.2b	0.72b	16.1a	-	-
2016	9.76b	128.1a	75.0c	0.69d	105.8a	0.97a	13.9b	-	-
2017	12.45a	136.9a	88.1b	0.86d	102.1a	0.94a	15.0a	-	-
Significance									
Rootstock[a]	***	***	***	***	***	***	***	***	***
Year	***	***	***	***	***	***	***	-	-
R x Y	ns	ns	*	ns	***	ns	***	-	-

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively.
R x Y indicates irrigation x year interaction

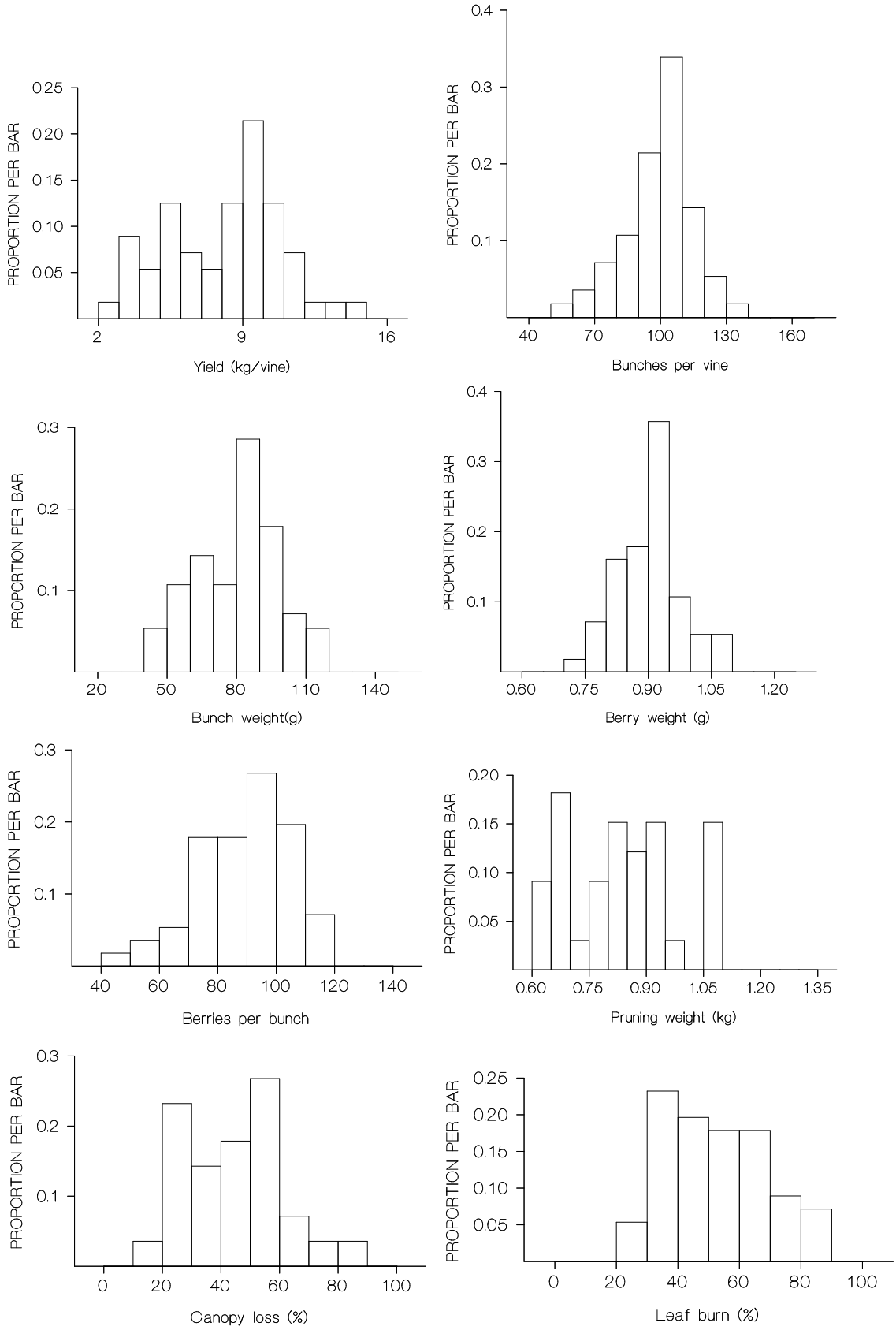


Figure 2.2.2.1. Distribution of mean values of yield and its components and pruning weight of 59 rootstock genotypes over five seasons (2013-17).

Correlation analyses were conducted using only the rootstock means to explore relationships between yield determinants and growth characteristics across the rootstock genotypes (Table 2.2.2.2). Bunch number, bunch weight and berry weight were significant contributors to yield variability. A highly significant multilinear regression ($p<0.001$) indicted that 97.2% of yield variation across the rootstock genotypes could be accounted for by inclusion of bunches, bunch weight and berry weight in the model. Bunch weight was positively correlated with both berry weight and berries per bunch and pruning weight. There were strong positive correlations between pruning weight and yield, bunch number, bunch weight, berry weight and berries per bunch. Canopy loss and leaf burn, measured in 2017, were negatively associated with mean values across seasons for yield, bunch number, bunch weight, berry weight berries per bunch and pruning weight. Such results indicate that crop development is more restricted with rootstock genotypes having lower conferred vigour and that these rootstocks are also more likely to be impacted by short term drought stress.

Table 2.2.2.2. Correlation matrix across rootstock means (n=56) describing relationships between rootstock growth characteristics of Shiraz over five seasons (2013-2017). Values retained are significant ($p<0.05$). Highly significant correlations ($p<0.001$) are in bold.

	Yield	***Bunches***	***Bunch weight***	***Berry weight***	***Berries per bunch***	***Pruning weight***
Bunches	**0.87**					
Bunch weight	**0.95**	**0.72**				
Berry weight	**0.81**	**0.67**	**0.78**			
Berries/ bunch	**0.87**	**0.64**	**0.95**	**0.58**		
Pruning weight	**0.88**	**0.77**	**0.83**	**0.80**	**0.71**	
Ravaz index	-0.30	**-0.42**	-0.26	-0.32	-0.21	**-0.56**
Canopy loss (%)	**-0.54**	**-0.47**	**-0.54**	**-0.65**	**-0.43**	**-0.53**
Leaf burn (%)	**-0.56**	**-0.51**	**-0.55**	**-0.62**	**-0.46**	**-0.57**

B. Fruit composition

Berry juice composition over five seasons (2013-17) for Shiraz across the 59 rootstock genotypes is presented in Table 2.2.2.3. GLM, ANOVA analysis of the data showed significant effects of Rootstock and Year for most variables. The results indicate that there were significant differences in maturity when samples were collected, ranging from a mean of 18.9 ^{o}Brix to 25.3 ^{o}Brix across the rootstocks. To test the effect associated with the differences in maturity, TSS was included as a covariate in the ANOVA analysis. There were significant effects of TSS as a covariate on pH, malate and tartrate (data not shown in detail).

The results show significant effects of season on all berry juice variables (Table 2.2.2.3). TSS was highest in 2015 and lowest in 2013. pH was highest in 2014 and lowest in 2013. TA was highest in 2015 and lowest in 2016. Malate was highest in 2013 and 2014 and lowest in 2016. Tartrate was highest in 2015 and lowest in 2016 and 2017. YAN was highest in 2016 and 2017 and lowest in 2015. Anthocyanin and phenolic substances were highest in 2016 and 2017 and lowest in 2014.

Rootstocks had significant effects on most juice compositional variables, except TA and tartrate (Table 2.2.2.3). The distribution of the berry composition variables across the 59 rootstock genotypes is shown in Figure 2.2.2.2. TSS of most genotypes fell between 22 and 24 ^{o}Brix. pH followed a relatively normal distribution with the greater proportion falling between pH 3.85 and 4.0. The highest proportion of rootstocks had TA levels between 3.4 and 3.5 g/L. The highest proportion of rootstocks had malate levels between 1.9 and 2.3 g/L. The highest proportion of rootstocks had tartrate levels between 5.7 and 5.9 g/L.

The highest proportion of rootstocks had YAN levels between 180 and 190 g/L. Anthocyanin concentrations largely fell in the 1.6 - 1.9 mg/g categories while the distribution of phenolic substances was broader with most in the 1.6 - 2.2 a.u./g) categories. Except for M 920 which had very low TSS (18.9 Brix), indicating delayed ripening, differences in TSS across the named rootstocks were small (22.6 - 24.0 ^{o}Brix). Dog Rridge and 1103 Paulsen had the highest pH while Merbein 5489, Merbein 5512, Merbein 6262 and M 920 had lower pH than own roots (3.86). Malate levels were highest with Dog Ridge and to a lesser degree, 1103 Paulsen and 101-14 while the Merbein rootstocks all had lower malate levels than own roots (1.88 g/L). YAN levels were

lowest in juice of own roots and M 920 and similar for the other named rootstocks (183-198 mg/L). Merbein 5489 and Merbein 5512 had significantly more anthocyanin and phenolic substances than all the other standard rootstocks. M920 and the lowest levels of berry anthocyanin.

Table 2.2.2.3. Mean juice TSS, pH, TA, Malate, Tartrate, YAN, anthocyanin and phenolic substances of Shiraz grafted on 51 CSIRO rootstock selections, eight standard rootstocks or grown as own roots over five seasons (2013-17). Means followed by the same letter are not significantly different ($p < 0.05$). A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***TSS (°Brix)***	***pH***	***TA (g/L)***	***Malate (g/L)***	***Tartrate (g/L)***	***YAN (mg/L)***	***Anth. (mg/g)***	***Phen. (a.u.)***
Own roots	22.7	3.86	3.59	1.88	5.48	157	1.76	1.70
Dog Ridge	23.7	4.07	3.41	2.59	5.90	187	1.71	1.79
1103 Paulsen	23.6	4.02	3.44	2.13	5.61	199	1.73	1.84
140 Ruggeri	23.8	3.98	3.34	1.95	5.92	194	1.69	1.78
101-14	24.0	4.01	3.46	2.16	5.87	186	1.83	1.72
Merbein 5489	22.9	3.82	3.85	1.80	6.23	188	1.95	2.01
Merbein 5512	23.2	3.80	3.74	1.59	6.14	183	2.00	1.91
Merbein 6262	22.6	3.79	3.56	1.70	5.42	198	1.76	1.79
M 920	18.9	3.79	3.59	1.94	4.76	149	1.44	1.73
Minimum	18.9	3.79	3.26	1.59	4.76	149	1.38	1.47
Maximum	25.3	4.14	3.94	2.59	6.09	229	2.23	2.26
Year								
2013	19.3d	3.75d	3.39c	2.59a	4.89c	138c	1.73b	1.84b
2014	24.3a	4.24a	3.58b	2.55a	6.61a	191b	1.40c	1.56c
2015	24.7a	3.80d	3.73a	1.85b	6.70a	171b	1.73b	1.82b
2016	23.8ab	4.02b	3.25c	1.62c	5.30b	205a	1.83a	1.94a
2017	21.9c	3.92c	3.68a	1.93b	5.39b	206a	1.82a	1.94a
Significance[a]								
Rootstock	***	***	ns	***	ns	***	***	***
Year	***	***	***	***	***	***	***	***
R x Y	ns	ns	***	***	***	ns	-	-
TSS covariate	-	***	ns	***	***	ns	ns	ns

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively.

[b] RxY indicates the rootstock x season interaction.

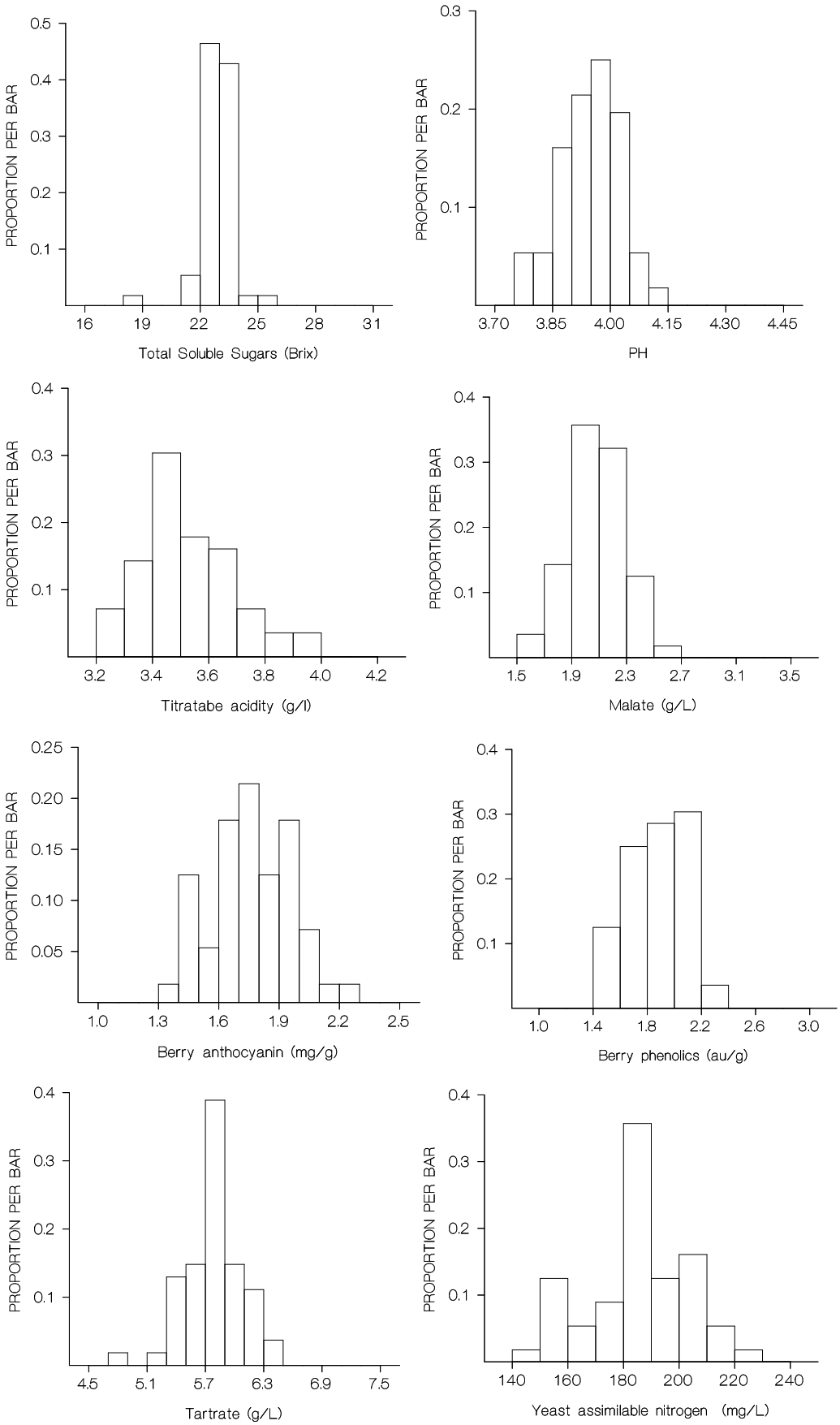


Figure 2.2.2.2. Distribution of mean fruit composition values 59 rootstock genotypes over five seasons (2013-17).

C. Juice nutrient status

In 2016, juice samples were analysed by ICP to determine nutrient status (Table 2.2.2.4). Rootstocks had a significant effect on all elements with 1.5-2.9 fold differences. Across the named rootstocks including own roots, B concentrations were highest with Merbein 5512 and lowest with Merbein 6262; Ca concentrations were highest with Merbein 6262 and lowest with own roots; K values were highest with Dog Ridge and lowest with Merbein 5489; Mg concentrations were highest with Merbein 6262 and lowest with Merbein 5489; Na concentrations were highest with M 920 and lowest with Merbein 5512; P concentrations were highest with M 920 and lowest with Merbein 5589; S concentrations were highest with Merbein 5512 and lowest with Merbein 5489. The distribution of the mineral elements across the 59 rootstock genotypes is shown in Figure 2.2.2.3. The pattern of B distribution across the rootstocks was slightly skewed toward lower concentrations with the highest proportion in the 5.5 - 5.8 mg/L category. Calcium was skewed to lower concentrations with the highest proportion in the 160 - 200 mg/L category. The distribution of K appeared to segregate with two peaks, i.e. the 1400 -1500 mg/L and the 1700-1800 mg/L categories. The distribution of Mg was skewed toward lower concentrations with the highest proportion in the 105 - 115 mg/L categories. Juice Na was widely distributed across the 59 rootstock genotypes. The distribution of P was skewed toward lower concentrations with the highest proportion in the 130 - 160 mg/L categories. The distribution of S was skewed toward higher concentrations with the highest proportion in the 85 - 90 mg/L category.

Table 2.2.2.4. Mean mineral element concentrations (mg/L) in juice of Shiraz grafted on 51 CSIRO rootstock selections, eight standard rootstocks or grown as own roots, season 2016. A summary of the significance values, generated from the GLM-ANOVA analysis is also presented.

Rootstock	***B***	***Ca***	***K***	***Mg***	***Na***	***P***	***S***
Own roots	5.31	177.5	1512	109.5	28.6	145.4	81.8
Dog Ridge	5.90	185.2	1795	116.4	23.7	131.2	83.8
1103 Paulsen	5.80	188.7	1479	125.7	27.6	134.6	89.4
140 Ruggeri	5.20	196.5	1421	131.4	34.1	139.6	93.1
101-14	5.90	193.6	1584	126.8	22.6	123.2	81.8
Merbein 5489	5.40	200.6	1224	107.2	20.6	115.6	77.9
Merbein 5512	6.17	227.4	1586	120.2	18.4	134.8	106.2
Merbein 6262	4.04	313.6	1615	135.3	28.3	147.2	84.4
M 920	4.65	157.6	1437	117.2	37.1	161.7	98.5
Minimum	4.04	147.5	1086	100.2	12.7	115.6	61.5
Maximum	9.00	313.6	2372	145.6	37.1	216.7	109.9
Significance[a]							
Rootstock	***	***	***	***	***	**	***
TSS covariate	***	**	***	*	ns	***	***

[a] *, **, *** represent significance values < 0.05, 0.01, 0.001 respectively.

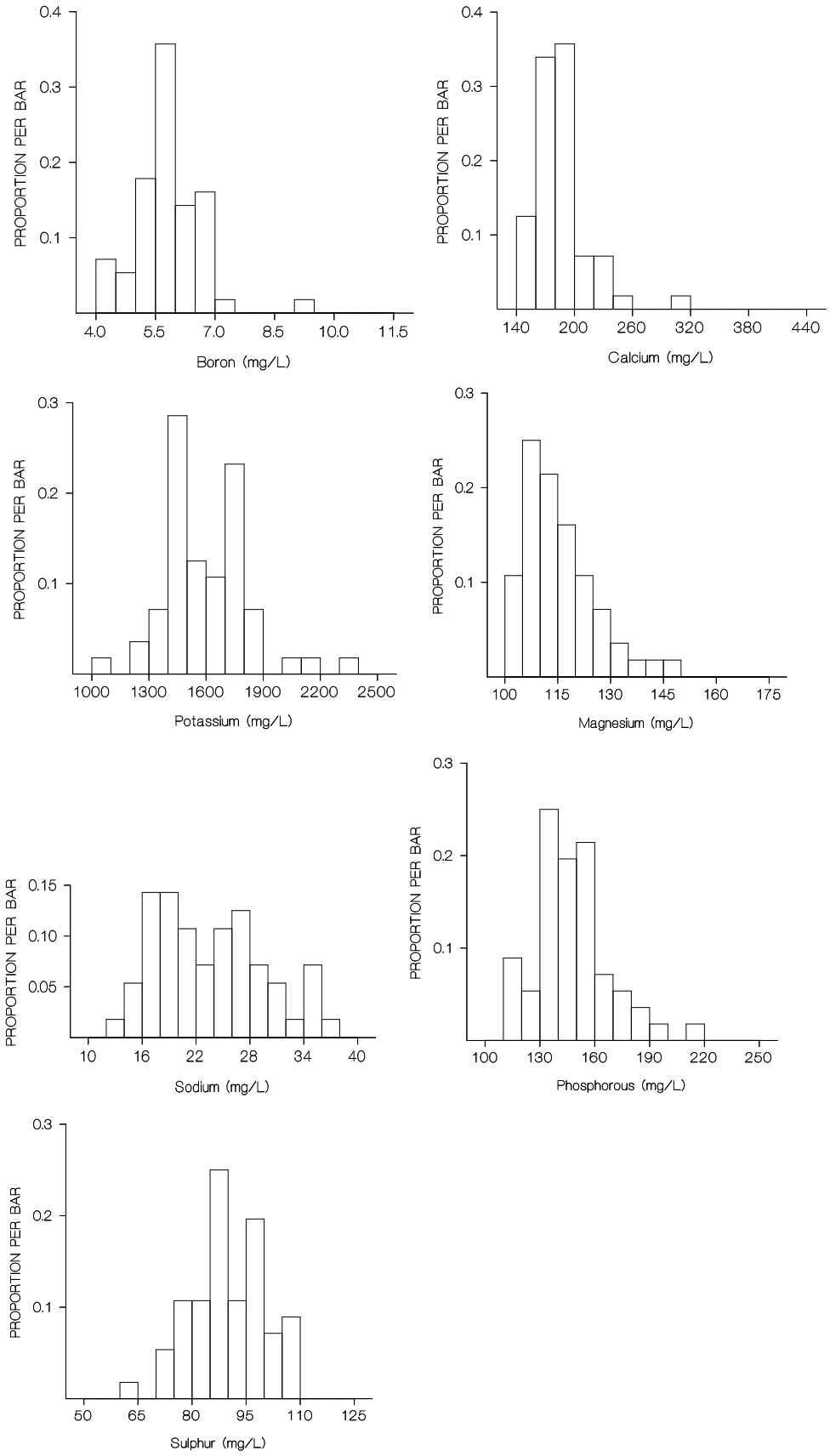


Figure 2.2.2.3. Distribution of mean mineral element concentrations of 59 rootstock genotypes, season 2016.

D. Relationships between growth characteristics and fruit composition

Correlation analyses were undertaken to explore relationships between Shiraz vine growth characteristics and berry juice composition using rootstock means with effects of irrigation and season removed (Table 2.2.2.5). Adjusted fruit composition means generated from the inclusion of the significant TSS covariate were also included to removes effects of fruit maturity. The results clearly show that rootstock effects on vine growth characteristics can have a significant influence on fruit composition. Yield was positively correlated with unadjusted and adjusted pH and malate and negatively correlated with unadjusted and adjusted TA, tartrate, anthocyanin and phenolics, Ca, Mg, Na and adjusted B, and S. Bunch number was positively correlated with unadjusted and adjusted pH and malate and negatively correlated with unadjusted and adjusted TA, anthocyanin and phenolics, Ca, Mg and Na. Bunch weight was positively correlated with unadjusted and adjusted pH and malate and negatively correlated with unadjusted and adjusted TA, tartrate, YAN, anthocyanin and phenolics, Ca, Mg, P, Na and adjusted B and S. Berry weight was positively correlated with unadjusted and adjusted pH and malate and negatively correlated with unadjusted and adjusted, tartrate, YAN, anthocyanin and phenolics, Ca, Mg, and S and adjusted B and S. Berries per bunch was positively correlated with unadjusted and adjusted malate, adjusted pH and negatively correlated with unadjusted and adjusted TA, tartrate, anthocyanin and phenolics, Ca, Mg, P, and Na.

The 8.5-fold difference in conferred vigour across the rootstock genotypes had highly significant influences on fruit composition (Table 2.2.2.5). Pruning weight was positively correlated with unadjusted and adjusted pH and malate and negatively correlated with unadjusted and adjusted, anthocyanin and phenolics, Ca, Mg and adjusted Na.
The Ravaz index was positively correlated with unadjusted and adjusted TA, Ca, and adjusted Mg and negatively correlated with juice TSS, unadjusted and adjusted pH and malate, B, K, Na and S.

Table 2.2.2.5. Correlation matrix, describing linear relationships between growth characteristics (yield component variables, pruning weight and the Ravaz index) and berry juice composition of Shiraz across rootstock genotypes (n=59). Values retained are significant ($p<0.05$). Highly significant correlations ($p<0.001$) are in bold. Note: adjusted means using the significant TSS covariate were also included in the analysis. Mineral element data for 2016 only

	Yield	***Bunches***	***Bunch weight***	***Berry weight***	***Berries per bunch***	***Pruning weight***	***Ravaz index***
TSS							-0.37
pH	0.22	0.21	0.20	0.24		0.27	**-0.43**
TA	-0.24	-0.23	-0.27		-0.33		0.23
Malate	**0.41**	0.34	0.38	**0.47**	0.30	**0.49**	-0.40
Tartrate	-0.22		-0.30	-0.25	-0.28		
YAN			-0.22	-0.27			
Anthocyanin	**-0.62**	**-0.46**	**-0.62**	**-0.72**	**-0.47**	**-0.53**	
Phenolics	**-0.67**	**-0.57**	**-0.61**	**-0.73**	**-0.46**	**-0.59**	
pH adj.	0.27	0.21	0.27	0.26	0.23	0.26	-0.29
TA adj.	-0.24	-0.24	-0.26		-0.32		0.23
Malate adj.	0.39	0.33	0.35	**0.45**	0.26	**0.47**	**-0.44**
Tartrate adj.	-0.24		-0.31	-0.30	-0.27		
YAN adj.			-0.22	-0.29			
Antho. adj.	**-0.63**	**-0.47**	**-0.62**	**-0.73**	**-0.48**	**-0.54**	
Phen. adj.	**-0.67**	**-0.57**	**-0.61**	**-0.73**	**-0.46**	**-0.59**	
B							**-0.53**
Ca	**-0.48**	**-0.42**	**-0.53**	**-0.56**	**-0.46**	**-0.46**	0.30
K						0.20	**-0.46**
Mg	-0.35	-0.21	**-0.46**	**-0.44**	**-0.44**	-0.35	
P			-0.20		-0.25		
Na	-0.27	-0.24	-0.22				-0.24
S				-0.29			-0.35
B adj.	-0.31		-0.34	-0.38	-0.27		-0.26
Ca adj.	**-0.53**	**-0.48**	**-0.57**	**-0.59**	**-0.50**	**-0.52**	0.37
K adj.							-0.27
Mg adj.	**-0.41**	-0.28	**-0.51**	**-0.48**	**-0.48**	**-0.41**	0.26
P adj.			-0.22		-0.26		
Na adj.	**-0.41**	-0.39	-0.34	-0.29	-0.30	-0.24	
S adj.	-0.24		-0.27	-0.39			-0.23

Multilinear regression techniques were used to identify the best combinations of growth traits with strong predictive value for Shiraz juice composition across the rootstock genotypes (n=56). They showed that a significant proportion of the variability in berry composition across could be accounted for based on adjusted R^2 values including:-

- 24% (p=0.000) of malate by pruning weight (+ve)
- 14% (p=0.008) of tartrate by bunch weight (-ve)
- 51% (p=0.000) of anthocyanin by berry weight (-ve)
- 54% (p=0.000) of phenolic substances by yield and berry weight (both -ve)

F. Relationships fruit composition variables

Correlation analyses were undertaken to explore relationships between Shiraz berry juice composition variables using rootstock means with effects of season removed (Table 2.2.1.6). Adjusted fruit composition means generated from the inclusion of the significant TSS covariate were also included to remove effects of fruit maturity. Across the rootstocks, there were significant effects of fruit maturity (TSS) on fruit composition described by the significant positive correlations between TSS and pH, malate, tartrate, YAN, anthocyanin, B, K, Mg and S. Across the rootstocks genotypes, pH was positively correlated with malate, tartrate and YAN. However, when adjusted for TSS the correlation with tartrate disappeared. Juice pH was also positively correlated with B, K (unadjusted and adjusted) and Na and negatively correlated with TA (unadjusted and adjusted) and Ca adjusted. TA was positively correlated with unadjusted and adjusted tartrate but not malate and negatively correlated with adjusted pH, K and S. Juice malate was positively correlated with YAN (unadjusted and adjusted), B and Na and K (adjusted) and negatively correlated with unadjusted and adjusted anthocyanin, phenolics and Ca. Juice tartrate was positively correlated with unadjusted and adjusted YAN, anthocyanin, phenolics, B, K, Mg and adjusted TA and tartrate. Juice YAN, was positively correlated with unadjusted and adjusted anthocyanin and phenolics, B, K, Mg and S, unadjusted Ca and Na and adjusted malate and tartrate. There were strong positive correlations between anthocyanin and unadjusted and adjusted phenolics, B, Mg and S and adjusted tartrate and YAN and negatively correlations with adjusted pH and malate. Phenolic substances were positively correlated with adjusted tartrate, YAN, anthocyanin and phenolics, unadjusted and adjusted CA, Mg and S and adjusted Na and negatively correlated with adjusted malate.

Table 2.2.2.6. Correlation matrix, describing linear relationships between Shiraz juice composition variables across rootstock (n=59). Values retained are significant ($p<0.05$). Highly significant correlations ($p<0.001$) are in bold.

	TSS	***pH***	***TA***	***Malate***	***Tartrate***	***YAN***	***Antho.***	***Phen.***
pH	**0.54**							
TA		-0.35						
Malate	0.24	**0.79**						
Tartrate	**0.53**	0.27	**0.43**					
YAN	**0.55**	0.39		0.22	**0.68**			
Anthocyanin	0.22			-0.39	0.36	0.34		
Phenolics				-0.31	0.25	0.28	**0.92**	
pH adj.		**0.87**	-0.38	**0.81**			-0.29	
TA adj.		-0.36	**1.00**		**0.42**			
Malate adj.		**0.84**		**0.98**	0.27	0.32	-0.32	-0.29
Tartrate adj.			**0.49**		**0.97**	**0.61**	0.34	0.27
YAN adj.		0.37		0.22	**0.66**	**1.00**	0.33	0.28
Antho. adj.				-0.40	0.34	0.33	**1.00**	**0.92**
Phen. adj.				-0.31	0.26	0.28	**0.92**	**1.00**
B	**0.51**	0.35		0.21	**0.50**	**0.41**	0.29	
Ca				-0.36		0.23	**0.42**	0.35
K	0.26	**0.56**	-0.21	**0.51**	0.35	0.36		
Mg	0.24				0.35	**0.50**	0.24	0.22
P								
Na		0.25		0.21		0.25		
S	0.23		-0.21			**0.41**	0.35	0.32
B adj.					**0.46**	0.40	**0.41**	0.37
Ca adj.		-0.25		-0.39			**0.42**	0.37
K adj.		**0.48**		**0.46**	0.28	0.33		
Mg adj.					0.32	**0.47**	0.24	0.24
P adj.								
Na adj.								0.25
S adj.						0.39	0.37	0.38

G. Wine 2017

Growth characteristic and fruit composition data based on the Shiraz means over seasons (2013-16) were sorted to identify the 10 most promising new CSIRO selections with potential for future release. Key criteria used in the selection were yield and conferred vigour, pH, malate and berry anthocyanin. Wines were made from these selections and the five standards, including own roots in season 2017. The results of growth characteristics over all seasons (2013-17) results are presented in Table 2.2.2.7.

The CSIRO selection M 230-70 had the highest yield associated with high bunch numbers and development of large bunches with a high number of large berries per bunch compared to the highest yielding standard rootstock, 1103 Paulsen. It also had the highest conferred vigour of all the rootstock genotypes. Selection 230-80 had a similar yield to 1103 Paulsen. M 230-10, M 230-45, M 231-30 and M 235-01 had higher yields than the own roots or the other standard rootstocks. All other selections had similar yields to own roots and the other standard rootstocks (i.e. 8.9 - 9.8 kg/vine). Overall, because of the selection process, differences in bunch number (90 - 116), bunch weight (79.3 - 110.9 g), berry weight (0.86 - 1.06 g) and berries per bunch (86.2 - 117.0) were small. However, there was almost a three-fold difference in conferred vigour, ranging from 0.67 - 1.73 kg/vine. Consequently, vine balance determined from the Ravaz index varied from 7.9 to 20.2.

Table 2.2.2.7. Mean yield, bunches per vine, bunch weight, berry weight, berries per bunch, pruning weight and Ravaz index of Shiraz grafted on 10 CSIRO rootstock selections showing promise, four standard rootstocks or grown as own roots over five seasons (2013-2017).

Rootstock	***Yield (kg/v)***	***Buns./ vine***	***Bunch weight (g)***	***Berry weight (g)***	***Berrs./ bunch***	***Prun. Wt. (kg/v)***	***Ravaz Index***
Shiraz	9.8	111	88.3	0.97	93.6	1.05	10.9
140 Ru	9.6	116	81.0	0.93	88.2	0.77	16.3
1103P	11.2	102	101.5	0.93	107.6	0.89	14.8
M 5489	9.1	107	79.3	0.96	86.2	0.60	20.2
101-14	9.5	105	82.2	0.90	91.9	0.70	16.4
M 230-10	10.6	102	110.9	0.95	117.0	0.82	16.5
M 230-30	9.1	95	89.1	0.88	103.8	0.81	13.3
M 230-45	10.2	116	85.8	0.98	87.7	0.83	15.5
M 230-70	13.8	116	116.3	1.06	112.6	1.73	9.6
M 231-30	10.4	107	98.9	1.03	96.3	1.14	10.6
M 231-51	9.0	99	88.4	0.86	109.9	0.67	14.3
M 233-80	11.2	116	97.8	0.97	102.3	1.28	10.4
M 234-27	9.3	108	85.9	0.93	91.0	1.23	10.0
M 235-01	10.3	103	99.1	0.95	105.3	1.51	7.9
M 235-70	8.9	90	101.5	0.91	112.7	0.75	14.2

Fruit composition data for the CSIRO selections and standard rootstocks over all years are presented in Table 2.2.1.8. Due to the selections process, overall differences in fruit maturity were small, with mean TSS ranging from 22.6 - 24.0 ^{o}Brix. Of the standard rootstocks, Merbein 5489 and own roots had the lowest pH while1103 Paulsen and 101-14 had high pH levels. Juice pH of a number of CSIRO selections was close to Merbein 5489 and own roots and lower than the other standard rootstocks. Differences in TA (3.34 - 3.85 g/L), malate (1.80 - 2.33 g/L) and tartrate (5.43 - 6.24 g/L), anthocyanin (1.44 - 1.95 mg/g) and phenolics (1.53 - 2.06 a.u.) across the rootstock genotypes were small. YAN levels of all rootstock genotypes were above the 150 mg/L recommended for fermentation.

Table 2.2.2.8. Mean juice TSS, pH, TA, Malate, Tartrate, YAN, anthocyanin and phenolic substances of Shiraz grafted on 10 CSIRO rootstock selections showing promise, four standard rootstocks or grown as own roots over five seasons (2013-2017).

Rootstock	***TSS (ºBrix)***	***pH***	***TA (g/L)***	***Malate (g/L)***	***Tartrate (g/L)***	***YAN (mg/L)***	***Anth. (mg/g)***	***Phen. (a.u.)***
Shiraz	22.7	3.86	3.59	1.88	5.48	157	1.76	1.70
140 Ru	23.8	3.98	3.34	1.95	5.92	194	1.69	1.78
1103P	23.6	4.02	3.44	2.13	5.61	198	1.73	1.84
M 5489	22.9	3.82	3.85	1.80	6.23	188	1.95	2.01
101-14	24.0	4.01	3.46	2.16	5.87	186	1.83	1.72
230-10	23.9	4.07	3.48	2.33	6.21	204	1.49	1.55
230-30	23.1	4.03	3.58	2.32	5.98	200	1.78	1.95
230-45	23.6	4.02	3.49	2.13	5.77	215	1.46	1.54
230-70	23.0	3.92	3.63	2.21	5.83	188	1.44	1.53
231-30	22.8	3.94	3.42	1.99	5.43	157	1.60	1.84
231-51	22.9	3.97	3.48	2.19	5.82	208	1.92	2.06
233-80	22.6	3.90	3.67	2.24	5.78	182	1.79	1.93
234-27	23.3	3.99	3.68	2.24	6.06	207	1.81	2.01
235-01	23.2	4.03	3.55	2.25	6.24	207	1.81	1.86
235-70	22.7	3.90	3.39	1.85	5.44	185	1.77	1.97

As indicated above wines were produced from these selections in 2017, harvested 11 April. The actual mean data for the growth characteristics from 2017 is presented in Table 2.2.2.9. As indicated above, crop development was impacted by the short term drought during the early stages of ripening. Yields across the rootstock genotypes in 2017 ranged from 13.1-23.2 kg/vine with 1103 Paulsen, M 230-10 and M 231-51 having the highest yields and 140 Ruggeri, M 230-45, M 231-30, producing the lowest yield. Bunch numbers ranged from 113-170 per vine, with 140 Ruggeri, Merbein 5489 and M 233-80 having the highest number and 1103 Paulsen, M 230-10, M 230-70 and M 235-70 having the lowest number. Bunch weights ranged from 80-176 g with 1103 Paulsen and M 230-10 having the largest bunches and 140 Ruggeri and M 230-45 having the smallest bunches. Berry weight ranged from 0.72 - 1.05 g, with largest berries produced by 140 Ruggeri, 1103 Paulsen, M 231-30 and M 230-45 and smallest berries produced by Merbein 5489 and M 231-51. Berries per bunch ranged from 82 - 228 g with M 231-51 having the highest number and 140 Ruggeri and M 230-45 having the lowest number of berries per bunch.

Conferred vigour in 2017 ranged from 0.68 - 1.80 kg/vine, with highest pruning weights recorded for M 235-01 and lowest values recorded by 101-14, Merbein 5489, M 230-10, M230-30 and M 231-51 (Table 2.2.2.9). As a consequence of the high yields recorded in

2017, vine balance, as determined by the Ravaz index, of most rootstock genotypes was high, ranging from 9.1-37.9 with M 235-01 having the lowest value and 101-14 and M 230-10 the highest values. All selections displayed a higher level of short term drought tolerance than the most sensitive rootstock variety, 101-14. Selections M 230-10, M 231-30, M 235-01 and M 235-70 displayed a high level of short term drought tolerance that was better than that of 140 Ruggeri, the best of the standard rootstocks.

Table 2.2.2.9. Mean yield, bunches per vine, bunch weight, berry weight, berries per bunch, pruning weight and Ravaz index of Shiraz grafted on 10 CSIRO rootstock selections showing promise, four standard rootstocks or grown as own roots in 2017.

Rootstock	*Yield (kg/v)*	*Buns. /vine*	*Bunch weight (g)*	*Berry weight (g)*	*Berrs./ bunch*	*Prun. Wt. (kg/v)*	*Ravaz Index*	*Can. loss (%)*	*Leaf burn (%)*
Shiraz	15.6	163	96	0.91	106	1.20	13.9	30.5	43.3
140 Ru	14.0	167	80	0.97	82	1.05	14.3	26.7	28.3
1103P	22.4	126	168	0.98	169	1.16	19.4	30.0	40.0
M 5489	19.2	169	116	0.79	139	0.85	20.2	50.0	62.5
101-14	21.6	160	130	0.81	157	0.75	33.4	57.5	70.0
M 230-10	23.2	125	176	0.93	184	0.68	37.9	20.0	33.3
M 230-30	17.0	131	125	0.83	151	0.83	23.9	50.0	52.0
M 230-45	13.1	155	81	0.99	86	0.96	11.4	30.0	44.0
M 230-70	15.5	119	122	0.96	129	1.56	12.2	35.0	35.0
M 231-30	13.9	132	102	1.05	104	1.31	11.2	15.0	36.3
M 231-51	21.3	139	158	0.72	228	0.77	27.2	50.0	64.0
M 233-80	15.7	170	92	0.94	97	1.47	10.9	30.0	42.0
M 234-27	14.4	162	90	0.82	111	1.33	10.7	55.0	55.0
M 235-01	16.2	152	106	0.93	116	1.80	9.1	26.7	30.0
M 235-70	17.8	113	175	0.91	193	0.86	22.3	23.3	23.3

Mean data of fruit composition collected at harvest in 2017 is presented in (Table 2.2.2.10). TSS of all rootstock genotypes was below the 24 ^{o}Brix desired for winemaking although differences were small, ranging from 21.3-23.8 ^{o}Brix with own roots and Merbein 5489 the least ripe and M 230-10 producing the ripest fruit. pH ranged from 3.79 - 4.09 with own roots, Merbein 5489 and M 233-80 having the lowest pH and 1103 Paulsen, 101-14 and M 230-10 having the highest pH. TA across the rootstock genotypes ranged from 3.44 - 4.16 g/L for 101-14 and M 234-27, respectively. Malate levels ranged from 1.33 - 1.97 g/L being highest for1103 Paulsen, M 230-10, M230-30 and 231-51 and lowest for 140 Ruggeri and M 235-70. Differences in tartrate levels across the rootstock genotypes ranged from 4.87 - 6.23 g/L with Merbein 5489, M 230-10, M 231-51, M 233-80, M 234- 27 and 235-01 having the highest levels and own roots and M 235-70 the

lowest levels. YAN levels were all above the desired level for fermentation of 150 mg/L. Berry anthocyanin ranged from 0.73 - 1.99 mg/g, with Merbein 5489 having the highest level and M 230-10 the lowest level. Phenolics ranged from 0.78 - 2.07 a.u. with highest levels recorded by Merbein 5489, M 230-30 and M 234-27 while M 230-10 had the lowest levels of phenolics.

Table 2.2.2.10. Mean juice TSS, pH, TA, Malate, Tartrate, YAN, anthocyanin and phenolic substances of Shiraz grafted on 10 CSIRO rootstock selections showing promise, four standard rootstocks or grown as own roots in 2017.

Rootstock	***TSS (ºBrix)***	***pH***	***TA (g/L)***	***Malate (g/L)***	***Tartrate (g/L)***	***YAN (mg/L)***	***Anth. (mg/g)***	***Phen. (a.u.)***
Shiraz	21.3	3.84	3.75	1.46	5.14	205	1.65	1.64
140 Ru	22.7	3.93	3.59	1.41	5.43	210	1.73	1.80
1103P	22.1	4.03	3.80	1.86	5.37	287	1.76	1.77
M 5489	21.3	3.82	4.19	1.70	6.05	286	1.99	2.07
M 101-14	22.5	4.01	3.44	1.68	5.85	232	1.62	1.57
M 230-10	23.8	4.09	3.65	1.97	6.23	232	0.73	0.78
M 230-30	21.6	3.99	3.90	1.90	5.84	246	1.78	2.01
M 230-45	22.3	3.97	3.79	1.78	5.94	266	1.15	1.20
M 230-70	22.2	3.93	3.83	1.80	5.68	220	1.32	1.45
M 231-30	22.4	3.88	3.88	1.75	5.65	191	1.58	1.77
M 231-51	22.7	3.95	4.01	1.90	6.18	343	1.73	1.87
M 233-80	22.3	3.79	4.10	1.76	6.02	233	1.79	1.92
M 234-27	21.8	3.91	4.16	1.78	6.10	287	1.74	2.00
M 235-01	21.6	3.91	3.92	1.73	6.00	260	1.73	1.86
M 235-70	22.7	3.85	3.48	1.33	4.87	204	1.67	1.92

Must composition, total tartaric acid addition to adjust must and wine pH and wine data are presented in Table 2.2.2.11. In general, TSS levels were close to the desired level for winemaking of 24 ^{o}Brix. Must TSS levels were highest for 140 Ruggeri and M 230-10 and lowest for own roots. Must pH was highest for 140 Ruggeri and M 235-01 and lowest for Merbein 5489. Merbein 5489 had the highest and 140 Ruggeri the lowest TA, respectively. Merbein 5489 and own roots had the lowest and M 230-10 the highest malate levels. Differences in YAN were small (data not shown), ranging from 199 - 266 mg/L, well above the desired level of 150 mg/L for wine fermentation.

Across the rootstock genotypes, the amount of acid addition required for pH adjustment ranged from 2 - 4.5 g/L with own roots, Merbein 5489, 101-14 and M 235-70 having the lowest requirement and 140 Ruggeri, M 230-45, M 230-70, M 231-30, M 233-80 and

M235-01 the highest requirements (Table 2.2.2.11). Following pH adjustment, final wine pH values were similar, ranging from 3.43 - 3.56 with own roots, Merbein 5489 and M 231-51 having the lowest wine pH and 1103 Paulsen, 101-14, M 230-30 and M 235-70 the highest wine pH. Differences in wine TA were small, ranging from 5.4 - 6.5 g/L. Wine alcohol ranged from 12.8-15.1 % , being lowest for own roots and highest for 140 Ruggeri and M 230-10. Wines from all rootstocks had lower wine Cl than own roots. Wines from selections M 230-30, 230-70 and M 235-70 had lower Cl than the standard rootstocks. Rootstock wines from 101-14, M 230-10, M 234-27 and M 233-80 had the highest Cl.

Table 2.2.2.11. Must composition (TSS, pH, TA, malate), total acid addition for pH adjustment and wine data (pH, TA, alcohol and Cl) of Shiraz grafted on 10 CSIRO rootstock selections showing promise, four standard rootstocks or grown as own roots in 2017.

	Must data					***Bottling data***			***Cl***
	TSS (°Brix)	**pH**	**TA** (g/L)	**Mal** (g/L)	**TA added** (g/L)	**pH**	**TA** (g/L)	**Alc.** (%)	mg/L
Shiraz	21.8	3.77	3.7	1.3	2.0	3.45	5.5	12.8	230
140 Ru	25.1	4.12	2.7	1.5	4.5	3.49	6.0	15.0	110
1103 P	24.0	3.96	3.7	1.7	3.3	3.54	5.8	14.2	107
M 5489	23.0	3.60	4.0	1.2	2.0	3.44	5.8	13.8	106
101-14	23.9	3.82	3.6	1.6	2.0	3.55	5.7	13.8	123
M 230-10	25.8	4.05	3.3	2.0	3.3	3.50	6.2	15.1	125
M 230-30	23.1	3.87	3.6	1.8	3.3	3.56	5.7	13.7	97
M 230-45	24.0	4.08	3.1	1.7	4.5	3.48	6.0	14.0	117
M 230-70	23.7	4.06	2.9	1.6	4.5	3.50	5.9	14.4	97
M 231-30	24.2	4.08	2.9	1.7	4.5	3.49	6.1	14.7	141
M 231-51	23.3	3.79	3.7	1.4	3.3	3.43	6.1	13.5	106
M 233-80	23.8	4.04	2.9	1.8	4.5	3.48	6.2	14.4	126
M 234-27	23.3	4.00	2.9	1.4	4.0	3.47	6.5	13.5	133
M 235-01	24.4	4.15	2.9	1.6	4.5	3.53	6.5	14.3	109
M 235-70	23.3	3.74	3.6	1.4	2.0	3.56	5.4	13.6	96

The spectral properties for each of the Shiraz wines are reported in Table 2.2.2.12. Colour density was highest in wines of own roots, Merbein 5489 and M 231-51 and lowest in 101-14 and M 235-70. Brightest wines with lowest hue were produced by own roots, Merbein 5489 and M 231-51. Total anthocyanin in wines ranged from 616 - 810 mg/L, with M 230-30 having the highest levels and 101-14 and M 235-70 the lowest levels. Ionised anthocyanin ranged from 61 - 101 mg/L with own roots and Merbein 5489 having the highest levels and 101-14, M 230-10 and M 235-70 the lowest levels. Wine phenolics

ranged from 39.2 - 60.0 a.u with 101-14 having the lowest and M 230-30 having the highest levels.

Table 2.2.1.12. Wine spectral properties of Shiraz grafted on 10 CSIRO rootstock selections showing promise, four standard rootstocks or grown as own roots in 2017.

	CD *(a.u).*	***Hue***	***Anthocyanin*** *(mg/L)*	***Ionised Anthocyanin*** *(mg/L)*	***Phenolics*** *(a.u).*
Shiraz	9.70	0.48	747	101	47.2
140 Ru	8.16	0.54	675	71	50.1
1103 P	8.49	0.50	782	83	50.6
M 5489	9.71	0.47	659	101	45.6
101-14	6.05	0.49	629	61	39.2
230-10	7.98	0.54	764	60	58.3
230-30	9.05	0.52	810	87	60.0
230-45	7.92	0.54	722	71	56.4
230-70	7.47	0.55	658	65	49.5
231-30	7.46	0.55	634	63	47.7
231-51	9.57	0.48	699	97	50.9
233-80	9.34	0.53	757	83	57.8
234-27	7.96	0.55	676	66	54.0
235-01	7.85	0.57	675	64	53.0
235-70	6.71	0.56	616	62	42.9

Correlation analyses were undertaken to explore relationships between vine growth characteristics and must and wine attributes across the rootstock genotypes (Table 2.2.1.13. Yield was positively correlated with berry juice pH, must TA and negatively correlated with wine hue. Bunch number was negatively correlated with wine pH. Bunch weight was positively correlated with berry juice TSS, pH and must TA. Berry weight was positively correlated with must pH and malate and wine alcohol and hue and negatively correlated with berry YAN, must TA and wine ionised anthocyanin. Berries per bunch was positively correlated with must TA and negatively correlated with must pH. Conferred vigour (pruning weight) was positively correlated with must pH and wine alcohol and negatively correlated with berry TSS and pH, and must TA. The Ravaz index was positively correlated with berry juice TSS and pH and must TA and negatively correlated with berry TA. Canopy loss was positively correlated with berry YAN and anthocyanin but negatively correlated with must TSS and pH, wine alcohol and hue. Leaf burn was positively correlated with berry tartrate and YAN and must TA and wine ionised anthocyanin and negatively correlated with must pH, wine alcohol and hue.

Multilinear regression techniques were used to identify the best combinations of growth traits with strong predictive value for Shiraz must and wine composition across the rootstock genotypes (n=15). They showed that a significant proportion of the variability in must and wine composition across rootstocks could be accounted for, based on adjusted R^2 values, including:-

- 41% (p=0.006) of must pH by berry weight (+ve)
- 51% (p=0.005) of must TA by bunch weight and leaf burn (both +ve)
- 46% (p=0.010) of must TA by yield (+ve) and pruning weight (-ve)
- 28% (p=0.026) of wine alcohol by berry weight (+ve)
- 49% (p=0.002) of wine hue by leaf burn (-ve)

Table 2.2.2.13. Correlation matrix, describing linear relationships between growth characteristics (yield component variables, pruning weight and the Ravaz index) and must and wine attributes of Shiraz grafted on 10 CSIRO rootstock selections showing promise, four standard rootstocks or grown as own roots in 2017 (n=15). Values retained are significant correlations (p<0.10). Significant correlations (p<0.05) are in bold.

	Yield	***Buns***	***Bunch weight***	***Berry weight***	***Berries per bunch***	***Prun. wt.***	***Ravaz index***	***Can. Loss %***	***Leaf Burn %***
Berry									
TSS			**0.48**			-0.41	**0.55**		
pH	**0.53**		0.46			-0.41	**0.61**		
TA							-0.43		
Mal									
Tart									**0.50**
YAN				**-0.63**				**0.57**	**0.58**
anth								0.41	
phen									
Must									
TSS								-0.44	
pH				**0.67**	**-0.51**	**0.56**		**-0.50**	**-0.54**
TA	**0.65**		**0.56**	**-0.57**	**0.64**	**-0.63**	**0.57**		**0.50**
Mal				0.46					
Wine									
pH		**-0.49**							
TA						**0.48**			
Alcohol				**0.57**				**-0.54**	**-0.49**
CD									
Hue	**-0.49**			**0.57**		**0.49**		**-0.53**	**-0.73**
T. Anth									
I. Anth				-0.45					**0.45**
Phen									
Cl									

Correlation analyses were undertaken to explore relationships between measurements of berry composition and must and wine attributes across the rootstock genotypes and irrigation treatments (Table 2.2.2.14, n=15). Berry TSS was positively correlated with berry pH, must TSS and malate and wine alcohol and negatively correlated with berry TA, anthocyanin and phenolics and wine colour density and ionised anthocyanins. There were positive correlations between berry pH and berry malate, must TSS and malate and negative correlations with berry TA, anthocyanin and phenolics. Berry TA was positively correlated with berry malate, tartrate, YAN and phenolics and wine TA and colour density and negatively correlated with wine pH. Berry malate levels were strongly correlated with berry tartrate and YAN, must malate and wine TA and total anthocyanin. Berry tartrate was positively correlated with berry YAN and wine TA. Berry YAN was positively correlated with wine colour density and ionised anthocyanin and negatively correlated with hue. Berry anthocyanin was positively correlated with berry phenolics and wine ionised anthocyanin but negatively correlated with must TSS, pH and malate and wine alcohol. Berry phenolics were positively correlated with wine ionised anthocyanin and negatively correlated with must TSS, malate and wine alcohol. Berry K, measured only in 2016, was positively correlated with berry pH and malate, must malate and wine TA.

Multilinear regression techniques were used to identify the best combinations of berry composition traits with strong predictive value for Shiraz must and wine composition across the rootstock genotypes (n=15). They showed that a significant proportion of the variability in must and wine composition across rootstocks could be accounted for, based on adjusted R^2 values, including:

- 50% (p=0.002) of must TSS by berry juice TSS (+ve)
- 71% (P=0.001) of must malate by berry pH and malate (both +ve) and YAN (-ve)
- 28% (P=0.026) of wine pH by berry TA (-ve)
- 47% (P=0.003) of wine TA by berry tartrate (+ve)
- 30% (p=0.020) of wine alcohol by berry TSS (+ve)
- 39% (P=0.008) of wine colour density by juice TA (+ve)
- 22% (p=0.043) of ionised anthocyanin by berry TSS (-ve)
- 29% (p=0.022) of phenolics by must malate (+ve)

Table 2.2.2.14. Correlation matrix, describing linear relationships between berry composition (TSS, pH, TA, Malate, tartrate, YAN, anthocyanin, phenolics and K) and must and wine attributes of Shiraz across 15 rootstock genotypes. Values retained are significant ($p<0.10$). Significant correlations ($p<0.05$) are in bold.

	TSS	***pH***	***TA***	***Mal***	***Tart***	***YAN***	***anth***	***phen***	***K***
pH	**0.51**								
TA	**-0.51**	-0.41							
Mal		**0.54**	0.45						
Tart			**0.56**	**0.76**					
YAN			**0.55**	**0.53**	**0.56**				
anth	**-0.67**	**-0.58**							
phen	**-0.62**	**-0.61**	0.45				**0.96**		
K		0.47		0.42					
Must									
TSS	**0.73**	**0.54**					**-0.55**	**-0.53**	
pH							-0.42		
TA									
Mal	**0.52**	**0.57**		**0.57**			**-0.60**	**-0.55**	**0.48**
Wine									
pH			**-0.57**						
TA			**0.52**	**0.48**	**0.71**				0.44
Alc	**0.59**						-0.43	-0.40	
CD	-0.41		**0.66**			0.42			
Hue						-0.40			
T. Anth				**0.49**					
I. Anth	**-0.53**		**0.48**			0.43	**0.49**	0.40	
T. phen			0.42	**0.59**	**0.49**				
Cl									

Correlation analyses were undertaken to explore relationships between must composition and wine attributes across the rootstock genotypes (Table 2.2.1.15, n=15). Must TSS was positively correlated with must pH and malate but negatively correlated with TA. Must TSS was positively correlated with wine TA, alcohol and hue, but negatively correlated with colour density, ionised anthocyanin and chloride. Must pH was positively correlated with malate, wine TA, alcohol, hue and total phenolic substances. Must TA was positively correlated with wine ionised anthocyanin and negatively correlated with wine TA, alcohol and hue. Must malate was positively correlated with wine pH, alcohol, hue, total anthocyanin and phenolics and negatively correlated with ionised anthocyanins.

Table 2.2.2.15. Correlation matrix, describing linear relationships between must and wine composition variables (must TSS, pH, TA and malate) and wine pH, TA, alcohol, spectral properties and Cl of Shiraz across 15 rootstock genotypes. Values retained are significant ($p<0.10$). Significant correlations ($p<0.05$) are in bold.

	TSS	***pH***	***TA***	***Mal***
Must				
pH	**0.67**			
TA	**-0.51**	**-0.89**		
Malate	**0.65**	**0.62**		
Wine				
pH				0.44
TA	**0.48**	**0.66**	**-0.65**	
Alcohol	**0.92**	**0.69**	**-0.59**	**0.64**
Colour Density	-0.40			
Hue	**0.49**	**0.73**	**-0.78**	0.40
Total Anthocyanin				0.50
Ionised Anthocyanin	**-0.65**	**-0.60**	**0.60**	-0.45
Total phenolics		**0.51**		**0.60**
Cl	-0.42			

H. Discussion and Summary

The Shiraz trial was designed to evaluate the field performance in Padthaway of the best performing rootstock genotypes from a single family tested for drought tolerance, under nursery conditions in the previous project (CSP 0503). It also included own roots and five commercial rootstocks (Dog Ridge, 140 Ruggeri, 1103 Paulsen, 101-14, Merbein 5489Φ, Merbein 5512Φ, Merbein 6262Φ) and M920, a cross involving *Vitis rotundifolia.* A systematic approach was used to select the most promising rootstocks genotypes based on their field performance and fruit composition. Key criteria used in this process were ability of rootstock genotypes to maintain acceptable productivity under supplementary irrigation with moderately saline water with low pH and malate levels and high berry anthocyanin. In 2017, small-scale wines were produced from the 10 most promising selections with the aim to identify selections for further development. For Shiraz vines growing in the Padthaway region this unique study has identified a number of promising new rootstock genotypes that warrant further development, it has also identified key relationships relating vine growth characteristics to fruit and wine composition.

Major findings from this study included:

- Confirmation that rootstocks provide a powerful tool to manipulate vine growth characteristics with implications for fruit and wine composition and carbon and water use efficiency. Across the 59 rootstock genotypes there were 8.5-fold differences in conferred vigour (pruning weight), 6.7 fold differences yield, 3.3-fold differences in the Ravaz index (yield to pruning weight ratio), 2.2-fold differences in bunch number, 2.8-fold differences in bunch weight and 1.5-fold differences in berry weight.
- Fruit composition variables were significantly impacted by rootstock genotype, except tartrate. The results showed significant potential to select rootstocks with lower pH, malate and YAN than most standard rootstocks currently grown with high levels of anthocyanin and phenolics. This was confirmed in winemaking studies undertaken in 2017.
- Confirmation that rootstocks are a powerful tool to manipulate berry juice mineral ion content with 1.5-3 fold differences in B, Ca, K, Mg, P, Na and S. This is particularly relevant for K, the dominant ion in juice which has been linked to negative wine attributes (see below) as many of the CSIRO selections had concentrations below the most widely adopted standard rootstocks including 1103 Paulsen, 140 Ruggeri and 101-14.
- Demonstrated that short term drought tolerance of rootstock genotypes during ripening, determined by canopy loss and leaf burn in 2017, was positively linked to conferred vine vigour and yield. This suggests that rootstocks with high vigour are likely to have deeper root systems that can access water deeper in the profile, despite having higher potential transpiration associated with larger canopies.
- Demonstrated that vine growth characteristics have a major impact on fruit and wine composition. Consequently, rootstocks which have significant effects on vine growth characteristics, provide growers with powerful tools to enhance wine quality attributes. Outcomes from key relationships in order of importance show that:
    - rootstocks with large berries are likely to have high berry juice pH and malate and lower tartrate, YAN, anthocyanin, phenolics, Ca, Mg and S; high must pH, and malate and low TA; high wine alcohol and hue (less bright) and low ionised anthocyanin.

- rootstocks with high conferred vigour are likely to have high berry juice pH, malate and K and low anthocyanin, phenolics, Ca, Mg and Na; high must pH and low TA; high wine TA and hue.
- rootstocks with large bunches are likely to have high berry juice pH and malate and low TA, tartrate, YAN, anthocyanins, phenolics, Ca, Mg, P and Na and high must TA.
- high yielding rootstocks are likely to have higher pH and malate and low TA, tartrate, anthocyanin, phenolics, Ca, Mg and Na; high must TA and low wine hue (i.e. brighter wine).
- rootstocks with high bunch numbers are likely to have higher pH and malate and low TA, anthocyanin, phenolics, Ca, Mg and Na and wine pH.
- vine balance, as determined by the Ravaz index (yield/pruning weight) was a useful indictor of fruit composition as it was the only parameter linked with fruit maturity with a negative association with TSS. It was also positively associated with juice TA, Ca and must TA. Interestingly the Ravaz index was not associated with berry anthocyanin or phenolics or wine spectral properties.
- Drought sensitive rootstocks, which had high canopy loss during ripening in 2017 were likely to have high YAN and anthocyanin in berries, low must pH and and low wine alcohol and hue. Similarly, leaf burn was associated with high berry tartrate and YAN, low must pH and high TA and in wine, low alcohol and hue and high ionised anthocyanin.

- Identified key relationships between fruit and wine composition variables across rootstock genotypes. These included:
  - Strong positive associations between berry juice TSS and pH, malate, tartrate, YAN, and anthocyanin, B, K, Mg and S. Such results that TSS should be included as a covariate in ANOVA analysis of rootstock effects on fruit composition to remove maturity effects. Across genotypes selected for winemaking in 2017, TSS was negatively associated with berry anthocyanin and phenolics; positively correlated with must TSS and malate and wine alcohol but negatively correlated with colour density and ionised anthocyanin.
  - pH was positively associated across rootstock genotypes with malate (very strong, $r = 0.79$), tartrate, B, K, Na and YAN and negatively associated with TA. The linkage with tartrate disappeared when corrected for TSS. Across genotypes selected for winemaking in 2017, juice pH was positively associated

with berry malate and K and negatively associated with TA, anthocyanin and phenolics and positively correlated with must TSS and malate.

- TA was positively associated across rootstock genotypes with tartrate rather than malate and negatively associated with pH (TSS adjusted), K and S which both disappeared when TSS adjusted. Across genotypes selected for winemaking in 2017, berry juice TA was positively correlated with berry malate, tartrate, YAN, phenolics and wine TA, colour density, ionised anthocyanin and total phenolics, Berry TA was negatively linked to wine pH.
- Malate was positively associated across rootstock genotypes with YAN, TSS adjusted pH, B, K and Na and negatively correlated with anthocyanin and phenolics and Ca. Across genotypes selected for winemaking in 2017 juice malate was positively correlated with must malate and in wine TA, total anthocyanin and total phenolics.
- Tartrate was positively associated across rootstock genotypes with YAN, anthocyanin and phenolics, B, K and Mg. Across genotypes selected for winemaking in 2017, juice tartrate was positively correlated with wine TA and total phenolics.
- YAN was positively associated across rootstock genotypes with anthocyanin and phenolics, B, Ca, K, Mg, Na and S. Across genotypes selected for winemaking in 2017, YAN was positively correlated with wine colour density and ionised anthocyanin and negatively correlated with hue.
- Positive associations between K, the dominant cation and TSS, pH, malate, tartrate and YAN which were retained after TSS adjustment. This result indicates that the relationships can be attributed to direct rootstock effects rather than to differences in maturity. Berry K was also positively correlated with must malate and wine TA.
- Anthocyanin and phenolics were negatively associated with malate but positively associated with tartrate and YAN. These relationships warrant further investigation to explore linkages with organic acid composition and berry anthocyanins and phenolics. Across genotypes selected for winemaking in 2017, berry anthocyanin and phenolics were negatively linked to must TSS, pH (anthocyanin only) and malate and wine alcohol but positively correlated with ionised anthocyanin in wine.

- Adoption of a systematic screening approach, based on performance and fruit composition over four seasons, successfully identified 10 new rootstock genotypes, capable of acceptable productivity, fruit and wine composition compared to most standard rootstocks when grown in the Padthaway region. Across the 10 selections there was a range in conferred vigour (0.7- 1.5 kg/v). Pruning weights of five selections were higher than own roots, which had the highest pruning weight. Pruning weights of the other five selections were all higher than Merbein 5489, which had the lowest pruning weight and similar to the other standard rootstocks. Yields of the selections identified for winemaking were in a similar range to own roots and the standard rootstocks, although yield selection M 230-70 (13.8 kg/v) was higher than 1103 Paulsen (11.2 kg/v). Almost all selections displayed a high degree of short term drought tolerance, particularly when compared to 101-14 which is widely planted in the region. Wine of all rootstocks, including the 10 selections had lower levels of Cl than own roots.

In summary it can be concluded that rootstock growth characteristics in the Padthaway region can be linked to must and wine quality attributes. In particular, the study has demonstrated negative effects of berry weight and to a lesser degree, conferred vigour and bunch weight on fruit composition. Rootstocks with large berries are likely to have high berry and must pH and malate and low tartrate, anthocyanin and phenolics and produce dull high hue wines with low ionised anthocyanin. The results show significant potential to identify new rootstocks with acceptable productivity, fruit and wine composition with good salt and short term drought tolerance. Further research is required to understand the effects of rootstock genotype on ripening processes and their effects on relationships between fruit composition parameters including mineral element and ion balance and the role of organic acids in berries.

2.3. Assessment of early screening methods

The aim of this project component was to assess the predictive value of simple traits measured previously with grafted vine performance in field trials. It aimed to test the efficacy of early screening assays undertaken in the previous project (CSP 0503) for vine growth characteristics, mineral element composition, in particular potassium uptake and response to drought and deficit irrigation. For this purpose, the study focussed on:

1. The performance of genotypes from a single family, F86 (a cross involving *V. cineria, V. champini, V. riparia* and *V. rupestris)* grafted with Shiraz which were evaluated as subsets of the trials involving well-watered and deficit irrigation treatments in a hot irrigated region at Irymple and at Padthaway, under milder conditions with supplemental irrigation described in 2.2.1 and 2.2.2 above. There were 35 genotypes of F86 common to the three sites. The deficit irrigation treatment received 45% of the well-watered control. This study only included data collected over four seasons, 2013-2016 because of the conversion to mechanical hedging in winter 2016 at the Irymple sites and potential issues with the short term drought stress at the Padthaway site in 2017.

2. The performance of genotypes from a single family involving a *V. cineria* x Riesling test population (F34) which was genotyped by next generation sequencing (see below) as part of this project; included in glasshouse K screening studies, and established in the field as ungrafted vines in a replicated trial at Irymple with well-watered control and deficit irrigation treatments. The deficit irrigation treatment received 45% of the well-watered control. Genotypes from this family were also included in abiotic stress screening studies established as part of CSP 1302.

2.3.1. Studies involving family F86

2.3.1.1. Linkages between simple traits and grafted field performance across three sites.

Mean data collected across the three trial sites (well-watered control and deficit irrigation at Irymple and at Padthaway) over four seasons (2013-2016) was used in this combined analysis. The results have been presented and published at the GiESCO symposium in Mendoza, Argentina, November 2017 by Clingeleffer, P., Morales, N and Smith, H. with the following title:

‘**Simple trait measurements across rootstock genotypes indicates performance as field grown, grafted vines.’** (Clingeleffer et al. 2017)

The GiESCO publication is included below.

Abstract

CSIRO rootstock breeding aims to provide industry with enhanced material to meet challenges associated with the Australian environment, future climate scenarios (high temperatures, limited water supply and drought) and soil-borne pests (phylloxera and root knot nematodes). In this study, analysis of results from three long term field trials with Shiraz grafted on genotypes from a single family involving *Vitis cineria*, *V. champini*, *V. riparia* and *V. rupestris* has shown that vine performance and fruit composition can be linked to simple traits, measured on field grown ungrafted seedlings or as ungrafted or grafted nursery grown plants. The field trials included full irrigation and deficit irrigation in a hot climate and supplementary irrigation in cooler region. The simple traits linked to vine performance in these trials included pruning weight and canopy condition after one or two seasons without irrigation and canopy height of field grown seedling vines; and for vines grown in a high density nursery shoot length, pruning weight, leaf transpiration efficiency, conductance, leaf temperature and ICP mineral element analysis of dried petioles. Across the three trials, significant multilinear regressions which included the measured traits accounted for a significant proportion of the variation across the rootstock genotypes including TSS (75%), malate (68%), pruning weight (66%), yeast assimilable nitrogen (62%), titratable acidity (51%), pH (38%), bunch number (38%), bunch weight (38%), tartrate (34%), berry weight (29%), anthocyanin (25%) and yield (25%). The results indicate that measurement of simple traits may provide tools for early screening of rootstock genotypes without establishment of large field trials. Together, the use of molecular markers for discrete traits combined with simple trait measurement tools provide breeders with a rapid evaluation system to breed new rootstocks.

Keywords: rootstocks, growth characteristics, fruit composition, root architecture, drought tolerance, deficit irrigation

Introduction

New rootstocks are required to meet the challenges associated with the Australian environment, future climate scenarios (high temperatures, limited water supply and drought) and soil-borne pests (phylloxera and root knot nematodes) (Clingeleffer et al.

2011). Rootstock development is both resource intensive and long term, because material must be screened for desired traits and their long term impact on vine performance and fruit and wine composition assessed in replicated field trials. Trait assessment may involve field assessment of seedlings for vigour and mineral status (Clingeleffer and Smith 2011), application of laboratory and glasshouse screens (e.g. for rooting ability, Smith et al. 2013, phylloxera resistance, Korosi et al. 2011, nematode resistance, Smith et al. 2014 or potassium uptake, Ruhl 1989, Smith et al. 2014). Standard and rapidly emerging molecular techniques such as genotyping-by-sequencing offer significant potential to develop marker-assisted selection (MAS) to accelerate plant breeding (He et al. 2014). However, molecular markers for the range of traits required for new rootstocks will require development and the approach may not be suited to complex traits. This study will assess the potential to measure simple traits to provide tools for early screening of rootstock genotypes, which in combination with future molecular marker selection may speed up the screening process and limit the number of genotypes to be evaluated in replicated field trials.

Materials and Methods

Vine performance data of grafted Shiraz was collected over four seasons (2013-16) from replicated trials planted in 2010 at three contrasting sites to assess new rootstock genotypes, including 35 genotypes from a single family involving *Vitis cineria*, *V. champini*, *V. riparia* and *V. rupestris*. Two sites were located in a hot irrigated region near Mildura, Victoria and irrigated with either 5.5 or 2.5 ML/ha/pa. The third site located in the cooler Padthaway region in South Australia was supplied with 1.0ML/ha/pa supplementary irrigation. Prior to the establishment of the trials, data had been collected for a range of simple traits from ungrafted field grown seedlings or from ungrafted and grafted vines in a replicated nursery trial at the former CSIRO Merbein site. Correlation and multilinear regression techniques were used to determine the predictive value of these simple traits with vine performance across the three trials (Table 1).

Traits measured for field grown seedlings, planted in 2002 and trained on a 1.0 m high bi-lateral cordon included:

1. Visual assessment of leaf loss (LL, 0-100%) across the season after one or two seasons without irrigation in 2008/09 and 2009/10.
2. Mid- summer canopy height (Ht) measured in 2007, range 1.1-1.8 m.

3. Dormant pruning weight (PW) measured in winter 2007 and 2008 (range 0.05-5.8 kg), after one or two seasons of drought in 2009 (0.02-6.0 kg) and 2010 (0.0-3.4 kg) respectively, and after recovery from drought in 2011 (0.04-7.7 kg).

Traits measured in the nursery trial, planted in spring 2006 and managed with vertical shoot positioning, included:

1. Carbon isotope discrimination (△) to assess transpiration efficiency of mature leaves (Gibberd et al. 2001) of ungrafted rootstock genotypes in 2007 (△, -27.6 to -30.2)
2. Shoot length (SL_{2007}) of ungrafted rootstock genotypes in 2007 (56.5-214.3 cm)
3. Shoot length (SL_{2008}) of grafted rootstock genotypes in 2008 (75.8-199.7 cm)
4. Dormant pruning weight of grafted rootstock genotypes with well-watered (PW_{ww}, 10.2-399.6 g) or with a water deficit irrigation (PW_{wd}, 15.0-76.0 g) in 2009.
5. Stomatal conductance (g_s) of grafted rootstock genotypes (g_s, 14.2-33.7 mmol $m^{-2}s^{-1}$), leaf temperature (LT, 22.1-33.7 ^oC), and leaf temperature differential from ambient (LTD, -4.6 to + 2.0 ^oC) measured with a Delta-T porometer for well-watered and deficit irrigation in 2009.
6. Dried petiole mineral analysis determined by ICP for ungrafted plants (2007), well-watered and deficit irrigation (2009). Ranges recorded for each element (mg/g) were B (0.04-0.58), Ca (7.4-19.3), K (11.0-68.3), Mg (1.67-10.3), Na (0.06-1.25), P (1.01- 6.21), S (0.49-2.98).

Results and discussion

Mean vine performance of grafted Shiraz, across the three sites and four seasons, showed significant differences associated with rootstock genotype in vigour (x6), yield (x3), bunch number (x2) and fruit composition (Table 1). Simple traits, which correlated with vine performance included late season LL, Ht, PW_{2010} and PW_{2011} determined for ungrafted seedlings (Table 2); $△_{ung}$, SL_{2007} SL_{2008}, PW_{ww}, PW_{wd}, gs_{ww}, LT_{ww}, LT_{wd} and LTD_{wd} (Table 3) and plant mineral status, B_{ung}, K_{ung}, Mg_{ung}, P_{ung}, K_{ww}, Na_{ww}, B_{wd}, Ca_{wd},, K_{wd} (Table 4), all measured for nursery plants.

Stepwise multilinear regression equations described the best combinations of the simple traits with strong predictive value of grafted vine performance. They showed that a significant proportion of the variability across the rootstock genotypes could be accounted for including:

- 75% ($p<0.000$) of TSS by $LL_{29/04/09}$, PW_{2011}, PW_{ww} and LT_{ww} and K_{ung} and K_{wd}

- 68% ($p<0.000$) of malate by $LL_{28/04/10}$, Ht, gs, LT_{ww} and K_{wd}
- 66 % ($p<0.000$) of pruning weight by $LL_{28/04/2010}$, Ht and gs_{ww}
- 62% ($p<0.000$) of YAN by gs_{ww}, $-LT_{wd}$, K_{ung} and K_{wd}
- 51% ($p<0.000$) of TA by Ht, gs_{ww} and $-Mg_{ww}$
- 38 % of pH by SL_{2008}, K_{ung} and K_{wd}
- 38% ($p=0.004$) of bunch number by $\triangle$, $-Ca_{ww}$ and $-Ca_{wd}$
- 38% ($p=0.004$) of bunch weight by gs_{ww}, Na_{ung} and B_{wd}
- 34% ($p=0.003$) of tartrate by $LL_{28/04/2010}$ and gs_{ww}
- 29% of berry weight by Na_{ww} and K_{wd}
- 25% ($p=0.011$) of anthocyanin by PW_{2010} and $-\triangle$
- 25% ($p=0.012$) of yield by $\triangle$ and $-Ca_{wd}$.

Of the traits measured, stomatal conductance of well-watered grafted vines in the nursery was the most powerful, being linked to 9/14 measures of vine performance (Table 3). While it has been reported that rootstocks may significantly affect g_s (Edwards et al. 2014), further physiological research is required to elucidate the positive links with conferred vigour leading to high pruning weight and larger bunches, and early ripening (high TSS and pH) and high and increased TA, malate, tartrate, and YAN. Leaf temperature, measured concurrently with g_s, also added value with LT_{ww} linked positively with TSS and malate and LT_{wd} linked negatively to YAN, presumably due to effects of rootstock on transpirational cooling. Transpiration efficiency, $\triangle$, shown previously to vary across rootstocks (Gibberd et al. 2001, Edwards et al. 2014) proved to be useful being the only trait, other than Ca, linked to yield and bunch number. $\triangle$ was also linked negatively with anthocyanin. The positive correlation between late season leaf loss and TSS may, at least in part be explained by effects of rootstock on precocity however the links with pruning weight and related conferred vigour effects on malate and tartrate (Walker and Clingeleffer 2009, 2016) require further investigation. Of the vigour related traits measured, nursery pruning weights, particularly with deficit irrigation were useful predictors of vine performance being linked to 8/14 performance parameters (Table 3) including those associated with conferred vigour (pruning weight and berry weight) and related effects of vigour on fruit composition (Clingeleffer, 2000, Walker and Clingeleffer 2009, 2016). This was not unexpected as nursery pruning weights had been linked to vine performance in other studies (Clingeleffer and Smith 2011). However, in the multilinear regression analyses, other vigour related traits were stronger factors, including canopy Ht (linked to pruning weight, malate and TA),

PW_{2011} (linked to TSS), PW_{2010} (linked to anthocyanin) and SL_{2008} (linked to pH). The importance of vine K status (K_{ung} and K_{wd}), linked to berry weight, TSS, pH, malate, YAN, were not unexpected as vine K status has previously been identified as playing a key role in fruit composition (Clingeleffer 1996, Clingeleffer 2000, Clingeleffer and Smith 2011, Walker and Clingeleffer 2016). The negative relationships with Ca (Ca_{ww} and Ca_{wd}) and bunch number (and yield), Mg_{ww} and TA, and positive relationships between Na_{ung} and B_{wd} and bunch weight and Na_{ww} warrant further investigation.

Conclusions

The results of this study indicate that measurement of simple traits may provide tools for early screening of rootstock genotypes. Measurement of these simple traits, in combination with future molecular marker selection, should provide a basis for rapid, efficient screening of rootstock genotypes and limit the number of genotypes to be evaluated in replicated field trials.

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Table 1. Performance of Shiraz grafted on 35 rootstock genotypes, based on means across three sites over four seasons (2013-2016).

	Overall mean	minimum	maximum	variation
Yield (kg/vine)	6.72	3.32	9.81	3-fold
Bunches per vine	72.6	50.1	97.4	2-fold
Berry weight (g)	1.14	1.03	1.23	23%
Bunch weight (g)	92.8	70.7	114.8	62%
Berries per bunch	81.5	61.3	96.7	58%
Pruning weight (kg)	1.98	0.63	3.51	6-fold
TSS (°Brix)	24.7	22.8	26.1	14%
pH	4.16	3.99	4.28	27%
Titratable acidity (g/L)	3.50	3.07	3.96	29%
Malate (g/L)	3.19	2.38	4.00	68%
Tartrate (g/L)	5.77	5.16	6.15	19%
YAN (mg/L)	207	153	273	78%
Anthocyanin (mg/g)	1.43	1.27	1.67	31%
Phenolics (au)	1.70	1.52	1.89	24%

Table 2. Correlation coefficients (r) between field assessment of ungrafted rootstock genotypes and vine performance and fruit composition of grafted Shiraz (n=35, correlations, $p < 0.05$ shown in bold).

	Leaf loss (LL)			Height	Pruning Weight	
	29/04/09	15/03/10	28/04/10	2007	2010	2011
Yield						
Bunches						
Berry weight	0.29		**0.35**			
Bunch weight				**0.42**		
Berry/bunch						
Pruning weight			**0.33**	**0.40**		
TSS	**0.49**		**0.32**			0.30
pH	**0.41**					
TA				**0.45**		
Malate	**0.37**	**0.33**	**0.46**	**0.36**		
Tartrate		**0.32**				
YAN	**0.34**		**0.39**			
Anthocyanin					**0.37**	
Phenolics					**0.34**	0.28

Note: For simplification, cells and columns have been removed where correlations were not significant ($p > 0.1$) including other columns for leaf loss and pruning weight (2007, 2008, 2009).

Table 3. Correlation coefficients (r) between nursery assessment of ungrafted and grafted rootstock genotypes and vine performance and fruit composition of grafted Shiraz (n=35, significant correlations, p< 0.05 shown in bold).

	Δ[1]	*Shoot length (SL)*		*Pruning weight (PW)*		*g_s*	*Leaf Temperature*		*Temp. diff.*
	2007	2007	2008	2009		2009	2009		2009
	ungrafted					grafted			
Irrigation	**ww**	**ww**	**ww**	**ww**	**wd**	**ww**	**ww**	**wd**	**wd**
Yield	**0.33**								
Bunches	**0.34**								
Berry wt.					**0.43**				
Bunch wt.						**0.40**			
Berr/bun									
Prun. wt.				**0.52**	**0.67**	**0.66**	0.28	-0.31	0.31
TSS		**0.33**	0.31	**0.35**	**0.49**	**0.48**	**0.35**		0.31
pH			0.32		0.32	**0.33**			**0.37**
TA				**0.34**	**0.35**	**0.45**		**-0.33**	
malate					**0.48**	**0.49**	0.29		0.28
tartrate					**0.41**	**0.49**			0.29
YAN					**0.44**	**0.57**	0.34	-0.29	
anthocyanin phenolics	-0.31	0.28				0.28			

[1] Carbon isotope discrimination. Note: For simplification, cells and columns have been removed where correlations were not significant (i.e. p >0.1). These included columns for stomatal conductance under deficit irrigation (wd) and temperature differential for the well-watered (ww) irrigation treatment.

Table 4. Correlation coefficients (r) between mineral element composition under nursery conditions of ungrafted and grafted rootstock genotypes and vine performance and fruit composition of grafted Shiraz (n=35, significant correlations, p< 0.05 shown in bold).

	Ungrafted 2007				*Well-watered 2009*			*Water Deficit 2009*		
	B	**K**	**Mg**	**P**	**Ca**	**K**	**Na**	**B**	**Ca**	**K**
Yield	-0.28				**-0.36**			0.30	**-0.41**	
Bunches					**-0.35**				**-0.44**	
Bunch wt.	-0.29				-0.31		**0.33**	**0.33**		
Berry wt.						**0.42**	**0.37**			0.29
Berr/bun								**0.36**	-0.28	
Prun. wt.	**-0.33**									0.28
TSS		**0.46**				0.32		-0.32		**0.37**
pH		**0.42**				**0.34**		-0.28		**0.39**
TA			**0.33**	**0.35**						
Malate		0.30				**0.38**	0.28			**0.44**
Tartrate	0.29					**0.34**	0.28	**-0.36**		
YAN		**0.37**				**0.39**		-0.29		**0.37**
anthocyanin phenolics							-0.30	**-0.33**		

2.3.1.2. Comparison of rootstock genotype performance between sites to assess Genotype x Environment (GxE) interactions with grafted Shiraz

Mean, minimum and maximum values for vine growth characteristics and fruit composition of Shiraz grafted on F86 genotypes at individual sites is shown in Table 2.3.1.2.1. The results highlight the effect of different environments on vine growth characteristics and fruit composition across the rootstock genotypes. Mean, minimum and maximum yield at site 1with control irrigation and site 3 in Padthaway, were similar, but lower at site 2 with the deficit irrigation treatment. Mean bunch numbers were highest at site 3 and lowest at site 1, although the maximum and minimum bunch numbers were recorded at site 2. This may be due to enhanced shoot fruitfulness associated with improved light environment of vines with higher conferred vigour under the deficit treatment. Mean, minimum and maximum berry weights were highest at site 1 and lowest at Padthaway, site 3. Mean bunch weights were similar at site 1 and 3 but lower at site 2 although both the minimum and maximum bunch weights were highest at site 1. Mean berries per bunch were similar across the three sites, although the mean and maximum berries per bunch were recorded at site 3. Mean pruning weight was highest at site 1 and lowest at site 3 in Padthaway which also had the lowest minimum and maximum values. Compared to the control irrigation (site 1), deficit irrigation (site 2) had lower mean, minimum and maximum pruning weights. Fruit maturity, determined by berry juice TSS was highest at site 2 and lowest at site 3 for the mean, minimum and maximum values. Mean pH was highest at site 2 and lowest at site 3 although the maximum and minimum pH values were recorded at site 1. Mean TA was highest at site 2 and lowest at site 3, although site 1 had the maximum value and site 2 the lowest minimum value. Mean malate levels were highest at site 1 and lowest at site 3. The minimum and maximum malate levels were also highest at site 1. Mean, minimum and maximum tartrate levels were similar at all sites, indicating a strong genetic component unaffected by environment. Mean YAN levels were highest at site 1 and lowest at 3. The minimum values at all sites, equal to or below 150, indicate potential issues with fermentation and a requirement for addition of diammonium phosphate (DAP). Site 1 had the maximum level of YAN. Mean, minimum and maximum anthocyanin and phenolics were highest at site 3 and lowest at site 1, despite the higher yields at site 3.

Table 2.3.1.2.1. Minimum, maximum and mean growth characteristics and berry composition data of Shiraz grafted on F86 rootstock genotypes at three sites (well-watered control and deficit irrigation treatments in a hot climate and at Padthaway) over four seasons (2013-2016).

	Site 1 (Control)			***Site 2 (Deficit)***			***Site 3 (Padthaway)***		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
Yield (kg)	3.4	12.5	**6.7**	2.1	10.7	**6.2**	2.9	13.6	**6.9**
Bunches	30	105	**62**	26	121	**67**	56	117	**87**
Berry wt. (g)	1.09	1.42	**1.30**	0.88	1.31	**1.20**	0.71	1.09	**1.17**
Bunch wt. (g)	78	130	**108**	44	119	**92**	36	117	**110**
Berr/bun	62	100	**83**	51	102	**77**	41	110	**82**
Prun. wt. (kg)	0.77	6.88	**2.97**	0.17	4.24	**2.15**	0.14	1.85	**0.78**
TSS (^{o}Brix)	21.6	27.4	**24.9**	22.2	28.9	**26.0**	20.8	25.9	**23.2**
pH	3.80	4.54	**4.21**	4.12	4.47	**4.30**	3.83	4.20	**3.97**
TA (g/l)	3.09	5.35	**3.80**	2.13	3.86	**4.33**	3.12	3.82	**3.44**
Malate (g/L)	2.54	5.64	**3.94**	2.52	4.33	**3.40**	1.75	2.61	**2.21**
Tartrate (g/L)	4.69	6.81	**5.61**	4.68	6.64	**5.85**	5.13	6.86	**5.83**
YAN (mg/L)	138	396	**236**	150	274	**214**	135	232	**171**
Antho. (mg/g)	0.86	1.53	**1.17**	0.95	1.84	**1.31**	1.32	2.24	**1.82**
Phen. (a.u.)	1.18	1.93	**1.52**	1.44	2.00	**1.66**	1.33	2.25	**1.94**

In the study, Shiraz vine performance traits (i.e. means of growth characteristics and fruit composition across four seasons) were measured for the 35 F86 rootstock genotypes at the three sites. Correlation analyses were conducted to compare vine performance of the rootstock genotypes at each site and identify stable traits under genetic control that are less likely to be impacted by the environment. The comparison of sites 1, with well-watered control irrigation, and site 2 with deficit irrigation were conducted under the same climatic conditions, hence differences in vine performance can only be attributed to the effect of irrigation treatment. Significant correlations for vine performance traits between site 1 and site 2 indicate that the effects of rootstock genotype on yield, bunch number, conferred vigour (pruning weight), TSS, malate, tartrate and YAN were relatively stable across the different irrigation treatments (Table 2.3.1.2.1). The highly significant correlation for malate ($p<0.001$) and significant correlations for pruning weight and TSS ($p<0.010$) indicate strong genetic control of these traits. The absence of significant correlations for berry weight, bunch weight, berries per bunch, pH, TA, anthocyanin and phenolics indicate that these traits were significantly impacted by the irrigation treatment. The comparison between site 1 and site 3, where the vines were grown under different climatic and management conditions indicate that only pruning weight and malate were less likely to be impacted by the environment and under strong genetic control. Similarly, the comparison of site 2 and site 3, with different climate and management but both

having limited water supply (i.e. deficit irrigation at site 2 and supplementary irrigation at site 3) has shown that pruning weight and malate were again strongly correlated across sites and likely to be under strong genetic control. Furthermore, the significant correlations for berry weight and pH between these lower vigour sites suggest some genetic control possibly associated directly with response to stress or indirectly to an improved light environment.

In summary it can be concluded that most vine performance traits are significantly impacted by environmental conditions. Two performance traits, pruning weight (conferred vigour) and malate, which are highly generally highly correlated appear to be under strong genetic control as they were stable across environments, a factor which warrants further investigation with ramifications for future rootstock selection and development.

Table 2.3.1.2.2. Correlations between sites for vine growth characteristics and fruit composition across F36 genotypes (n=35). Values retained are significant (p>0.05).

	Correlations between sites		
	Site 1 and 2	Site 1 and 3	Site 2 and 3
Yield	**0.39**		
Bunches	**0.33**		
Berry wt.			**0.40**
Bunch wt.			
Berr/bun			
Prun. wt.	**0.47**	**0.51**	**0.45**
TSS	**0.46**		
pH			**0.40**
TA			
malate	**0.62**	**0.46**	**0.51**
tartrate	**0.33**		
YAN	**0.34**		
anthocyanin			
phenolics			

2.3.1.3. Site 1. Correlations of grafted Shiraz vine performance with simple traits measured in previous projects for Family 86

The performance of the Shiraz grafted on 36 F86 rootstock genotypes was assessed, at site 1 under well-watered conditions over four seasons (2013-2016). A summary of the mean data is provided in Table 2.3.1.2.1 above. Mean vine performance of grafted Shiraz at site 1, showed significant differences associated with rootstock genotype in yield (3.7-fold), bunch number (3.5-fold), berry weight (30%), bunch weight (1.7-fold), berries per bunch (61%), pruning weight (8.9-fold), TSS (21.6 - 27.4 ^{o}Brix), pH (3.80 - 4.54), TA (1.7-fold), malate (2.2-fold), tartrate (1.5-fold), YAN (2.9-fold), anthocyanin (1.8-fold) and phenolic substances (1.6 fold).

Under the well-watered conditions at site 1 there were few, but weak correlations with canopy condition at different stages of drought stress and grafted vine performance (Table 2.3.1.3.1). In the first season of drought, leaf loss of ungrafted vines on 01/03/2009 correlated negatively with mean berry weight, anthocyanin and phenolics and on 29/04//09 correlated positively with bunch weight and TSS. In the second season of drought, leaf loss on 15/03/2010 correlated positively with bunch weight and berries per bunch and on 28/04/2010 with bunch weight, pruning weight, malate and YAN.

Table 2.3.1.3.1. Correlation coefficients (r) between field assessment of canopy condition of ungrafted rootstock genotypes and vine performance and fruit composition of grafted Shiraz grown under well-watered conditions (n=36). Correlations, $p< 0.05$ shown in bold. Non significant correlations have been removed ($p>0.1$)

	Canopy leaf loss (%)			
	01/03/09	29/04/09	15/03/10	28/04/10
Yield				
Bunches				
Berry wt.	-0.28			
Bunch wt.		0.26	**0.34**	0.27
Berr/bun			0.28	
Prun. wt.				0.29
TSS		0.28		
pH				
TA				
malate				**0.34**
tartrate				
YAN				0.32
anthocyanin	-0.29			
phenolics	-0.31			

At site 1, there were few, but weak correlations with root architecture, pruning weight, trunk diameter or canopy height of ungrafted seedling vines established in the field and grafted vine performance (Table 2.3.1.3.2). With respect to root architecture, root diameter was positively correlated with pH and root angle was negatively correlated with TSS and anthocyanins of the grafted Shiraz. Pruning weight in 2007 was positively correlation with pH and in 2011, post recovery from two years without irrigation, with berry weight, TSS, anthocyanin and phenolics. Trunk diameter, recorded in 2011, was positively correlated with pH. Canopy height recorded in 2007 was positively correlated with TSS, pH, malate and YAN.

Table 2.3.1.3.2. Correlation coefficients (r) between field assessment of root architecture, pruning weight, trunk diameter and canopy height of ungrafted rootstock genotypes and vine performance and fruit composition of grafted Shiraz grown under well-watered conditions (n=36). Correlations, $p < 0.05$ shown in bold. Non significant correlations removed ($p > 0.1$).

	Root architecture			***Pruning weight***		***Trunk diameter***	***Canopy height***
	type	diameter	angle	2007	2011	2011	2007
Yield							
Bunches							
Berry wt.					**0.31**		
Bunch wt.							
Berr/bun							
Prun. wt.							0.30
TSS			-0.26		0.30		
pH		0.30		**0.36**		**0.31**	0.30
TA							
malate							0.29
tartrate							
YAN							0.28
anthocyanin			-0.27		0.30		
phenolics					0.27		

At site 1 there were significant correlations with the assessments conducted in the nursery environment and grafted vine performance across the 36 genotypes (Table 2.3.1.3.3). Carbon isotope discrimination, a measure of water use efficiency conducted on ungrafted plants in 2007 was positively correlated with yield, bunch number, berries per bunch and negatively correlated with pruning weight, TA, malate and YAN. There were no correlations of ungrafted shoot length in 2007 and field performance, whereas shoot length of grafted plants in 2008 was positively correlated with TSS, anthocyanin and

phenolic substances. Conductance measured in 2009 for well-watered vines in the nursery was a very useful trait being positively correlated with pruning weight ($p<0.001$), TSS, TA ($p<0.001$), malate ($p<0.01$), tartrate ($p<0.001$), YAN ($p<0.01$), anthocyanin ($p<0.010$) and phenolics and negatively correlated with yield and bunch number. There were no correlations with conductance of the deficit treatment and field performance as conductance across the genotypes were all low (data not shown). Leaf temperature, particularly under deficit irrigation in the nursery, was also a very useful trait. Under well-watered conditions, leaf temperature was positively correlated with pruning weight, TSS, TA, malate, tartrate and YAN and negatively correlated with yield and bunch number. In contrast, under deficit irrigation leaf temperature was positively correlated with yield, bunch number and berries per bunch and negatively correlated with berry weight, pruning weight, TA ($p<0.010$), malate, tartrate, YAN ($p<0.01$) and anthocyanin. Pruning weight of grafted vines in the nursery was also a useful trait, particularly with the deficit treatment. Under well-watered conditions, pruning weight was positively correlated with pruning weight of the grafted vines in the field ($p<0.010$) and TSS and negatively correlated with yield and bunch number. With the deficit treatment pruning weight was positively correlated with pruning weight of the grafted vines in the field ($p<0.001$), TSS, TA, malate ($p<0.001$), tartrate, YAN ($p<0.01$), anthocyanin ($p<0.01$) and phenolics ($p<0.01$) and negatively correlated with yield and bunch number.

Table 2.3.1.3.3. Correlation coefficients (r) between a range of nursery assessments of ungrafted and grafted rootstock genotypes and vine performance and fruit composition of grafted Shiraz grown under well-watered conditions (n=36). The assessments conducted in the nursery included carbon isotope discrimination (delta) of ungrafted plants in 2007, shoot length of ungrafted and grafted plants in 2007 and 2008, and conductance, leaf temperature and pruning weight, of well-watered or deficit plants in 2009. Correlations, $p < 0.05$ are shown in bold. Non significant correlations removed ($p > 0.1$).

	Delta	***Shoot Length***		***Conductance***	***Leaf temp.***		***Pruning wt.***	
Year	2007	2007	2008	2009	2009	2009	2009	2009
Irrigation	Full	Full	Full	Full	Full	Def.	Full	Def.
Yield	**0.36**			**-0.37**	**-0.32**	0.31	**-0.34**	**-0.36**
Bunches	**0.32**			**-0.39**	-0.28	0.27	**-0.36**	**-0.38**
Bunch wt.								
Berry wt.						-0.26		
Berr/bun	**0.35**					**0.37**		
Prun. wt.	**-0.32**			**0.57**	0.31	**-0.43**	**0.49**	**0.66**
TSS			0.29	**0.33**	**0.33**		**0.32**	0.30
pH								
TA	-0.28			**0.54**	0.30	**-0.44**		**0.40**
malate	**-0.36**			**0.46**	0.30	**-0.39**		**0.52**
tartrate				**0.56**	0.30	-0.31		**0.34**
YAN	**-0.36**			**0.49**	**0.35**	**-0.44**		**0.48**
anthocyanin			**0.39**	**0.44**		**-0.32**		**0.45**
phenolics			**0.40**	**0.38**				**0.44**

At site 1 there were significant correlations with leaf mineral concentrations measured in the nursery environment and grafted vine performance across the 36 genotypes (Table 2.3.1.3.4). With ungrafted plants in 2007, Ca was negatively correlated with yield, bunch number and berries per bunch, K was positively correlated with TSS, Mg was negatively correlated with yield and bunch number and P was positively correlated with TA and negatively correlated with bunch weight and berry weight. With grafted plants under well-watered conditions in the nursery in 2009, Ca was negatively correlated with yield, bunch weight, berry weight and berries per bunch, K was positively correlated with bunch weight, berry weight, malate, tartrate and YAN. With grafted plants under deficit conditions in the nursery in 2009, B was negatively correlated with pruning weight, TA, malate, tartrate and YAN, K was positively correlated with berry weight, pruning weight, malate and YAN, Mg was negatively correlated with berry weight and Na was positively correlated with pH.

Table 2.3.1.3.4. Correlation coefficients (r) between a leaf nutrient status of ungrafted and grafted rootstock genotypes in the nursery and vine performance and fruit composition of grafted Shiraz grown under well-watered conditions (n=36). Correlations, $p < 0.05$ are shown in bold. Non significant correlations removed ($p > 0.1$).

	Grafted control 2009		*Grafted deficit 2009*				*Ungrafted 2007*			
	Ca	K	B	K	Mg	Na	Ca	K	Mg	P
Yield	-0.27						-0.30		-0.30	
Bunches							-0.29		-0.28	
Bunch wt.	**-0.42**	**0.33**								-0.29
Berry wt.	-0.26	**0.35**		0.28	**-0.39**					**-0.42**
Berr/bun	-0.28						-0.27			
Prun. wt.			-0.30	**0.36**						
TSS								**0.38**		
pH						**0.36**				
TA			**-0.32**							0.27
malate		**0.32**	-0.27	**0.49**						
tartrate		**0.45**	**-0.53**							
YAN		**0.32**	**-0.37**	**0.48**						
antho. phenolics										

Multilinear regression equations which described the best combinations of the simple traits with strong predictive value of grafted vine performance were developed. They showed that a significant proportion of the variability across the 36 rootstock genotypes of F86 could be accounted for using adjusted R^2 values including:

- 36% ($p=0.004$) of yield by leaf loss (29/04/2009) and in the nursery, leaf temperature under deficit irrigation, shoot length of grafted vines in 2008 and leaf K of ungrafted plants (all +ve)
- 41% ($p=0.005$) of bunch weight by leaf loss (29/04/2009), canopy height in 2007, and in the nursery leaf temperature and B with deficit irrigation and leaf P of ungrafted plants (all +ve) and trunk girth in the field in 2007 (-ve)
- 49% ($p<0.001$) of berries per bunch by leaf loss (29/04/2009), canopy height in 2007, and in the nursery, leaf temperature and B with deficit irrigation and leaf P of ungrafted plants (all +ve) and trunk girth in 2007 in the field and P of ungrafted plants in the nursery (both -ve)
- 61% ($p<0.001$) of pruning weight by conductance of well-watered plants, and K with deficit irrigation (both +ve) and leaf temperature with deficit irrigation (-ve) of nursery grown plants

- 37% (p=0.003) of TSS by leaf loss (28/04/10) and pruning weight 2011 of field grown plants and K of ungrafted plants and leaf temperature of well-watered vines in the nursery (all +ve)
- 15% (p=0.028) of pH by field pruning weight in 2007 and K of ungrafted plants in the nursery
- 60% (p<0.001) of TA by leaf loss (15/03/2010) and nursery conductance of well-watered vines, p of ungrafted plants (all +ve) and leaf temperature of deficit vines (-ve),
- 50% (p<0.001) of malate by conductance and K of deficit vines (both +ve) and leaf temperature of deficit vines (-ve) of nursery vines
- 50% (p<0.001) of tartrate by conductance (+ve) and B and leaf temperature of deficit vines (both -ve) of nursery vines
- 70% (p<0.001) of YAN by conductance of well-watered vines, K of ungrafted vines and K of deficit vines (all +ve) and B and leaf temperature and B of deficit vines (both -ve) in the nursery
- 56% (p<0.001) of anthocyanin by shoot length of grafted vines, and conductance of well-watered vines, (both +ve) and K of well- watered vines and leaf temperature of deficit vines (both -ve) in the nursery
- 26% (p=0.006) of phenolics by shoot length of grafted vines and conductance of well-watered plants in the nursery (both +ve).

2.3.1.4. Site 2. Correlations of grafted Shiraz vine performance with simple traits measured in previous projects for Family 86

The performance of the Shiraz grafted on 36 F86 rootstock genotypes was assessed under deficit irrigation over four seasons (2013-2016) at site 2 (Table 2.3.1.2.1 above). Mean vine performance of grafted Shiraz at site 2, showed significant differences associated with rootstock genotype in yield (5.1-fold), bunch number (4.6-fold), berry weight (1.5-fold), bunch weight (2.7-fold), berries per bunch (2-fold), pruning weight (25-fold), TSS (22.2- 28.9 ^{o}Brix), pH (4.10-4.57), TA (1.8-fold), malate (1.7-fold), tartrate (1.4-fold), YAN (1.8-fold), anthocyanin (1.9-fold) and phenolic substances (1.4 fold).

Under the deficit treatment at site 2, there were a number of strong correlations with canopy condition at different stages of drought stress and grafted vine performance (Table 2.3.1.4.1). At the end of the first season (29/04/2009), leaf loss of ungrafted vines correlated positively with TSS ($p<0.001$), pH, malate, tartrate and anthocyanin and negatively with yield and bunch number. In the second season of drought, leaf loss on 15/03/2010 correlated positively with TSS, malate, tartrate and YAN. On 28/04/2010 in that season leaf loss correlated positively with pruning weight, TSS, pH, malate, tartrate and YAN. These results, showing effects of leaf loss on fruit maturity and composition suggest that leaf loss may not only be associated with stress from deficit irrigation but also on precocity, leading to earlier ripening.

Table 2.3.1.4.1. Correlation coefficients (r) between field assessment of canopy condition of ungrafted rootstock genotypes and vine performance and fruit composition of grafted Shiraz grown with a water deficit (n=36). Correlations, $p< 0.05$ shown in bold. Non significant correlations have been removed ($p>0.1$)

	Canopy leaf loss (%)		
	29/04/09	15/03/10	28/04/10
Yield	-0.29		
Bunches	**-0.33**		**-0.37**
Berry wt.			
Bunch wt.			
Berr/bun			
Prun. wt.			0.26
TSS	**0.58**	**0.31**	**0.43**
pH	**0.37**		0.26
TA			0.29
malate	**0.41**	**0.42**	**0.55**
tartrate	**0.33**	**0.42**	**0.49**
YAN		**0.33**	**0.42**
anthocyanin	0.27		
phenolics			

At site 2 there were few but significant correlations with root architecture, pruning weight, trunk diameter or canopy height of ungrafted seedling vines and grafted vine performance (Table 2.2.1.4.2). Root type was positively correlated with yield and bunch number and negatively correlated with tartrate and YAN of grafted Shiraz. Root diameter was positively correlated with bunch number and pruning weight. Root angle was positively correlated with yield and negatively correlated with pH. Root number was positively correlated with pruning weight, TSS, and malate. Pruning weight in 2010, after two

seasons without irrigation, was positively correlated pruning weight, anthocyanin and phenolics. Trunk diameter in 2011 was negatively correlated with tartrate. Canopy height proved to be a useful trait, being positively correlated with bunch weight, pruning weight, TA ($p<0.01$) and malate.

Table 2.2.1.4.2. Correlation coefficients (r) between field assessment of root architecture, pruning weight, trunk diameter and canopy height of ungrafted rootstock genotypes and vine performance and fruit composition of grafted Shiraz grown with a water deficit (n=36). Correlations, $p< 0.05$ shown in bold. Non significant correlations removed ($p>0.1$).

	Root architecture				*Pruning weight*	*Trunk diameter*	*Canopy height*
	type	diameter	angle	number	2010	2011	2007
Yield	0.24		0.27				
Bunches	0.30	0.28					
Berry wt.							
Bunch wt.							0.30
Berr/bun							
Prun. wt.		0.27		0.26	0.30		**0.35**
TSS				0.26			
pH			**-0.34**				
TA							**0.51**
malate				**0.39**			**0.38**
tartrate	**-0.32**					**-.33**	
YAN	**-0.34**						
anthocyanin					0.29		
phenolics					**0.33**		

At site 2, with deficit irrigation, there were significant correlations with the assessments conducted in the nursery environment and grafted vine performance across the 36 genotypes (Table 2.2.1.4.3). Carbon isotope discrimination, a measure of water use efficiency conducted on ungrafted plants in 2007 was positively correlated with yield, bunch weight and berry weight and negatively correlated with anthocyanin. Ungrafted shoot length in 2007 was positively correlated with TSS and negatively correlated with yield, berry weight and berries per bunch. Shoot length of grafted plants in 2008 was positively correlated with pH and negatively correlated with bunch weight and berry weight. Conductance measured in 2009 for well-watered vines in the nursery was a very useful trait being positively correlated with berry weight, pruning weight, TSS, malate, tartrate and YAN. There were no correlations with conductance of the deficit treatment and field performance as conductance across genotypes was low (data not shown). Leaf

temperature was not a useful trait as a predictor of vine performance under deficit irrigation as it was only negatively correlated with berries per bunch under well-watered conditions. Under well-watered conditions in the nursery, pruning weight was positively correlated with bunch weight, pruning weights, pH, malate and tartrate. With the deficit treatment, pruning weight was positively correlated with pruning weight of the grafted vines in the field, TSS, pH, malate and tartrate.

Table 2.2.1.4.3. Correlation coefficients (r) between a range of nursery assessments of ungrafted and grafted rootstock genotypes and vine performance and fruit composition of grafted Shiraz grown with a water deficit (n=36). The assessments conducted in the nursery included carbon isotope discrimination (delta) of ungrafted plants in 2007, shoot length of ungrafted and grafted plants in 2007 and 2008, respectively, and conductance, leaf temperature and pruning weight, with or without deficit treatments in 2009. Correlations ($p < 0.05$) are shown in bold. Non significant correlations have been removed ($p > 0.1$).

	Delta	***Shoot Length***		***Conductance***	***Leaf temp.***		***Pruning wt.***	
year	**2007**	**2007**	**2008**	**2009**	**2009**	**2009**	**2009**	**2009**
Irrigation	**Full**	**Full**	**Full**	**Full**	**Full**	**Def.**	**Full**	**Def.**
Yield	**0.34**	**-0.31**						
Bunches								
Bunch wt.	**0.36**		-0.27				0.26	
Berry wt.	**0.32**	**-0.31**	-0.29	**0.35**				
Berr/bun		**-0.30**			-0.28			
Prun. wt.				**0.57**			**0.38**	**0.39**
TSS		0.30		**0.50**			**0.37**	**0.53**
pH			**0.48**	**0.41**				**0.37**
TA								
malate				**0.47**			0.27	**0.32**
tartrate				**0.40**			**0.36**	**0.44**
YAN				**0.43**				
anthocyanin	**-0.32**							
phenolics								

At site 2 there were significant correlations with leaf mineral concentrations measured in the nursery environment and grafted vine performance across the 36 genotypes (Table 2.2.1.4.4). With ungrafted plants in 2007, B was negatively correlated with TSS, pH, and anthocyanins; K was positively correlated with pruning weight, pH, malate, tartrate and negatively correlated with yield, bunch number, berries per bunch, and TSS; Na was

positively correlated with bunch number and negatively correlated with TSS and P was positively correlated with pruning weight and TA. With grafted plants under well-watered conditions in the nursery in 2009, Ca was negatively correlated with yield; K was positively correlated with TSS, pH, malate and YAN and Na was positively correlated with bunch weight, berry weight, malate, tartrate and YAN. With grafted plants under deficit conditions in the nursery in 2009, B was positively correlated with yield, bunch weight and berries per bunch and negatively correlated with TSS, pH, and anthocyanin; Ca was negatively correlated with yield and bunch number; K was positively correlated with TSS and Na was positively correlated with yield, bunch number and negatively correlated with anthocyanins and phenolics.

Table 2.2.1.4.4. Correlation coefficients (r) between a leaf nutrient status of ungrafted and grafted rootstock genotypes in the nursery and vine performance and fruit composition of grafted Shiraz grown with a water deficit (n=36). Correlations, $p < 0.05$ are shown in bold. Non significant correlations have been removed ($p>0.1$).

	Grafted control 2009				*Grafted deficit 2009*				*Ungrafted 2007*		
mean (n=36)	Ca	K	Na	B	Ca	K	Na	B	K	Na	P
Yield	**-0.29**			0.26	**-0.38**		**0.32**		**-0.42**		
Bunches					**-0.38**		**0.31**		**-0.39**	**0.37**	
Bunch wt.			**0.29**	**0.38**							
Berry wt.			0.26								
Berr/bun				**0.55**					**-0.31**		
Prun. wt.									0.24		0.26
TSS		**0.38**		**-0.29**		**0.28**		**-0.34**	**-0.37**	**-0.29**	
pH		**0.35**		**-0.41**				**-0.37**	**0.46**		
TA											**0.31**
Malate		**0.34**	**0.32**						**0.35**		
Tartrate			**0.39**						**0.42**		
YAN		0.25	**0.29**						**0.41**		
Antho.				**-0.36**			**-0.43**	**-0.28**			
Phenolics							**-0.36**				

Multilinear regression equations which described the best combinations of the simple traits with strong predictive value of grafted vine performance were developed. They showed that a significant proportion of the variability across the 36 rootstock genotypes of F86 could be accounted for using adjusted R^2 values including:

- 37% ($p=0.001$) of yield by shoot length and K of ungrafted plants and Ca of deficit plants (all -ve) grown in the nursery

- 28% (p=0.007) of bunch number by pruning weight of well-watered and deficit vines (both +ve) and K of ungrafted plants and Ca of deficit plants (both –ve) grown in the nursery
- 55% (p<0.001) of berry weight by canopy height in 2007 and in the nursery, delta of ungrafted plants, conductance and K of well-watered plants (all +ve) and shoot length of grafted plants (-ve)
- 10% (p=0.037) of bunch weight by delta (+ve) of ungrafted plants in the nursery
- 43% (p<0.001) of berries per bunch bunch weight by delta (+ve) of ungrafted plants and B of deficit vines in the nursery (both +ve)
- 38% (p=0.001) of pruning weight by delta of ungrafted plants and conductance of well-watered plants (both +ve) and shoot length of grafted vines (-ve) of nursery grown plants
- 60% (p<0.001) of TSS by leaf loss (29/04/09) and pruning weight in 2010 and in the nursery, K of ungrafted and well-watered plants (all +ve).
- 50% (p<0.001) of pH by shoot length of grafted plants and K of ungrafted plants (both +ve) and B of deficit vines (-ve) in the nursery
- 64% (p<0.001) of malate by leaf loss (28/04/2010) and canopy height in 2007 and in the nursery, conductance of well-watered vines and K of ungrafted vines (all +ve)
- 35% (p=0.001) of tartrate by leaf loss (28/04/2010) and in the nursery K of ungrafted plants and Na of deficit plants (all +ve)
- 23% (p=0.011) of YAN by leaf loss (15/03/2010) and in the nursery conductance of well-watered vines
- 31% (p=0.004) of anthocyanin by pruning weight in 2010 (+ve) and canopy height in 2007 (-ve) and in the nursery, delta of ungrafted vines and Na of deficit plants (both -ve)
- 18% (p=0.024) of phenolics by pruning weight in 2007 (+ve) and canopy height in 2007 (-ve) and in the nursery, Na of deficit plants (-ve).

2.3.1.5. Site 3. Correlations of grafted Shiraz vine performance with simple traits measured in previous projects for Family 86

The performance of the Shiraz grafted on 51 F86 rootstock genotypes was assessed over four seasons (2013-2016) at site 3, in the cooler Padthaway region where supplementary

irrigation with moderately high salt levels is applied during crop development. Mean vine performance of grafted Shiraz at site 3 (Table 2.3.1.2.1 above), showed significant differences associated with rootstock genotype in yield (4.7-fold), bunch number (2.1-fold), berry weight (1.5-fold), bunch weight (3.3-fold), berries per bunch (2.7-fold), pruning weight (13.2-fold), TSS (20.8-25.9 ^{o}Brix), pH (3.83-4.20), TA (1.2-fold), malate (1.5-fold), tartrate (1.3-fold), YAN (1.7-fold), anthocyanin (1.7-fold) and phenolics (1.7-fold).

At Padthaway, there were few but weak correlations with canopy condition at different stages of drought stress and grafted vine performance (Table 2.2.1.5.1). In the second season of drought, leaf loss on the 14/03/2010 correlated negatively tartrate, anthocyanin and phenolics; on 15/03/20010 leaf loss correlated negatively with anthocyanin while on 28/04/2010 leaf loss correlated positively with berry weight and bunch weight and negatively with tartrate, anthocyanin and phenolics.

Table 2.2.1.5.1. Correlation coefficients (r) between field assessment of canopy condition of ungrafted rootstock genotypes and vine performance and fruit composition of grafted Shiraz established in Padthaway (n=51). Significant correlations ($p< 0.05$) shown in bold. Non significant correlations have been removed ($p>0.1$)

	Canopy condition (% leaf loss)		
	14/01/10	15/03/10	28/04/10
Yield			
Bunches			
Berry wt.			0.25
Bunch wt.			0.22
Berr/bun			
Prun. wt.			
TSS			
pH			
TA			
malate			
tartrate	-0.25		-0.25
YAN			
Anthocyanin	**-0.32**	-0.24	**-0.38**
Phenolics	-0.27		**-0.33**

At site 3, there were few but weak correlations with root architecture, pruning weight, trunk diameter or canopy height of ungrafted seedling vines and grafted vine performance (Table 2.2.1.5.2). Root type was negatively correlated with berry weight, pH and malate; root diameter was positively correlated with pruning weight and root angle was positively correlated with anthocyanin and phenolics and negatively correlated with berry weight, bunch weight, pruning weight and malate of the grafted Shiraz.

Pruning weight in 2008 was positively correlated with TSS and YAN; after one season of drought in 2009 with pruning weight; after two seasons of drought in 2010 with pruning weight and anthocyanin, and after recovery from drought in 2011 with pruning weight (Table 2.2.1.5.2).

Table 2.2.1.5.2. Correlation coefficients (r) between field assessment of root architecture, pruning weight, trunk diameter and canopy height of ungrafted rootstock genotypes and vine performance and fruit composition of grafted Shiraz of grafted Shiraz established in Padthaway (n=51). Significant correlations ($p < 0.05$) shown in bold. Non significant correlations have been removed ($p > 0.1$).

	Root architecture			*Pruning weight*			
	type	diameter	angle	2008	2009	2010	2011
Yield							
Bunches							
Berry wt.	-0.25		-0.25				
Bunch wt.			-0.23				
Berr/bun							
Prun. wt.		**0.29**	-0.23		0.26	**0.28**	**0.30**
TSS				0.23			
pH	-0.24						
TA							
malate	-0.24		-0.26				
tartrate							
YAN				**0.29**			
anthocyanin			0.24			0.22	
phenolics			**0.31**				

At site 3 in Padthaway, there were significant correlations with the assessments conducted in the nursery environment and grafted vine performance across the 51 genotypes (Table 2.2.1.5.3). Carbon isotope discrimination, a measure of transpitation efficiency conducted on ungrafted plants in 2007 was positively correlated with bunch weight and berries per bunch. Shoot length of ungrafted plants in 2007 was positively correlated with

anthocyanin and phenolics. Shoot length of grafted plants in 2008 was positively correlated with pruning weight, TA and malate.

Conductance measured in 2009 for well-watered vines was positively correlated with pruning weight, and pH and negatively correlated with TA. There were no correlations with conductance of the deficit treatment and field performance as conductance across the genotypes was low (data not shown). Leaf temperature under a deficit irrigation treatment was positively correlated with pruning weight. Pruning weight of grafted vines in the nursery was also a useful trait with the deficit treatment. Under well-watered conditions pruning weight was positively correlated with bunch number. In contrast for deficit plants in the nursery there were positive correlations with yield and all its components, pruning weight, pH and malate.

Table 2.2.1.5.3. Correlation coefficients (r) between a range of nursery assessments of ungrafted and grafted rootstock genotypes and vine performance and fruit composition of grafted Shiraz of grafted Shiraz established in Padthaway (n=51). The assessments conducted in the nursery included carbon isotope discrimination (delta) of ungrafted plants in 2007, shoot length of ungrafted and grafted plants in 2007 and 2008, respectively, and conductance, leaf temperature and pruning weight, with or without deficit treatments in 2009. Significant correlations ($p < 0.05$) are shown in bold. Non significant correlations removed ($p > 0.1$).

	Delta	***Shoot Length***		***Conductance***	***Leaf temp.***		***Pruning wt.***	
year	**2007**	**2007**	**2008**	**2009**	**2009**	**2009**	**2009**	**2009**
Irrigation	**Full**	**Full**	**Full**	**Full**	**Full**	**Def.**	**Full**	**Def.**
Yield								**0.44**
Bunches							0.27	**0.43**
Bunch wt.	0.24							**0.29**
Berry wt.								**0.42**
Berr/bun	0.24							**0.44**
Prun. wt.			0.26	**0.36**		0.24		**0.50**
TSS								
pH				**0.35**				**0.30**
TA			0.27	**-0.31**				
malate			**0.33**					**0.39**
tartrate								
YAN								
anthocyanin		**0.35**						
phenolics		0.25						

For site 3 site in Padthaway, there were significant correlations with leaf mineral concentrations measured in the nursery environment and grafted vine performance across the 51 genotypes (Table 2.2.1.5.4). With ungrafted plants in 2007, K was positively correlated with pH and negatively correlated with TA and Mg was positively correlated with TA and tartrate. With grafted plants under well-watered conditions in the nursery in 2009, B was positively correlated with TA and negatively correlated with pH; Ca was negatively correlated with yield, bunch weight and berry weight; Na was positively correlated with yield, bunch weight, berry weight and pruning weight and malate and negatively correlated with anthocyanin and phenolics; and P was negatively correlated with tartrate and YAN. With grafted plants under deficit conditions in the nursery in 2009, K was positively correlated with yield and all its components, pruning weight, pH and malate and negatively correlated with TA; Mg was positively correlated with TA, Na was positively correlated with pruning weight and malate and negatively correlated with YAN, anthocyanin and phenolics.

Table 2.2.1.5.4. Correlation coefficients (r) between a leaf nutrient status of ungrafted and grafted rootstock genotypes in the nursery and vine performance and fruit composition of grafted Shiraz of grafted Shiraz established in Padthaway (n=51). Significant correlations ($p < 0.05$) are shown in bold. Non significant correlations removed ($p > 0.1$).

	Grafted control 2009				*Grafted deficit 2009*			*Ungrafted 2007*	
mean (n=51)	B	Ca	Na	P	K	Mg	Na	K	Mg
Yield		**-0.28**	0.24		**0.33**				
Bunches		**-0.32**			**0.33**				
Bunch wt.		**-0.28**	0.27		**0.33**				
Berry wt.		**-0.35**	**0.34**		**0.29**				
Berr/bun					**0.31**				
Prun. wt.			0.25		**0.32**		**0.32**		
TSS									
pH	**-0.29**				**0.46**			**0.33**	
TA	**0.28**				**-0.30**	0.28		-0.26	0.28
Malate			**0.31**		**0.39**		0.24		
Tartrate				-0.27					**0.39**
YAN				**-0.39**			-0.29		
Antho.			**-0.42**				**-0.31**		
Phenolics			**-0.35**				-0.26		

Multilinear regression equations which described the best combinations of the simple traits with strong predictive value of grafted vine performance were developed. They showed that a significant proportion of the variability across the 36 rootstock genotypes of F86 could be accounted for using adjusted R^2 values including:

- 35% ($p<0.001$) of yield by nursery measurement of pruning weight of deficit vines and Ca of well-irrigated vines (both +ve)
- 36% ($p<0.001$) of bunch number by nursery assessment of pruning weight of deficit plants and Ca of well-watered plants (both +ve)
- 20% ($p=0.002$) of berry weight by nursery measurement of pruning weight of deficit plants and Na of well-watered plants (both +ve)
- 32% ($p<0.001$) of bunch weight by nursery assessment of pruning weight of deficit plants (+ve) and Ca of well-watered plants (both -ve)
- 29% ($p<0.001$) of berries per bunch by nursery assessment of pruning weight of deficit plants (+ve) and Ca of well-watered plants (-ve)
- 37% ($p<0.001$) of pruning weight by nursery assessment of pruning weight and Na of deficit plants (both +ve)
- 41% ($p<0.001$)) of pH by nursery assessment of conductance of well-watered plants and K of deficit plants (both +ve)
- 46% ($p<0.001$) of TA by nursery assessment of shoot length of grafted plants (+ve) and, conductance and Ca of well-watered plants and K of deficit plants and Na of well-watered plants (all -ve)
- 25% ($p<0.001$) of malate by nursery assessment of pruning weight and K of deficit plants and Na deficit vines (all +ve)
- 20% ($p=0.002$) of YAN by nursery assessment of Na of deficit plants and P of well-watered plants (both -ve)
- 22% ($p=0.001$) of anthocyanin by leaf loss (28/04/10) and Na of well-watered nursery plants (both -ve)
- 16% ($p=0.006$) of phenolics by nursery assessment of pruning weight of deficit vines and Na of well-watered plants (both -ve).

2.3.1.6. Distribution of simple traits of F86 rootstock genotypes linked to grafted field performance of Shiraz across three sites

To enhance the understanding of the relationships between simple traits measured for F86 which have been linked to the performance of grafted field vines at three sites, distributions for each trait across the population are provided below (n=59), Figures 2.3.1 - 2.3.6.

2.3.1.6.1. Field measurements of ungrafted single seedling vines planted in the field

The distributions at different time points, across the F86 population of canopy leaf loss across two seasons without irrigation 2008/09 and 2009/10 are provided in Figure 2.3.1. At the end of the first season of drought (29/04/09), leaf loss displayed a skewed distribution toward high values, ranging from 30% - 90% with the greatest proportion in the 90% leaf loss category. Approximately 15% of the population retained more than 50% of their leaves. In contrast, in the second season in January (14/01/2010) the distribution was highly skewed toward low levels of leaf loss with the 70% of the population showing no leaf loss. Two months later, leaf loss was more normally distributed, ranging from 30% - 100% loss, with the greater proportion of the population falling in the 50% - 60 % loss categories. The small proportion with low leaf loss below 30% could be considered drought tolerant. Similarly, the small proportion with high leaf loss (90-100%) could be considered drought sensitive. At the end of the second season of drought (28/04/2010), the distribution of leaf loss was highly skewed to the higher end with more than 60% of the population having 100% leaf loss. Genotypes with lower levels of leaf loss in the 60 and 70% loss categories warrant further study with regard to their drought tolerance.

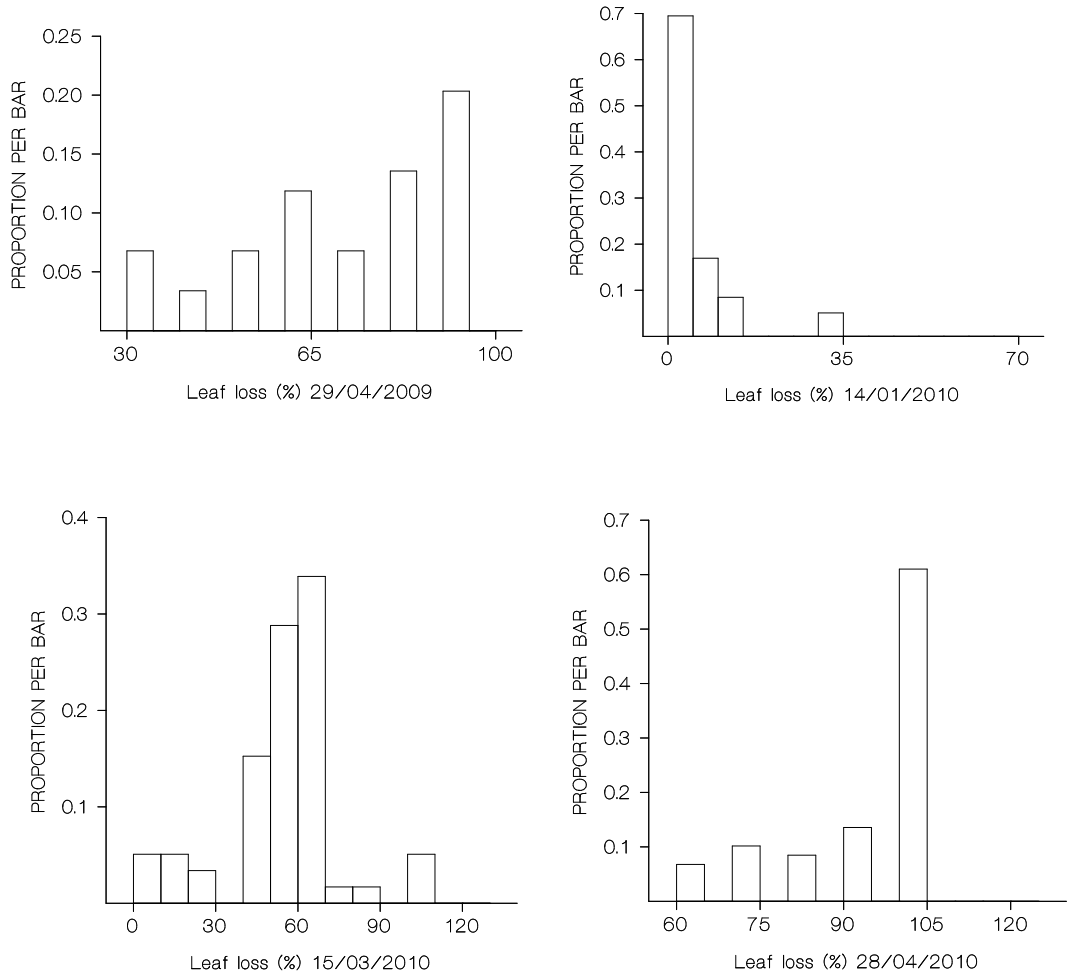


Figure 2.3.1. Distribution of leaf loss across 59 single vine F86 rootstock genotypes established in the field after one season of drought (29/04/2009) and during the second season of drought (14/01/2010, 15/03/2010 and 28/04/2010).

The distribution of pruning weight across single vine seedlings of 59 genotypes, measured prior to the commencement of drought (2008), after one or two seasons without irrigation (2009 and 2010) and after recovery from drought, 2011 is shown in Figure 2.3.2. It should be noted that in the 2010/11 growing season approximately 960 mm of rainfall was recorded. Prior to the imposition of the drought treatment, pruning weights in 2008 showed a skewed distribution with the greater proportion of the population having low pruning weights, the majority in the 0 - 0.5 kg category. A small proportion (10%) of the population had high pruning weights, > 5 kg. After one season without irrigation, the distribution was further skewed toward lower pruning weights with most (72%) in the 0-1.5 category with almost all below the 3.5 kg category. After two seasons without

irrigation, the skewed distribution was maintained with most of the population (68%) in the 0.5 kg category. The few genotypes falling into higher pruning weight categories (>2.0kg) could be considered as drought tolerant. After one season of high rainfall there was a significant increase in pruning weights with some genotypes falling in the 6 and 7 kg categories. While a skewed distribution was maintained the highest proportion of the population (30%) was 2 - 3 kg category.

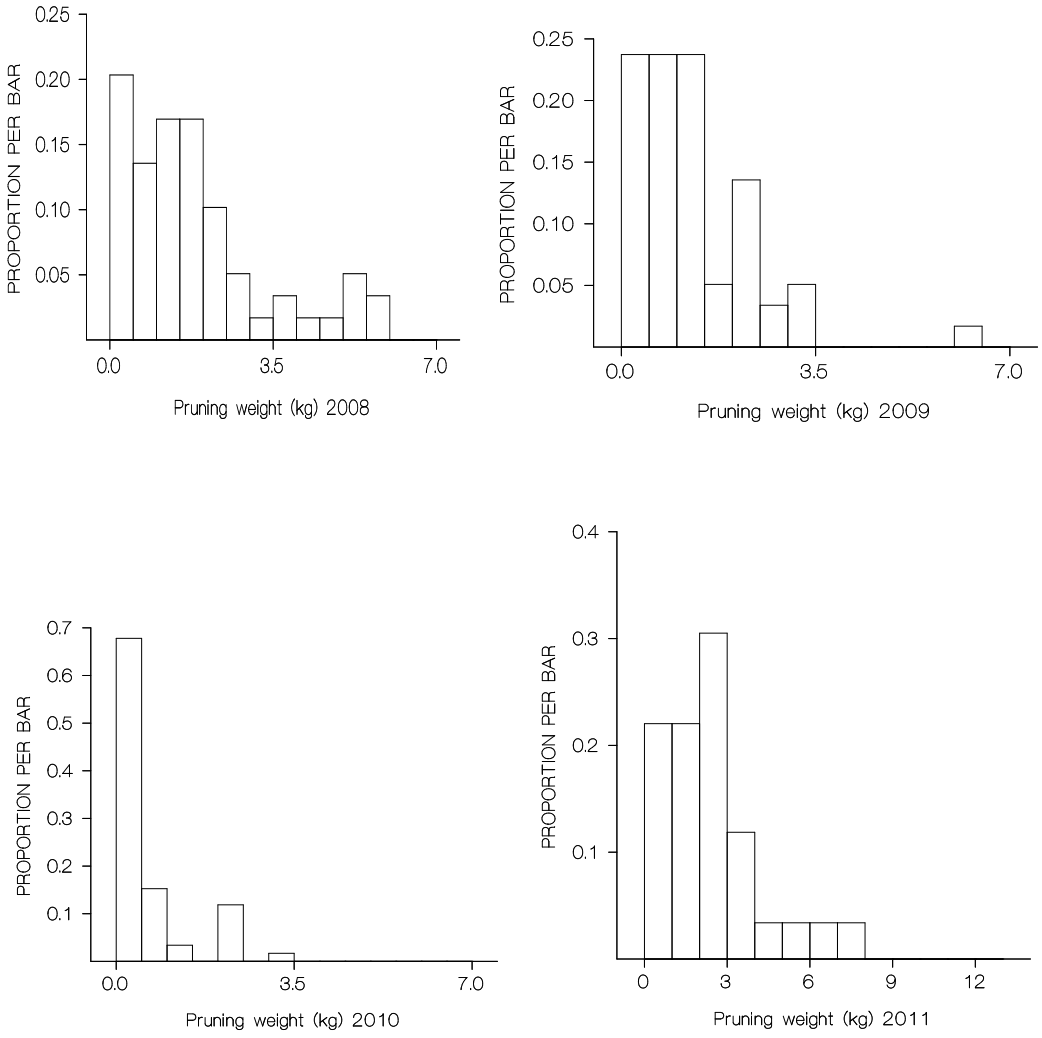


Figure 2.3.2. Distribution of pruning weight across 59 single vine F86 rootstock genotypes established in the field in seasons 2008 (no drought), after one season of drought (2009), after two seasons of drought (2010) and after recovery from drought (2011).

The distribution of canopy height and trunk diameter, recorded in 2007 and 2011, respectively is shown in Figure 2.3.3. The distribution of canopy height which ranged from 1.1m to 1.8 m was skewed to higher values with the highest proportion falling in the 1.7 m category. The distribution of trunk diameter followed a more normal distribution that was slightly skewed to higher values. The greatest proportion (40%) fell in the 30 and 35 cm categories.

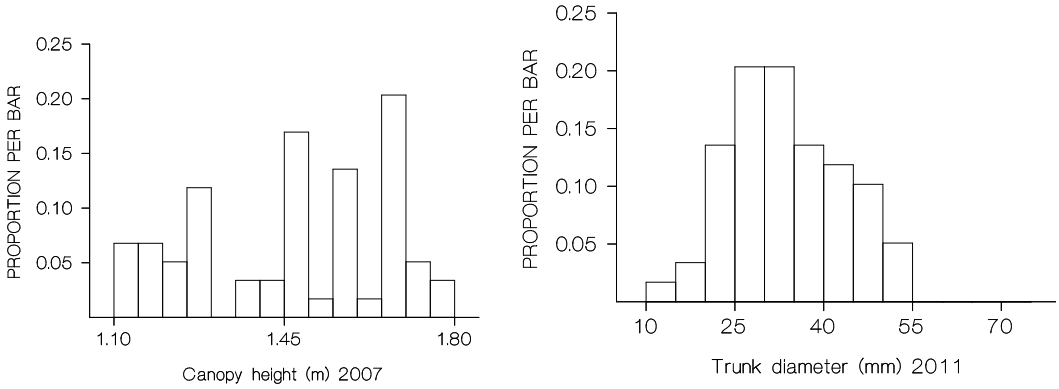


Figure 2.3.3. Distribution of canopy height (2007) and trunk diameter (2011) across 59 single vine F86 rootstock genotypes established in the field.

2.3.1.6.2. Replicated nursery trial assessment

Shoot length of ungrafted (2007) and grafted plants (2008) and pruning weight of well-watered and deficit grafted plants in the replicated nursery trial were important traits linked to field performance. Their distributions are shown in Figure 2.3.4. Ungrafted shoot length in 2007, which ranged from 40 - 220 cms showed a reasonably normal distribution with the highest proportion in the 140 -160 cm category. Grafted shoot length (2008), which ranged from 70-190 cm, also displayed a normal distribution with the highest proportion in the 130 - 140 cm category. It should be noted that vines were only grafted in spring 2007, hence the shoot length represents less than one full season of growth. The distribution of pruning weights of the well-watered grafted vines in 2009, which ranged from <50 - 400 g, was highly skewed to lower values with the highest proportion in the 50-100 g category. With the deficit treatment, the range in pruning weights was much smaller, ranging from 10-80 g with the distribution skewed toward lower values with the highest proportion in the 40-50 g category.

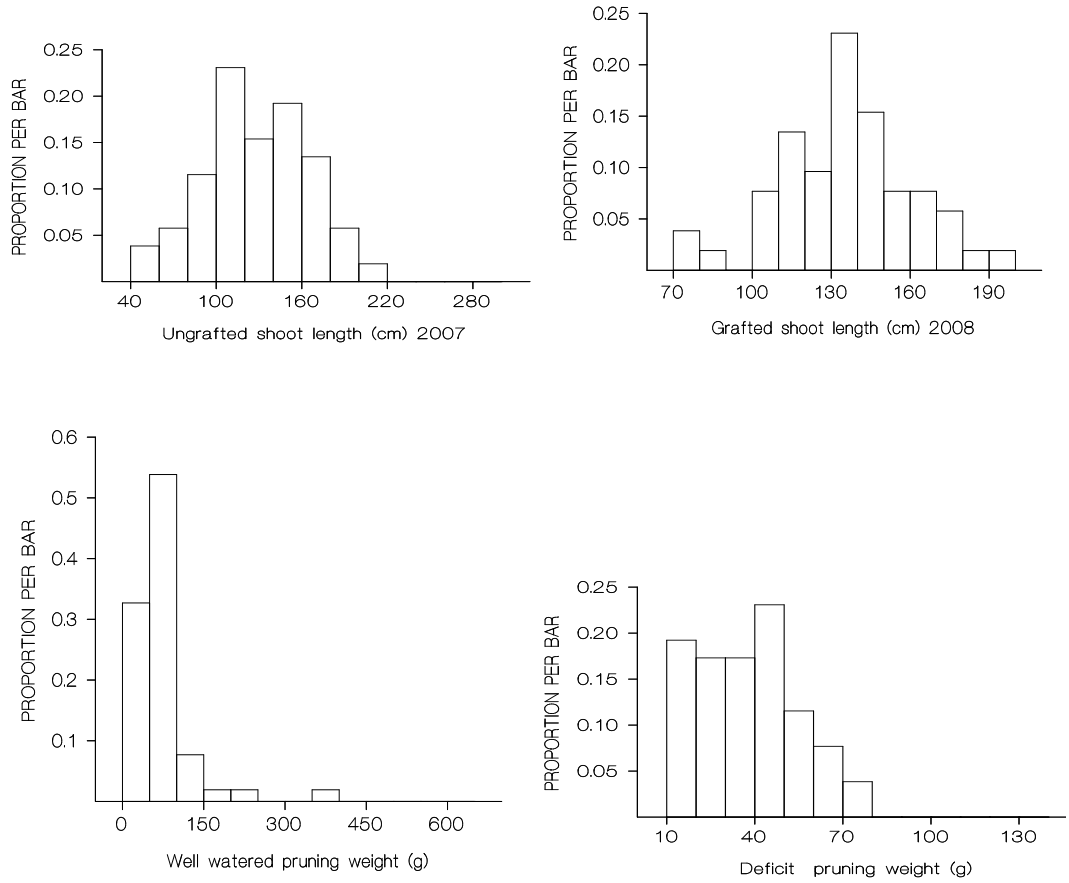


Figure 2.3.4. Distribution of ungrafted (2007) and grafted shoot length (2008), pruning weight under well-watered and deficit conditions (2009) across 59 F86 rootstock genotypes established in a replicated nursery trial.

Distribution of transpiration efficiency (delta) recorded in the nursery for ungrafted plants in 2007, conductance recorded for well-watered plants and leaf temperature of well-watered and deficit plants, all recorded in 2009 is shown in Figure 2.3.5. Delta showed a wide distribution (-30.2 to -27.6), skewed to lower negative values. Genotypes with more negative values are likely to have higher stomatal conductance (Edwards et al. 2014). Stomatal conductance (g_s) of well-watered plants in 2009, showed more than a 10-fold difference (<0.50 - 550 mmol m^{-2} s^{-1}) was normally distributed across the grafted population, with the highest proportion in the 250 - 300 mmol m^{-2} s^{-1} category. Leaf temperature of well-watered plants also showed a normal distribution, ranging from 22 - 30^{o}C, with the highest proportion falling in the 26 - 27^{o}C category. Leaf temperature of the deficit

treatment was higher and more uniformly spread across the population, ranging from 23 - 34ºC, with the highest proportion falling in the 28 - 29ºC category. Differences in leaf temperature between the well-watered and deficit treatments are likely to be due to reduced transpirational cooling with the deficit treatment compared to well-watered plants.

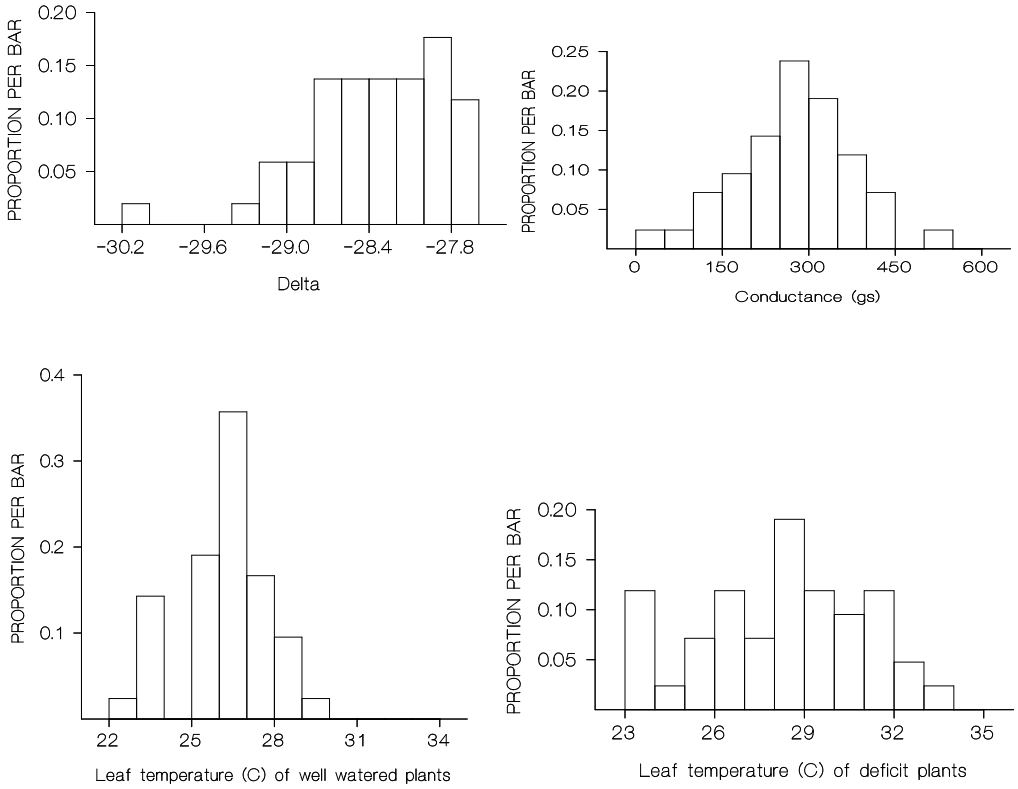


Figure 2.3.5. Distribution of transpiration efficiency (delta) of ungrafted plants (2007), and for grafted plants, conductance of well-watered plants, and leaf temperature of well- watered and deficit plants (2009) across 59 F86 rootstock genotypes established in a replicated nursery trial.

Distribution of key leaf mineral element data which correlated with grafted field performance of Shiraz is shown in Figure 2.3.6. Leaf K of ungrafted plants, determined in 2007, was normally distributed, range 10 - 19 mg/g with the highest proportion in the 15 - 16 mg/g category. Distribution of leaf K of well-watered grafted plants, range 35 - 65

mg/g, was skewed toward higher values with highest proportion in the 55 - 60 mg/g category. In contrast K of deficit plants, range 25 - 70 mg/g was normally distributed with a strong peak (48%) in the 55 - 55 mg/g category. Leaf Ca of well-watered plants, range 9-18 mg/g was skewed toward lower values, with the highest proportion in the 11 - 12 mg/g category. In contrast leaf Ca of deficit plants, range 7 - 18 mg/g was normally distributed with the highest proportion in the 13-14 mg/g range. Leaf B, range 0.06 - 0.22 was normally distributed with the highest proportion in the 0.10 - 0.12 mg/g category.

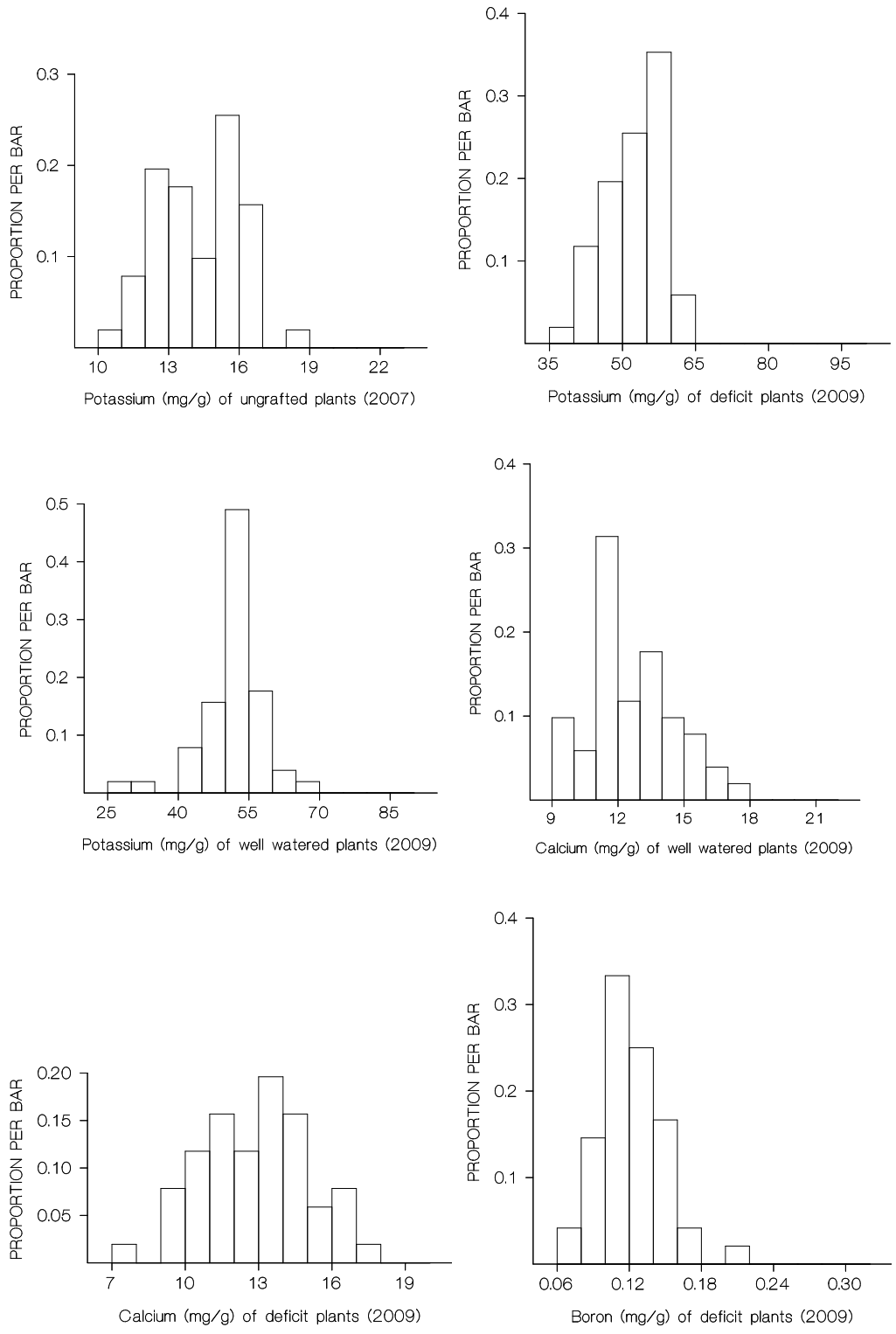


Figure 2.3.6. Distribution of leaf K of ungrafted plants (2007), K and CA of well-watered and deficit grafted plants (2009) and B of deficit plants (2009) across 59 F86 rootstock genotypes established in a replicated nursery trial.

2.2.1.6. Discussion and summary of F86 results

The aim of this project component was to assess the predictive value of simple traits measured previously with grafted vine performance in field trials. It aimed to test the efficacy of early screening assays undertaken in the previous project (CSP0503) for vine growth characteristics, mineral element composition, in particular potassium uptake and response to drought and deficit irrigation. For this purpose, the performance of genotypes from a single family, F86 (a cross involving *V. cineria*, *V. champini*, *V. riparia* and *V. rupestris)* grafted with Shiraz was assessed at three sites, which were evaluated as subsets of the trials involving well-watered (site 1) and deficit irrigation (site 2) treatments in a hot irrigated region at Irymple and at Padthaway (site 3), grown under milder conditions with supplemental irrigation. There were 35 genotypes of F86 common to the three sites. This study included data collected over four seasons, 2013-2016.

In summary, it can be concluded that most vine performance traits of the 35, F86 rootstock genotypes are significantly impacted by environmental conditions, indicative of strong genotype x environment (G x E) interactions. Consequently, the ability to predict grafted rootstock performance by measurement of simple traits prior to establishment of field trials is likely to be constrained by regional differences and management effects (e.g. irrigation). Nonetheless, two performance traits (i.e. pruning weight and berry juice malate) across the F86 rootstocks genotypes grafted with Shiraz were relatively stable across the three environments, indicative of strong genetic control. This gives merit to the classification of rootstocks based on conferred vigour despite the significant differences across sites for this trait (i.e. means of 2.97, 2.15, 0.78 kg/v for sites 1, 2 and 3, respectively). The strong genetic control of conferred vigour and malate, which are highly correlated warrants further investigation with ramifications for future rootstock selection and development. For rootstock genotypes growing under similar soil and climatic conditions but with different irrigation treatments significant correlations across sites indicate that yield, bunch number, TSS, tartrate and YAN were relatively stable and potentially under direct genetic control. Similarly, the comparisons between sites 2 and 3 where vines were grown with deficit or supplementary irrigation, gave significant correlations for berry weight (and pH) despite varying soil and climatic conditions, suggesting some genetic control possibly associated a direct response to stress on berry

development or indirectly to an improved light environment associated with their reduced pruning weight.

Links between simple traits and overall performance across sites

Despite the G x E interactions described above, the study was able to identify simple traits measured for field grown ungrafted individual seedling vines or established in replicated nursery trials as ungrafted or grafted vines that were linked to overall mean vine performance of grafted vines across the three sites. Of the traits measured, stomatal conductance (g_s) of well-watered grafted vines in the nursery was the most powerful, being linked to 9/14 measures of vine performance. While it has been reported that rootstocks may significantly affect g_s (Edwards et al. 2014), further physiological research is required to elucidate the positive links with conferred vigour leading to high pruning weight and larger bunches, and early ripening (high TSS and pH) and high and increased TA, malate, tartrate, and YAN. Leaf temperature, measured concurrently with g_s, also added value as under well-watered conditions in the nursery it was positively linked with TSS and malate and negatively to YAN under deficit conditions, presumably due to effects of rootstock on transpirational cooling. Measurement of transpiration efficiency using carbon isotype discrimination, shown previously to vary across rootstocks (Gibberd et al. 2001, Edwards et al. 2014), also proved to be useful being the only trait, other than leaf Ca, linked to yield and bunch number. It was also linked negatively with berry anthocyanin across the genotypes.

The positive correlation between late season leaf loss under drought conditions and TSS across the three sites may, at least in part, be explained by effects of rootstock on precocity however the links with pruning weight and related conferred vigour effects on malate and tartrate (Walker and Clingeleffer 2009, 2016) require further investigation. Of the vigour related traits measured, nursery pruning weights, particularly with deficit irrigation were useful predictors of vine performance being linked to 8/14 performance parameters including those associated with conferred vigour (pruning weight and berry weight) and related effects of vigour on fruit composition (Clingeleffer, 2000, Walker and Clingeleffer 2009, 2016). This was not unexpected as nursery pruning weights had been linked to vine performance in other studies (Clingeleffer and Smith 2011). However, in the multilinear regression analyses, other vigour related traits were stronger factors, including canopy height measured on ungrafted seedlings in 2007 which was linked to

pruning weight, malate and TA; pruning weight of seedling vines after recovery from drought in 2011 linked to TSS; pruning weight of ungrafted seedlings in 2010 after two seasons of drought, linked to berry anthocyanin, and shoot length of grafted vines in the nursery measured in 2008, linked to pH. The importance of vine K status measured for ungrafted and grafted vines, particularly under deficit conditions in the nursery was linked to berry weight, TSS, pH, malate, YAN. This was not unexpected as vine K status has previously been identified as playing a key role in fruit composition (Clingeleffer 1996, Clingeleffer 2000, Clingeleffer and Smith 2011, Walker and Clingeleffer 2016). The negative relationships with leaf Ca under both well-watered and deficits in the nursery and bunch number (and yield) warrant further investigation.

Links between simple traits and performance at individual sites

Genotype and environment (G x E) interactions were identified above for the F86 genotypes across the three sites which could impact grafted vine performance and the linkages with simple traits. The usefulness of late season leaf loss of ungrafted seedlings, after one and two seasons of drought as a predictor was largely limited to site 2, i.e. performance under deficit irrigation being negatively linked to bunch number and positively linked to pruning weight, TSS, pH, malate, tartrate and YAN. At the well-watered site 1, late season leaf loss in the second season was positively correlated with bunch weight, pruning weight, malate and YAN. At site 3, leaf loss was positively correlated with pruning weight after one season of drought and negatively with anthocyanin and phenolics after two seasons of drought. Given that end of season leaf loss trait was assessed as a response to drought in a hot climate, it is not unexpected that the linkages were most frequent and strongest for site 2 with deficit irrigation except for the stable performance traits identified above, i.e. conferred vigour and berry malate.

Pruning weight of ungrafted F86 genotypes measured following two seasons of drought in 2010 and after recovery in 2011 was linked to grafted vine performance. After two seasons of drought it was positively linked to pruning weight and anthocyanin at sites 2 and 3 but not to any measure of vine performance at site 1. After recovery from drought in 2011, pruning weight was positively correlated at site 2 with berry weight, TSS, anthocyanin and phenolics and with pruning weight at site 3. These results indicate that ungrafted seedling vigour, determined under drought or well-watered conditions will

impact differently on grafted vine performance. When determined under drought conditions in 2010, linkages with vine performance were found at sites under water stress from deficit (site 2) or supplementary irrigation (site 3) whereas when measured under well-watered conditions in 2011 linkages were found with vine performance of the well-watered (site 1). Trunk diameter of field grown seedlings was positively correlated with pH at site 1 and negatively correlated with tartrate at site 2. Seedling canopy height determined in 2007 was strongly correlated with bunch weight, pruning weight TA and malate at site 2 and to a lesser degree with pruning weight, pH and malate at site 1 but not to any performance measures at site 3.

Root architecture traits were not included in the combined analysis using means across sites described above as the correlations were generally weak. However, the results linking root architecture traits with grafted vine performance for individual sites provided some interesting contrasts. Root type was not correlated with any performance trait at the well-watered site1, positively correlated with yield and bunch number and negatively correlated with tartrate and YAN with deficit irrigation at site 2 and correlated negatively with berry weight, pH and malate at site 3. Root diameter was positively correlated with pH at site 1, bunch number and pruning weight at site 2 and pruning weight at site 3. Root angle was negatively correlated with TSS and anthocyanin at site 1, positively correlated with yield and negatively correlated with pH at site 2 and negatively correlated with berry weight pruning weight, malate and positively correlated with anthocyanin and phenolics at site 3. Root number was positively correlated with pruning weight, TSS and malate only at site 3. Overall the importance of root architecture traits as predictors of grafted vine performance varied across sites. Comparisons of sites 1 and 2 indicate that root architecture was of little importance when vines were grown under well-watered conditions whereas with the deficit treatment the root architecture of the genotypes was important to vine performance. Linkages between root architecture traits and grafted vine performance at the low vigour Padthaway site 3 were also important with root type negatively linked to berry weight, pH and malate; root diameter linked positively with pruning weight; root angle linked negatively with berry weight, pruning weight, malate and positively with anthocyanin and phenolics.

Traits measured for ungrafted and grafted F86 genotypes in the nursery situation were strongly linked to grafted vine performance indicating strong potential for rapid screening of rootstock genotypes to minimise numbers for evaluation in field trials. Transpiration efficiency, determined by carbon isotope discrimination of ungrafted plants was a very useful indicator of vine performance, particularly vine growth characteristics being negatively linked to pruning weight and positively linked to yield, bunch number, berries per bunch at the well-watered site 1; yield berry weight and bunch weight at site 2 with deficit irrigation and berry weight and berries per bunch at site 3. Transpiration efficiency was also negatively correlated with TA, malate and YAN at site 1 and anthocyanin at site 2.

Assessment of conductance of well-watered grafted plants was the most powerful trait linked to grafted vine performance being negatively correlated with yield and bunch number at site 1. It was positively correlated with pruning weight at sites, TSS at sites 1 and 2, pH at sites 2 and 3, TA at site 1, malate, tartrate and YAN at sites 1 and 2 and anthocyanin at site1 and negatively with TA at the low vigour Padthaway site 3. Conductance of the deficit treatment was low for all genotypes and did not correlate with any measure of vine performance

Leaf temperature measured under well-watered and deficit irrigation was a useful predictor of performance at site 1 under well-watered conditions but not at site 2 or 3. Leaf temperature of well-watered plants was negatively linked to yield and bunch number and positively linked to pruning weight, TSS, TA, malate, tartrate and YAN at site 1. In contrast, leaf temperature of the deficit treatment was positively correlated with yield, bunch number and berries per bunch negatively linked bunch weight, pruning weight, TA, malate, tartrate and YAN at site 1. These results indicate that rootstock genotypes under well-watered conditions that are able to maintain lower temperatures conditions through transpirational cooling in the nursery, are likely to have lower conferred vigour and be more fruitful, leading to higher yields with lower TSS, malate, tartrate and YAN when fully irrigated (site 1). Conversely, genotypes under deficit conditions in the nursery that can maintain low temperature are likely to have higher pruning weights, lower bunch number, yield and berries per bunch and higher higher TA, malate, tartrate, YAN and anthocyanin when fully irrigated (site 1). The underlying physiology leading to these

responses linking transpiration efficiency, conductance and leaf temperature require further investigation.

Pruning weight of grafted vines in the nursery with both well-watered and deficit treatments were very strong predictors of grafted vine performance at all three sites. In general, correlations with pruning weight and grafted vine performance, if common to both irrigation treatments, were stronger with the deficit treatment. Pruning weight of the deficit treatment was positively correlated with conferred vigour (pruning weight) of grafted vines at all three sites. Under full irrigation (site 1) pruning weight of the deficit treatment in the nursery was negatively linked to bunch number and yield whereas at the low vigour Padthaway site 3 it was positively correlated with not only with bunch number and yield, but also berry weight, bunch weight and berries per bunch. There were no significant correlations between nursery pruning weight and crop development at site 2. These results suggest that genotypes conferring high vigour when managed with full irrigation have reduced fruitfulness whereas at the low vigour site in Padthaway, high inherent vigour was required to produce high bunch numbers and larger bunches associated with larger berries and high berries per bunch. Pruning weight of the deficit treatment was also strongly linked to fruit composition at all sites, being positively correlated with TSS at sites 1 and 2, pH at sites 2 and 3, TA at site 1, malate at all sites, tartrate at sites 1and 2 and YAN, anthocyanins and phenolics at site 1. Further research to understand the linkages between inherent pruning weight of genotypes under deficit conditions in the nursery and conferred vigour, crop development and fruit composition is required.

Assessment of leaf K of ungrafted and grafted plants under well-watered and deficit treatments in the nursery also proved to be a useful indicators of grafted vine performance. These results were not unexpected as vine K status has previously been identified as playing a key role in fruit composition (Clingeleffer 1996, Clingeleffer 2000, Clingeleffer and Smith 2011, Walker and Clingeleffer 2016). In this study, leaf K of ungrafted vines was strongly linked to grafted vine performance at site 2, being positively correlated to conferred vigour (pruning weight), pH, malate, tartrate and YAN and negatively correlated to yield, bunch number, berries per bunch and TSS. K of ungrafted vines was linked positively to TSS at site 1 under full irrigation and positively with pH and negatively with TA at the low vigour site 3 in Padthaway. K of well-watered grafted plants was a strong indicator of grafted performance at site 1, being positively linked to berry weight, bunch weight, malate, tartrate and YAN. At site 2, K of well-watered grafted plants was positively linked to TSS, pH,

malate and YAN. It was not linked to any measure of vine performance at site 3. In contrast, K of deficit grafted plants was useful for predicting performance at the low vigour site 3, with positive links with conferred vigour, yield, bunch number, berry weight, bunch weight, berries per bunch and pH and negatively linked with TA. The underlying causes for these differences of K leaf status of ungrafted and well-watered and deficit grafted vines in the nursery and the different responses at each of the sites warrant further investigation.

Assessment of leaf Ca in the nursery was also useful as a predictor of crop development at each of the sites. In particular, Ca of the well-watered grafted plants was negatively linked to yield at all sites, bunch number at site 3, berry weight at site 1 and 3, bunch weight at site 1 and 3 and berries per bunch at site 1. Further study is required to understand the role of Ca in crop development.

Summary

Overall, the results across the three sites indicate that measurement of simple traits may provide tools for early screening of rootstock genotypes. Obviously, the aims of rapid screening of rootstock is to avoid field planting of seedlings and minimise the number of genotypes planted in replicated performance trials in the field. Based on the results of this study, measurement of simple traits under nursery conditions offers considerable promise for rapid screening of genotypes. It is suggested that future use of this approach, in combination with future molecular marker selection (e.g. for root knot nematode and phlloxera resistance), be used to provide a basis for rapid, efficient screening of rootstock genotypes. The nursery component should involve a bench grafting propagation step prior to establishment, to screen for rootability (Smith and Clingeleffer 2013) and graftability and once established, application of well-watered and deficit irrigation treatments. Measurements, as identified in this study would include simple growth traits (shoot length, pruning weight, trunk diameter) augmented by physiological traits (conductance, leaf temperature, transpiration efficiency by carbon isotope discrimination) and plant mineral element status. Because of the strong linkages between conferred vigour and YAN it is suggested that N status be included in such a screening approach, as Clingeleffer 1996, 2000 has previously established very strong associations between conferred vigour and N status. Furthermore, recent studies by Cochetel et. 2017 and Medici et al. 2017 have shown that rootstocks with high and low conferred vigour differ in genes that control nitrate uptake and transport. Once the nursery assessments are completed the vines could

be lifted for assessment of root architecture traits (type, diameter, number and angle). Further efficiencies could be expected if screening studies for mineral element status (e.g. K), drought tolerance and root architecture could be assessed under glasshouse conditions to reduce numbers established in the nursery (see later discussion in section 2.3.2 below).

2.3.2. Studies involving F34 (*V. cineria* x Riesling test population)

The performance of genotypes from a single family involving a *V. cineria* x Riesling test population (F34), which was genotyped by next generation sequencing (see nematode and phylloxera marker development in 2.4 below) as part of this project, included vigour assessment in a field nursery with a well-watered and a deficit irrigation treatment planted in spring 2014 and glasshouse K screening studies with limited K and high K treatments. The deficit irrigation treatment in the nursery received 45% of the well-watered control. In the previous project (CSP 0503) considerable information on field established seedling vines had been collected similar to that described for F86 above. Genotypes from this family were also included in abiotic stress screening studies established as part of CSP 1302.

2.3.2.1. Assessment of single vine seedlings of F34 in the field, 2007-2011

The distribution of leaf loss across the F34 population (114 genotypes) is presented as a time series over two seasons without irrigation (Figure 2.3.2.1). At the first time point (27/01/2009, not shown), the canopies of all genotypes were in excellent condition with less than 2% leaf loss. After a further six weeks without irrigation over the summer period (10/03/2009), there was a significant divergence in leaf loss, range 0 - 55%, with skewed distribution toward lower values, with 45% in the 10 - 15% leaf loss category. Genotypes in the higher leaf loss categories (30%), could be considered to be sensitive to short term drought. At the end of the season (29/04/2009), leaf loss ranged from 10 - 100% with a skewed distribution toward higher values with more than 50% of genotypes having more than 70% leaf loss, an indication of severe sensitivity to drought. There was a small proportion of genotypes (< 10%) which appeared to be drought tolerant with less than 20% leaf loss. In mid-January (14/01/2010) in the second season without irrigation leaf loss ranged from <10% to 100%, although the distribution was highly skewed toward lower values with 65% in the 0 - 10% leaf loss category. By mid-February (08/02/2010) there was a significant shift in the distribution, range <10% to 100% leaf loss, with almost 30% of the population falling in the 40% leaf loss category. More than 30% of genotypes had lost more than 50% of leaves. Leaf loss of almost 20% of genotypes was below 20%, an indication of drought tolerance. By the middle of March (15/03/2010) the distribution was skewed toward higher values with the highest proportion of genotypes (45%) in the 60 and 70% leaf loss categories. Interestingly, leaf loss of 10% of the population was in

the 0-10% category, indicative of drought tolerance. At the end of the season (29/04/2010) 70% of genotypes had 100% leaf loss. For a very few individual genotypes, leaf loss was less than 50%, an indication of potential high drought tolerance.

The distribution of pruning weight across 114 single vine seedlings, measured prior to the commencement of drought (2008), after one or two seasons without irrigation (2009 and 2010, respectively) and after recovery from drought, 2011is shown in Figure 2.3.2.2. It should be noted that in the 20/10/11 growing season approximately 960 mm of rainfall was recorded. Prior to the imposition of the drought treatment, pruning weights in 2008 showed a skewed distribution with the greater proportion of the population having low pruning weights, the majority in the 0 - 0.5 kg category. A small proportion (10%) of the population had pruning weights > 2 kg. After one season without irrigation, the distribution was further skewed toward lower pruning weights with most (55%) in the 0 - 0.5 kg category with almost all below the 4 kg category. After two seasons without irrigation, the skewed distribution was maintained with most of the population (70%) in the <0.5 kg category. The few genotypes (10%) falling into higher pruning weight categories (>1.0kg) could be considered as drought tolerant. After one season of high rainfall there was a significant increase in pruning weights with some genotypes falling in categories >3 kg. While a skewed distribution was maintained, the highest proportion of the population (42%) was in the 0 - 0.5 kg category. Approximately 45% of genotypes were in the 0.5 - 2.0 kg pruning weight categories. The distribution of trunk diameter in 2007, range 5 - 55 mm, was normally distributed with most genotypes in the 20 and 25 mm categories (Figure 2.3.2.3).

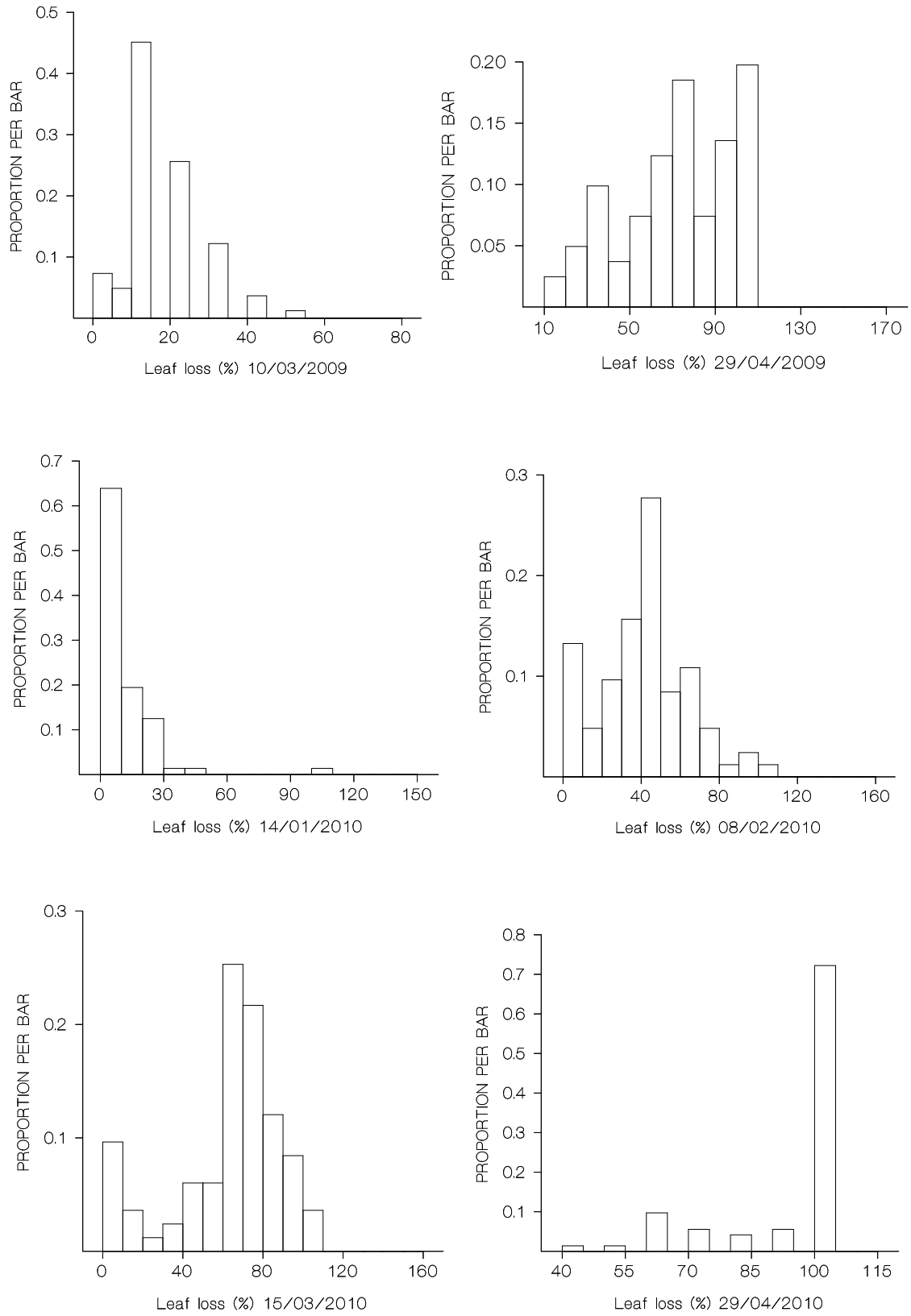


Figure 2.3.2.1. Distribution of leaf loss (%) across 114 single vine F34 rootstock genotypes established in the field at different time points after one and two seasons of drought.

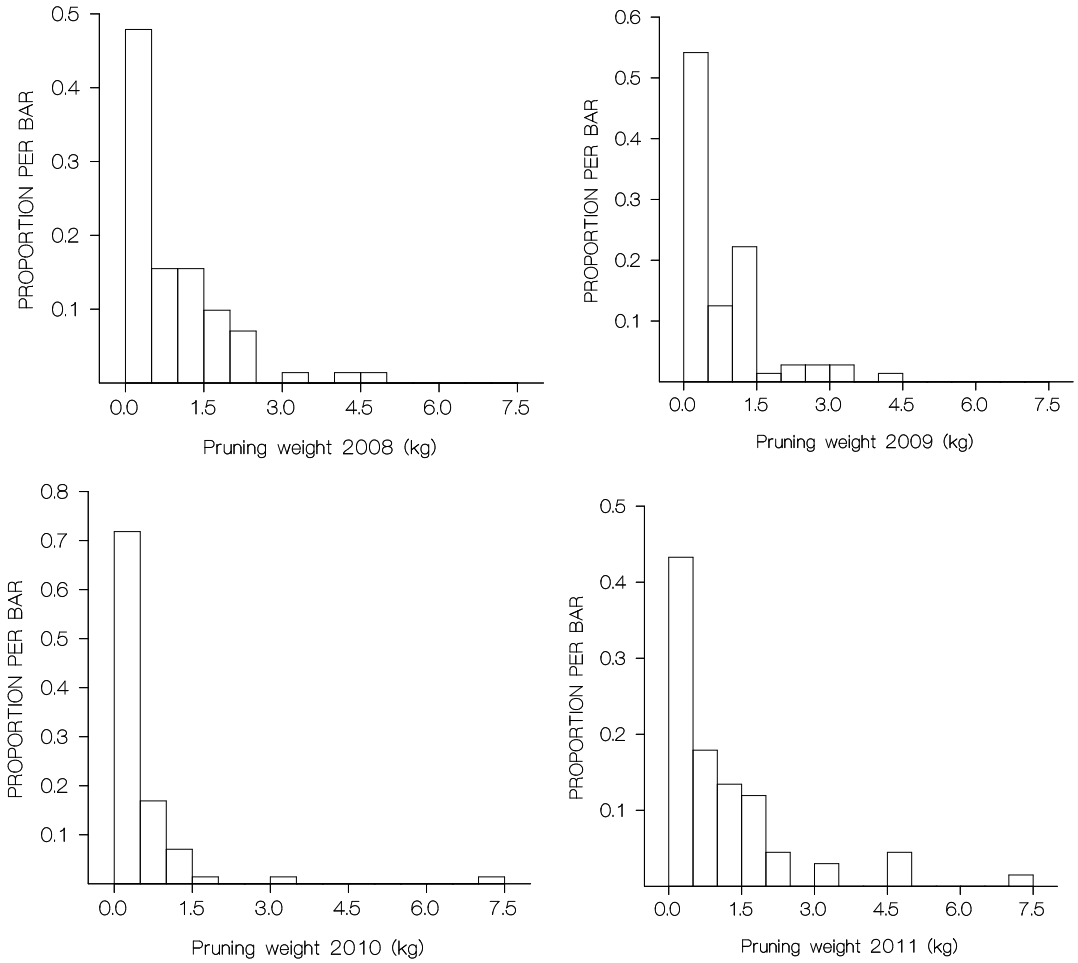


Figure 2.3.2.2. Distribution of pruning weight across 114 single vine F34 rootstock genotypes established in the field in seasons 2008 (no drought), after one season of drought (2009), after two seasons of drought (2010) and after recovery from drought (2011).

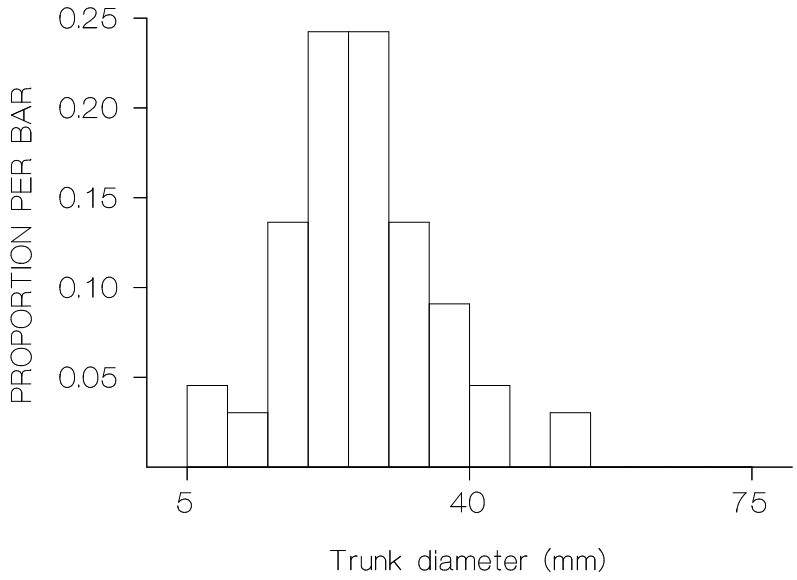


Figure 2.3.2.3. Distribution of trunk diameter measured in 2007 for 114 single vine F34 rootstock genotypes established in the field.

Distributions for root architecture traits across 65 F34 genotypes are presented in Figure 2.3.2.4. For the root type trait, 10 % of the genotypes had a branching rootstock system with development of fine roots (root type 1), 50% had a plunging tap root system (root type 3) with the remaining 40% a mixture of types 1 and 3. It should be noted that retention of the tap root from the seedling plant would not be reflected in cuttings used to propagate rootstocks hence caution should be used in studies involving this trait. Root number (range <10 - 80) was normally distributed with 55% of genotypes in the 10-30 root number categories with the highest proportion in the 20 roots category. Root diameter (range 4 - 32 cm) was normally distributed with the highest proportion (30%) in the 12 mm category. Root angle showed a broad range across the genotypes with the highest proportion in category 2, 22 - 45^{o}, from horizontal with 75% in categories 2 - 3.

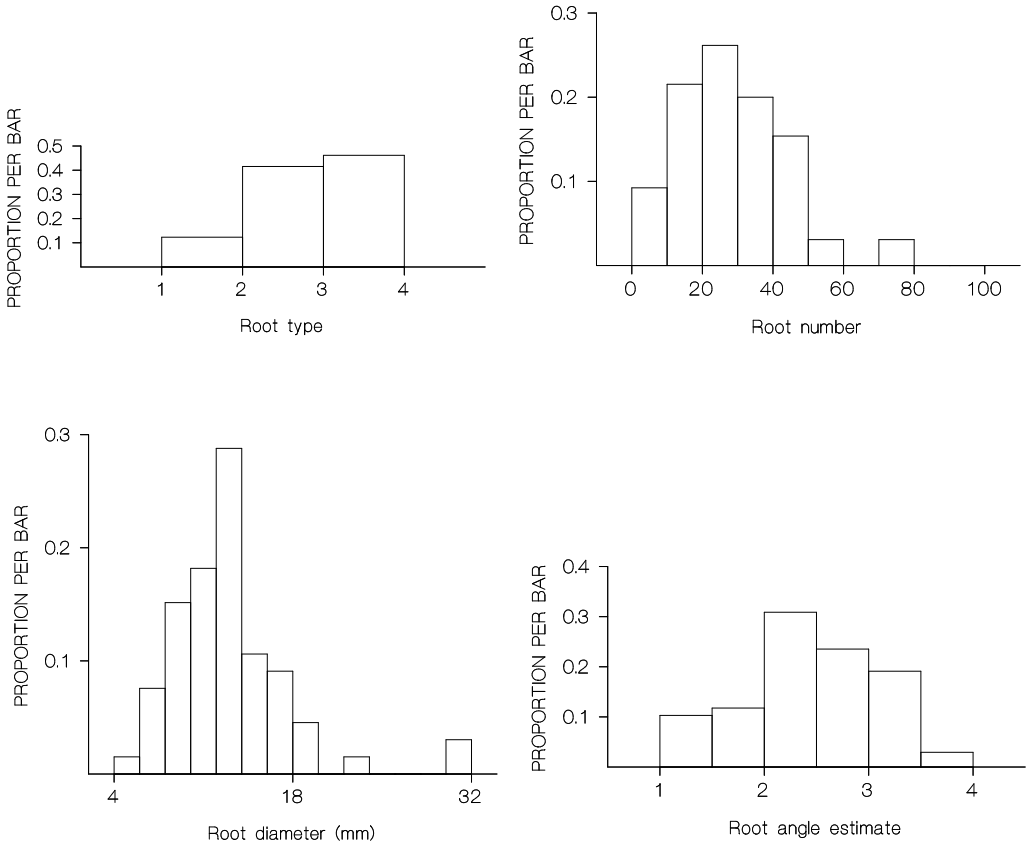


Figure 2.3.2.4. Distribution of root architecture traits across 65 single vine F34 rootstock genotypes established in the field and removed in 2011. (For root type, 1 has >90% branching with fine roots, 3 dominated by tap root, 2 mixture of 1 and 3. For root angle estimate, 1= 0-25^{o}, 2= 22.5-45.5^{o}, 3=45-67.5^{o} and 4= 67.5-90^{o} from horizontal.

2.3.2.2. Assessment of vine growth and canopy characteristics of F34 genotypes in the replicated nursery trial (2016-17)

GLM analyses of the pruning weight data collected in 2016 and 2017 from the high density replicated nursery under well-watered and deficit irrigation treatments showed highly significant effects ($p<0.001$) of season (0.62 and 0.53 kg in 2016 and 2017, respectively), irrigation (0.76 and 0.40 kg for well-watered control and deficit plants, respectively) and rootstock genotype. The irrigation x year interaction was not significant. The distribution of pruning weight across the rootstock genotypes in each season and for each irrigation treatment is shown in Figure 2.3.5. Mean pruning weight of the well-watered control in 2016 ranged from <0.2 - 1.8 kg in 2016 and <0.2 - 2.0 kg in 2017. In

both seasons the distribution was skewed to lower values with the highest proportion of genotypes falling in the 0.6 - 0.8 kg category in 2016 and the 0.4 - 0.6 kg category in 2017. Mean pruning weight of the deficit treatment 2016 ranged from <0.2 -1.2 kg in 2016 and <0.2 - 0.8 kg in 2017. In 2016, the distribution was skewed to lower values with the highest proportion of genotypes falling in the 0.4 - 0.6 kg category. In 2017 pruning weights of most genotypes (55%) were in the 0.2 - 0.4 category.

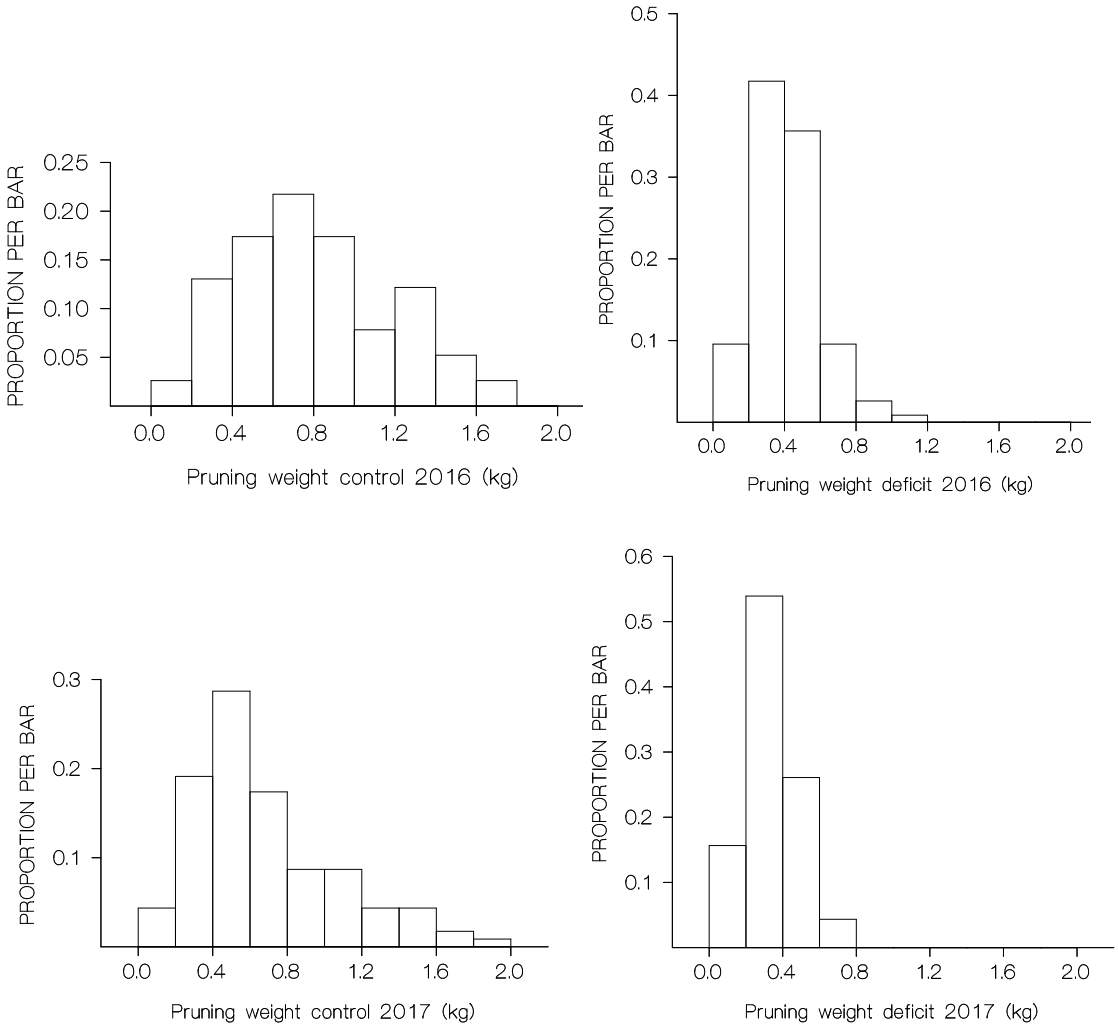


Figure 2.3.2.5. Distribution of pruning weight across 114 F34 rootstock genotypes in 2016 (Top) and 2017 (Bottom) for well-watered (Left) and deficit plants (Right)

In season 2017, canopy condition was assessed at the end of the season on 7 April and 1 May, for both irrigation treatments (Figure 2.3.2.6). GLM analyses showed significant effects of irrigation treatment at both time points on canopy condition (mean 1.77 and 2.46 in April for the control and deficit, respectively and 2.32 and 3.00 in May for the control and deficit, respectively). The effect of rootstock genotype, shown in Figure 2.3.2.6, was

significant in April ($p<0.05$) and highly significant in May ($p<0.001$). In April the distribution of canopy condition of the well-watered treatment (range 1 - 4) was skewed toward lower values, with approximately 80% of the population in categories 1 - 2.5, an indication that the canopies of most genotypes were still in good condition. In contrast, the distribution for the deficit treatment (range 1 - 4.5) was slightly skewed toward higher values, with the majority of the population (65%) in categories 2.0 - 3.5, an indication of some canopy damage for most genotypes. In May, canopy condition across the control treatment (range 1 - 4.5) displayed a normal distribution with the majority of the population (60%) in categories 2 and 3. Genotypes across the deficit treatment (range 1 - 5.5) also displayed a normal distribution, with 50% in categories 2.5 - 3.5. Canopy condition of more than 30% of the genotypes, falling in categories 3.5 - 5.5, was poor.

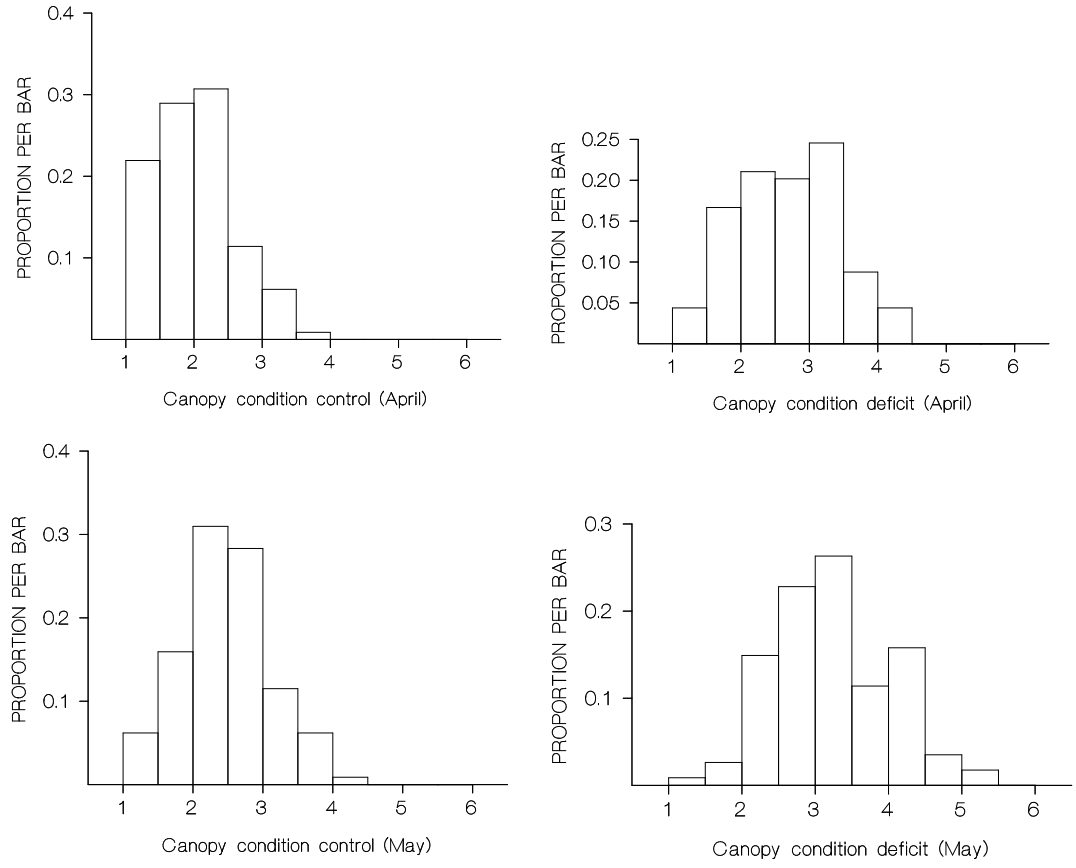


Figure 2.3.2.5. Distribution of the assessment of canopy condition across 114 F34 rootstock genotypes in 2016 (Top) and 2017 (Bottom) for well-watered (Left) and deficit plants (Right). (1 = 100% of leaves in good condition, 2 = minor marginal leaf burn, 3 = 40% of leaves with significant leaf burn, 4 = 60% of leaves with significant leaf burn, 5 50% leaf loss and significant burn, 6 = 100% leaf loss).

2.3.2.3. Results from potassium glasshouse screening of F34 genotypes

Glasshouse screening of 114 F34 genotypes was undertaken with 1.0 mmol /L (Low) and 10.0 mmol/L (High) solutions as recommended by Ruhl 1989 using a bed flow technique (Smith and Clingeleffer 2011). The study was replicated four times at different times of the season, leading to some differences in growing conditions. GLM analyses of the petiole K concentrations showed highly significant effects ($p<0.001$) of K treatment (mean 15.5 and 25.5 mg/g, for the low and high treatment respectively), rootstock genotype and time of season (replicate, range 17.2 - 23.9 mg/g). Petiole K concentration distribution across the genotypes for the two potassium treatments is shown in Figure 2.3.2.6. Petiole K of the low K treatment (range 6 - 22 mg/g) was normally distributed with 45% in mid-range categories (13 - 15 mg/g). Petiole K of the high K treatment (range 14 - 46 mg/g) was slightly skewed to lower values with 60% in mid-range categories (22 - 30 mg/g). Low petiole K in both treatments indicates potential for K exclusion by a number of genotypes. Conversely, high petiole K indicates potential for high K uptake by some genotypes. It should be noted that the correlation across the genotypes between the two treatments was not significant. This supports the concept that K uptake by different genotypes is controlled by two mechanisms, i.e. active transport in low K environments and passive uptake by mass flow in high K environments, proposed by Ruhl 1989.

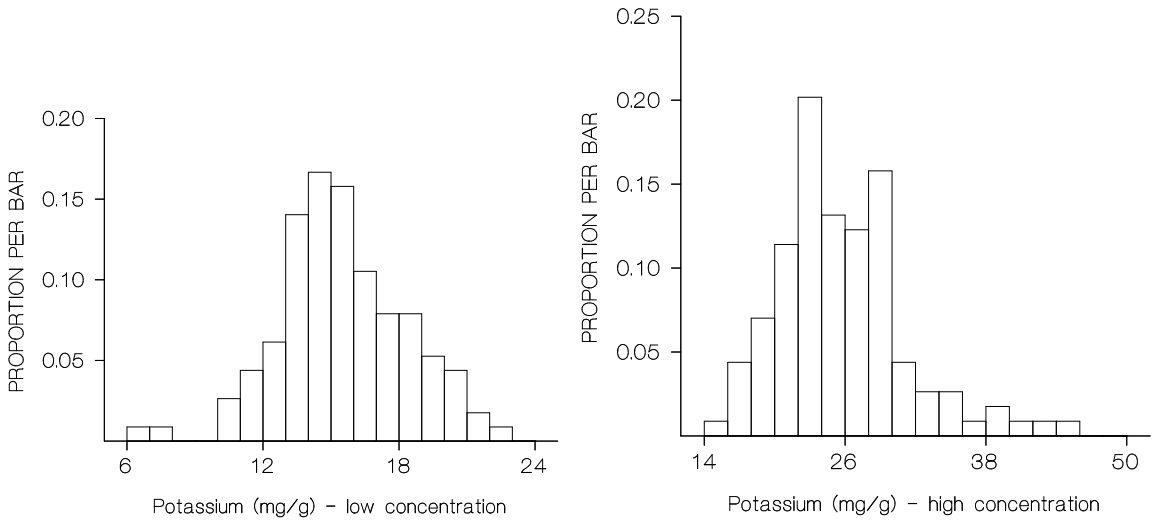


Figure 2.3.2.6. Distribution of mean petiole K concentration across 114 F34 rootstock genotypes for the low and high potassium treatments grown under glasshouse conditions.

2.3.2.4. Relationships between measured traits across F34 genotypes

There were significant correlations between traits measured for single vine seedlings of F34 genotypes established in the field and their growth performance as ungrafted vines in replicated nursery trials, glasshouse growth studies, and glasshouse screening of potassium uptake (Tables 2.3.2.4.1 - 2.3.2.5.1).

In the two-year drought study with seedling vines established in the field which were not irrigated for two seasons, there were significant correlations with leaf loss at different times points (Table 2.3.4.1). Despite low levels of leaf loss at the first time of measurement (27/01/09), there was still a significant correlation across the genotypes with leaf loss six weeks later, but not at other times of assessment over the two seasons, once the full effects of the drought treatment were evident. Leaf loss on the 27/01/09 was also positively correlated with petiole K under limited K supply and negatively correlated with pruning weights in the field trial under deficit conditions in both 2016 and 2017. Leaf loss on 01/03/2009 was positively correlated with leaf loss on the 29/04/09, 14/01/2010 and the 15/03/2010, indicating some commonality in drought response within and between seasons. Leaf loss at this time point also correlated positively with both the mean pruning weight and pruning weight of the well-watered control treatment in the nursery study.

There were strong positive correlations between leaf loss on 29/04/2009 and leaf loss at other time points in the second season of drought, indicative of consistent response to drought across the genotypes. It also suggests that only one season of drought study is required to identify sensitive and tolerant genotypes, based on canopy conditions. Furthermore, leaf loss on 29/04/2009 was negatively correlated with pruning weight in 2009, 2010 and 2011, trunk diameter in 2007 and root number and diameter which indicates that drought tolerance is linked to root architecture, specifically genotypes with more, larger roots are likely to retain canopy and have higher vigour under long term drought conditions. Similarly, leaf loss on 14/01/2010 was also positively correlated with leaf loss at other time points in that season, and negatively correlated with pruning weight in 2008, 2009 and 2011, trunk diameter, root number and diameter but also pruning weights in the field trial in 2016 and 2017 for both the well-watered control (2017 only) and the deficit plants. Leaf loss on 08/02/2010 and 15/03/ 2010 followed similar trends except that there were no significant correlations with pruning weight of deficit treatments in the nursery study in 2016 or 2017. Leaf loss at the end of the second season of drought, which was highly skewed toward high loss (Figure 2.3.2.2) was negatively correlated with

pruning weight in 2010 and 2011, root number, trunk diameter, and in the nursery trial, pruning weight of the well-watered control in 2017 and the deficit treatment in both 2016 and 2017.

Table 2.3.2.4.1. Correlation coefficients (r) between field assessment of canopy condition of ungrafted rootstock genotypes over two seasons without irrigation, 2008-09 and 2009-10, and measured traits (n= 65-83). Correlations retained are significant p< 0.05. Highly significant correlations (p<0.001) are shown in bold.

	Canopy condition (% leaf loss)						
	Season 2009			**Season 2010**			
	27/01	01/03	29/04	14/01	08/02	15/03	28/04
Leaf loss % (01/03/09)	0.31						
Leaf loss % (29/04/09)		0.29					
Leaf loss % (14/01/10)		0.23	**0.38**				
Leaf loss % (08/02/10)			**0.52**	**0.51**			
Leaf loss % (15/03/10)		0.23	**0.53**	**0.43**	**0.83**		
Leaf loss % (28/04/10)			**0.52**	0.27	**0.57**	**0.75**	
Pruning weight (2008)				-0.23	-0.24	-0.24	
Pruning weight (2009)			-0.26	-0.26	-0.31	-0.35	
Pruning weight (2010)			-0.32		**-0.40**	**-0.49**	**-0.45**
Pruning weight (2011)			-0.34	-0.26	-0.35	**-0.37**	-0.30
Root number			-0.25	-0.28	-0.34	-0.36	-0.29
Root diameter			-0.24	-0.35	-0.36	**-0.40**	
Trunk diameter			-0.35	**-0.37**	**-0.43**	**-0.44**	-0.28
Mean pruning wt. (2016)		0.28		-0.27	-0.26	-0.23	
Prun. wt, Control (2016)		0.29			-0.26	-0.23	
Prun. wt, Deficit (2016)	-0.26			-0.30			-0.23
Mean pruning wt. (2017)				-0.30	-0.32	-0.26	
Prun. wt, Control (2017)				-0.23	-0.33	-0.25	**-0.39**
Prun. wt, Deficit (2017)	-0.23			-0.24			-0.27
Glasshouse K low	0.25						

Pruning weight in 2008, prior to imposition of drought, was positively correlated with pruning weight in other seasons, trunk diameter, root number, root diameter and pruning weight of the mean and control treatment in 2016. After one season of drought, pruning weight in 2009 was positively correlated with pruning weight in 2010 and 2011, trunk diameter, root diameter and pruning weight in the nursery of both the well-watered control and deficit treatments in 2016. After two seasons of drought, pruning weight in 2010 was positively correlated with pruning weight in 2011, trunk diameter, root number and root diameter (all p<0.001) and with all measurements of pruning weight in the nursery study in both 2016 and 2017. After recovery from drought in 2011, pruning was positively correlated with trunk diameter, root number and root diameter (all p<0.001) and in the

nursery, pruning weight of the mean and well-watered control treatments, but not deficit treatments in 2016 and 2017 and negatively correlated with root angle. Trunk diameter in 2007 was positively correlated with root number and root diameter ($p<0.001$) and all measurements of pruning weight in the nursery trial in 2016 and 2017, ($p<0.001$, except deficit treatments) and negatively correlated with root angle.

It can be concluded that vine vigour of ungrafted seedlings (pruning weight and trunk diameter) of F34 genotypes can be linked to vine vigour in replicated nursery trials, but more importantly to root architecture. Interestingly, root angle was negatively correlated with pruning weight only under well-watered conditions in 2007, 2008 and 2011 but not under drought conditions in 2009 or 2010, indicating that genotypes with shallow roots are less vigorous under well-watered conditions. Overall the results highlight the strong relationship between genotype vigour and root diameter (all highly significant) under both well-watered and drought conditions. Root number was also related to a lesser extent with genotype seedling vigour. Highly significant linkages with pruning weight and trunk diameter indicates that it a very useful trait for predicting ungrafted vine vigour, and a useful surrogate for predicting root diameter. In previous studies with grafted vines (Smith and Clingeleffer 2011) trunk diameter has been linked to wood density, an indicator of xylem vessel size.

Table 2.3.2.4.2. Correlation coefficients (r) between pruning weight of ungrafted rootstock genotypes over four seasons 2008-2011 including two seasons without irrigation, 2008-09 and 2009-10, and measured traits (n= 65-83). Correlations retained are significant $p< 0.05$. Highly significant correlations ($p<0.001$) are shown in bold.

	Pruning weight				***Trunk Diameter***
	2008	2009	2010	2011	2007
Pruning weight (2009)	**0.74**				
Pruning weight (2010)	**0.48**	**0.54**			
Pruning weight (2011)	**0.69**	**0.63**	**0.60**		
Root number	0.23		**0.45**	**0.46**	**0.56**
Root diameter	**0.55**	**0.56**	**0.57**	**0.60**	**0.72**
Root angle	-0.29			-0.27	-0.29
Trunk diameter	**0.64**	**0.69**	**0.61**	**0.70**	
Mean pruning wt. (2016)	0.27	**0.36**	0.30	**0.39**	**0.44**
Prun. wt, Control (2016)	0.26	**0.37**	0.28	**0.39**	**0.41**
Prun. wt, Deficit (2016)		0.29	0.26		0.36
Mean pruning wt. (2017)			0.24	0.33	**0.40**
Prun. wt, Control (2017)			0.23	0.35	**0.37**
Prun. wt, Deficit (2017)			0.24		0.28

Significant correlations between root architecture assessments of F34 genotypes and measured traits are shown in Table 2.3.2.4.3. Root type was positively correlated with root diameter and negatively correlated with root number and canopy condition in the nursery in 2016 and 2017, suggesting that root systems with plunging roots and fewer branching fine roots are more likely to maintain canopies under deficit irrigation. Root number was negatively correlated with root angle but positively correlated with root diameter and all measurements of pruning weight in the nursery, except the control in 2017. Root angle was negatively correlated with all measurements of pruning weight in the nursery trial with highly significant correlations ($p<0.001$) for the deficit treatment in 2016, the mean and control treatment in 2017. These results indicate that rootstock genotypes with broader but shallower root systems are more likely have high vigour, under well-watered and deficit irrigation treatments. Root diameter was positively correlated with all measurements of pruning weight under both well-watered and deficit treatments in the nursery and also with petiole K of the high K treatment in the glasshouse study.

Table 2.3.2.4.3. Correlation coefficients (r) measurements of single vine F34 rootstock genotypes established in the field and removed in 2011 and measured traits (n=65). (For root type, 1 has >90% branching with fine roots, 3 dominated by tap root, 2 mixture of 1 and 3. For root angle estimate, 1= 0-25^o, 2= 22.5-45.5^o, 3=45-67.5^o and 4= 67.5-90^o from horizontal. Correlations retained are significant $p< 0.05$. Highly significant correlations ($p<0.001$) are shown in bold.

	Root architecture			
	type	number	angle	diameter
Root number	-0.30			
Root angle		-0.27		
Root diameter	0.23	0.36		
Canopy condition (07/4/17) Deficit	-0.35	0.23		
Canopy condition (01/5/17) Deficit	-0.25			
Mean pruning weight. (2016)		0.27	-0.36	0.33
Pruning weight, Control (2016)		0.23	-0.29	0.31
Pruning weight, Deficit (2016)		0.23	**-0.43**	0.30
Mean pruning weight. (2017)		0.23	**-0.47**	0.33
Pruning weight, Control (2017)			**-0.46**	0.30
Pruning weight, Deficit (2017)		0.30	-0.34	0.34
Glasshouse K, high treatment				0.24

Across the F34 genotypes in the nursery trial, pruning weight in both seasons for both the well-watered control and deficit treatments were all positively correlated (Table 2.3.2.4.4). In both seasons the weakest correlations, although significant, were found between the control and deficit treatments indicating different responses by the genotypes to the different irrigation treatments.

Table 2.3.2.4.4. Correlation coefficients (r) between pruning weight of ungrafted rootstock genotypes established in the nursery under well-watered control and deficit irrigation treatment over two seasons 2016 and 2017 (n= 114). Correlations retained are significant p< 0.05. Highly significant correlations (p<0.001) are shown in bold.

	Pruning weight						***Canopy condition***		
Year		**2016**			**2017**		**April 2017**		**May 2017**
Irrigation	**mean**	**con**	**def**	**mean**	**con**	**def**	**con**	**def**	**con**
Prun. wt., con (2016)	**0.93**								
Prun. wt., def (2016)	**0.59**	0.30							
Mean prun. wt. (2017)	**0.80**	**0.76**	**0.44**						
Prun. wt., con. (2017)	**0.75**	**0.78**	0.27	**0.96**					
Prun. wt., def (2017)	**0.48**	0.27	0.76	**0.56**	0.32				
Can. condition, May con						0.21	**0.68**		
Can. condition, May def							0.27	**0.76**	0.34

2.3.2.5. Relationships between measured traits of F34 genotypes and growth rate in the Plant Accelerator, measured as part of CSP 1302

The plant accelerator study involved measurement of both the relative growth rate and absolute growth rate of small rooted plants of F34 grown under well-watered control and deficit treatments (see final report for CSP 1302 for detail and results). Correlation studies were undertaken to compare the growth rates measured for these small plants with traits determined in the field for ungrafted seedling vines, in the nursery trial and glasshouse K screening of K uptake described above (Table 2.3.2.5.1). Under well-watered control conditions relative growth rate of the genotypes, where growth is adjusted for plant size, was negatively correlated with leaf loss of seedling vines on 08/02/2010 after two seasons without irrigation. Relative growth rate was positively correlated with pruning weights of seedlings in 2009, 2010 and 2011, root and trunk diameter and mean and control irrigation pruning weight in the nursery trial in 2017. Relative growth rate of the genotypes, measured under deficit conditions, was negatively correlated with leaf loss from seedlings in the second season of drought assessed on 08/02/2010, 15/03/2010 and

28/04/2010 and positively correlated with seedling pruning weights in 2008, prior to imposition of the drought treatment, after one and two seasons of drought in 2009 and 2010 and in 2011 after recovery from drought. Relative growth rate under deficit conditions was also positively correlated with root number, root diameter and trunk diameter (both $p<0.001$). There were no correlations with absolute growth rate of the well-watered control treatment except a weak, but significant correlation, with petiole K of plants under limited K supply in the low-K glasshouse experiment. Absolute growth rate was weakly correlated, negatively, across the genotypes with leaf loss (08/02/2010) and positively with seedling pruning weight in 2009 and 2011, root number and diameter, trunk diameter and in the nursery, the overall mean pruning weight over seasons and irrigation treatments. Absolute growth rate was also weakly correlated with mean petiole K in the glasshouse screening study. The latter relationship suggests a link between plant uptake of K and plant growth.

Overall, the results with the F34 family indicate that the assessment of relative growth rate shows considerable promise as a rapid screening tool to identify genotypes for drought tolerance, in this case based on leaf loss and pruning weight under well-watered and long term drought conditions. The study has also shown that relative growth under deficit conditions was strongly linked to root and trunk diameter, and to a lesser degree, with root number. The important relationships between the relative growth rate trait across the genotypes and leaf loss, pruning weight, root and trunk diameter are shown in Figure 2.3.2.7 for both the well-watered control and deficit treatments. In most cases they show lower values and tightening of the relationships with the deficit treatment compared to the control.

Key relationships between relative growth rate and traits measured for F34 genotypes planted as field grown seedlings are shown in Figure 2.3.2.7.

2.3.2.5.1. Relationships with vine growth characteristics, measured as part of CSP 1302, in the Plant Accelerator facility and screening traits measured in the field and glasshouse, (n=35 except for K, n=72). Significant correlations retained are significant ($p<0.05$). Highly significant correlations ($p<0.001$) are shown in bold.

	Relative Growth Rate		***Absolute Growth Rate***	
	Control	**Deficit**	**Control**	**Deficit**
Leaf loss (08/2/10)	-0.49	-0.50		-0.32
Leaf loss (15/3/10)		-0.36		
Leaf loss (28/4/10)		-0.33		
Pruning weight (2008)		0.35		
Pruning weight (2009)	0.43	**0.54**		0.32
Pruning weight (2010)	0.42	**0.59**		
Pruning weight (2011)	0.41	0.43		0.35
Root number		0.37		0.33
Root diameter	**0.52**	**0.57**		0.34
Trunk diameter	**0.56**	**0.59**		0.34
Mean pruning weight (2017)	0.34			
Pruning weight, con (2017)	0.32			
Pruning weight, overall mean, con				0.31
K mean-GH				0.23
K low- GH			0.25	

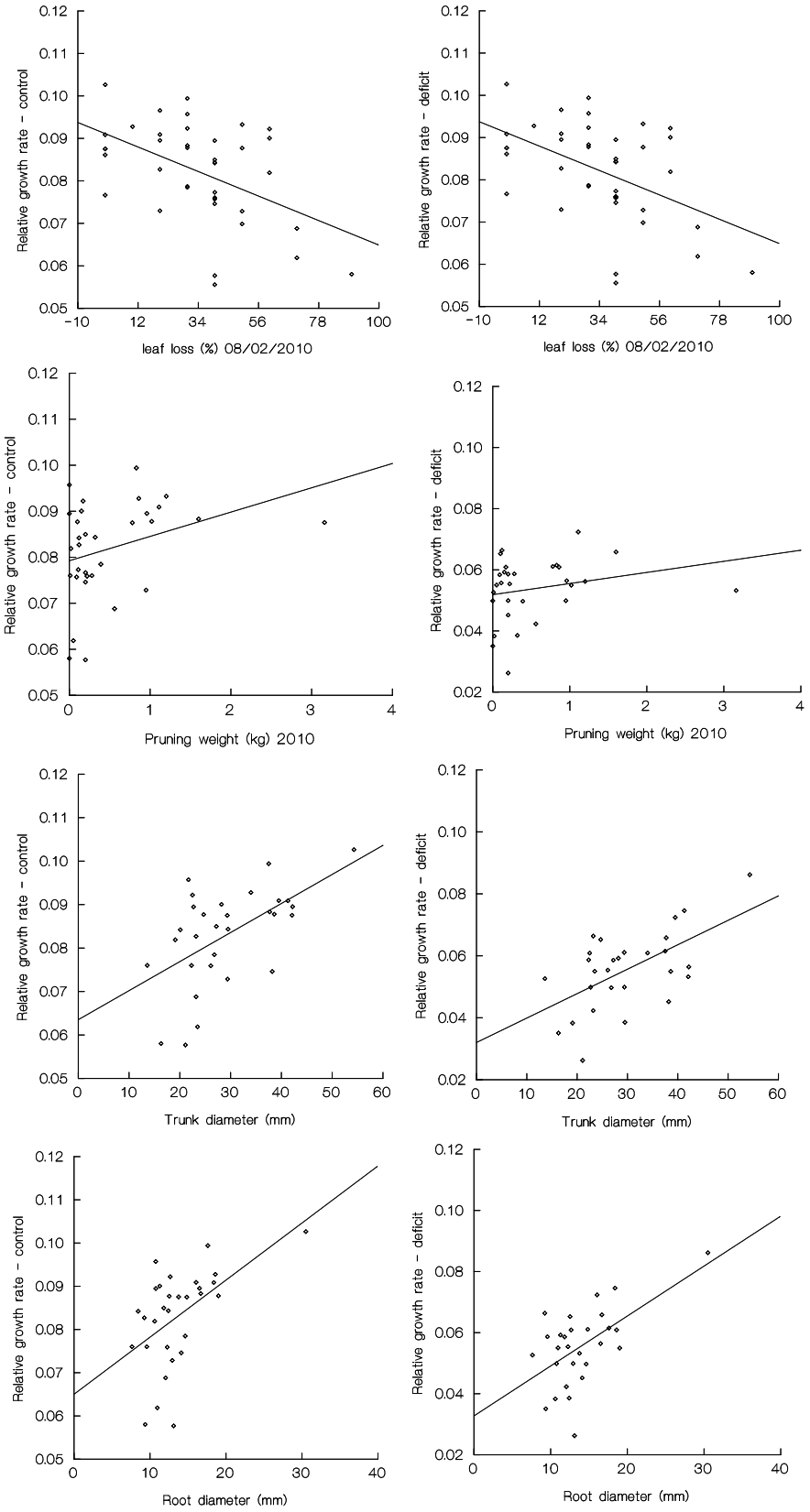


Figure 2.3.2.7. Relationships between relative growth rate of both the well-watered control and deficit treatments and measured traits (leaf loss 08/02/2010, pruning weight (2010), trunk diameter 2007 and root diameter across F34 genotypes (n=35).

2.3.2.5. Discussion and summary of F34 results

The main purpose of this study was to identify linkages between genotypic traits across a single family involving a *V. cineria* x Riesling test population (F34). In previous studies (CSP 0503) a significant amount of data had been collected for individual seedlings of F34 in the field, at the former CSIRO Merbein, including effects of long term drought where irrigation was withheld for two seasons (2009 and 2010) on leaf loss during the season and pruning weight and trunk diameter and root architecture. In this project, further data were collected for F34 genotypes in replicated trials on vine growth and canopy condition in a high density nursery trial planted in spring 2014 which included well-watered and deficit treatments and K uptake under limited and high K supply in glasshouse studies. Furthermore, vine growth data collected as part of abiotic stress screening studies for F34 in CSP 1302 was also included.

Studies in the nursery trial established highly significant effects of season, irrigation treatment and F34 genotype on pruning weight and canopy condition, assessed at the end of the season in 2017. In the case of pruning weight, there was two-fold reduction with deficit irrigation compared to the control and more than 10-fold difference across genotypes. In 2017, deficit irrigation increased leaf burn and leaf loss at the end of the growing season.

The K screening study showed significant effects of F34 genotype on petiole K under low and high K supply. Petiole K ranged from 6 - 22 mg/g with the low supply treatment and 14 - 46 mg/g with the high K supply treatment. There was no correlation across the genotypes between petiole K of the low and high K treatments, supporting the concept that K uptake is controlled by two mechanisms, i.e. active transport with low K supply and passive uptake by mass flow in high K environments (Ruhl 1989).

The results for F34 have shown large effects of genotype on all traits with distributions following normal or skewed patterns with little evidence of segregation across the population. Such results suggest that these traits are most likely to be controlled by more than a single gene and that development of molecular markers for these traits is likely to be problematic. Hence, simple trait measurement will be required for screening purposes if breeding efficiencies are to be improved and the number of individuals assessed either

as seedlings in the field or in replicated grafted trials is to be minimised. From this perspective, the results from this study are particularly encouraging as vine growth characteristics under well-watered and deficit conditions and under drought conditions could be linked to simple traits. These are discussed below.

1. Trunk diameter of young five year old seedlings in the field was a very useful trait, being negatively correlated with leaf loss during severe drought and highly correlated with pruning weight under both well-watered conditions and severe drought. It was also linked to vine vigour (pruning weight) of nursery grown vines under both well-watered and deficit treatments. Trunk diameter was also highly linked to root diameter and root number, also found to be useful traits. In previous studies (Smith and Clingeleffer 2011) trunk diameter of grafted vines has been linked to wood density, an indicator of xylem vessel size. To increase the value from a rootstock screening perspective, further study of trunk development should be undertaken to assess potential for assessment of seedlings at an early stage. Such an approach could be complemented by assessment of xylem vessel size, which also requires further investigation, in particular in relation to early measurement and to final grafted vine performance, in particular movement of water and nutrients through the plant.

2. Root diameter of field grown seedlings, assessed on removal when nine years old, was a very useful trait being negatively correlated with leaf loss during severe drought and highly correlated with pruning weight under both well-watered and severe drought. It was also linked to vine vigour (pruning weight) of nursery grown vines under both well-watered and deficit treatments. Trunk diameter was also linked to K uptake under high K supply in the glasshouse, suggesting a link to passive mass flow of K to upper parts of the plant (Ruhl 1989). Further study of root diameter development is required to assess if it can be determined at an early stage of seedling growth and used as a screening tool. It could be complemented by assessment of xylem vessel size at an early stage and to grafted vine performance, in particular movement of water and nutrients, including K, into the plant.

3. Root number of field grown seedlings, assessed on removal when nine years old, was a very useful trait being negatively correlated with leaf loss during severe

drought and correlated with pruning weight under both well-watered and severe drought. It was also linked to vine vigour (pruning weight) of nursery grown vines under both well-watered and deficit irrigation and also to canopy condition of deficit plants. Further study is required to determine if root number can be assessed at an early stage of seedling development. To facilitate this process and avoid complications associated with the development of a tap root identified above, it is suggested that the tap root be removed from seedlings at a very early stage of development in such studies.

4. The assessment of vigour and canopy condition of young plants in the nursery environment, under well-watered and deficit treatments has also proven to be very useful as they could be linked to seedling traits described above. Assessments in the nursery were able to describe significant effects on vigour of irrigation (two-fold) and genotype across the F34 population (six-fold). Interestingly the correlation for pruning weight between genotypes in the well-watered and deficit treatments, although significant were small, suggesting a strong involvement of different genes in response to the environmental cues, in this case restricted water supply. The results show that screening studies in the nursery environment can provide an early stage assessment of vine growth traits and response to irrigation treatment. Future studies could include grafted plants, from a conferred vigour perspective, and include nutrient status assessment of ungrafted and grafted vines.

5. The glasshouse screening for K uptake proved to be useful, enabling the identification of genotypes with low and high uptake under limited and excessive K supply. In general, the K uptake traits were independent of other traits, except leaf loss at the very early stage of drought in 2009 with K uptake under low supply; root diameter with K uptake under high supply; and absolute growth rate of well-watered plants with K uptake under low supply and with deficit plants, K uptake under high supply. The correlation across the genotypes between the two treatments was not significant, indicating that K uptake by different genotypes is controlled by two mechanisms, i.e. active transport in low K environments and passive uptake by mass flow in high K environments as proposed by Ruhl 1989. The links with root diameter under high K supply warrant further study, particularly the role of xylem vessel size in passive mass flow K transport. The

correlations with absolute growth rate warrant further study as they indicate relationships between K uptake and plant size which may possibly be attributed to transpiration and mass flow of K.

6. The assessment of growth rate of very young plants of the F34 genotypes produced from single node cuttings in the Plant Accelerator facility, in particular relative growth rate, offers considerable promise as a tool for early screening of rootstock seedlings for growth traits and drought tolerance. Under both well-watered and deficit treatments relative growth rate, in particular with the deficit treatment, was negatively correlated with leaf loss under drought conditions in 2010, positively correlated with seedling pruning weight, in particular under drought in 2009 and 2010 and positively correlated with trunk and root diameter and root number. More detailed studies should be undertaken to assess trunk diameter, root architecture and anatomy of the small plants to verify linkages with the traits measured in the field, to enhance the understanding of the physiological mechanisms involved and their genetic control.

2.3 Targeted selection to address gaps using molecular markers

2.3.1. Root knot nematode resistance in grapevine: screening, genetics and molecular markers development

(This study has been prepared and submitted as a research paper to a scientific journal)

Introduction

Plant parasitic nematodes cause extensive damage to a wide range of crops with an estimated cost of $80 billion (USD) per year (Davies and Elling, 2015). Greater than 4100 plant parasitic nematode species have been identified and they are classified as endoparasites (sedentary and migratory), semi-endoparasites or ectoparasites. In viticulture, rootstocks resistant to plant parasitic nematodes provide a sustainable approach to maintain productivity (Jones et al., 2013; Saucet et al., 2016).

The root knot nematodes species *Meloidogyne javanica*, *M. incognita M. hapla* and *M. arenaria* are sedentary endoparasites that cause extensive damage to a wide range of crop species including *V. vinifera* (Nicol et al., 1999; Williamson and Gleason, 2003). Root knot nematodes thrive in sandy soils and complete their life cycle by parasitising roots of susceptible plants. The root knot nematode life cycle begins after the eggs hatch and the root knot nematode juveniles migrate through the soil. Upon penetration of the root, the juvenile nematode migrates intracellularly toward the root meristem then turns and migrates up the vascular cylinder. A permanent root knot nematode feeding site is established in the zone of differentiation, in which effectors secreted by the nematode act to induce the formation of multinucleate ‘giant cells’. A gall is eventually formed when cells surrounding the giant cells undergo cell division and cell expansion. Root knot nematodes feed on the cytoplasm of the giant cells through their stylets. The root knot nematode cycle is completed after the juvenile nematode undergoes several molts and is transformed into an egg-laying female (Nicol et al., 1999; Williamson and Gleason, 2003).

Experimental results show that extensive gall formation and nematode feeding ultimately impairs grapevine root function, leading to a reduction in shoot and root growth and a marked decline in crop production (Anwar and Van Gundy, 1989; Nicole et al., 1999; Walker and Stirling, 2008). Moreover, tolerance to abiotic stresses and soil pathogens is substantially reduced as a result of root knot nematode feeding (Nicole et al., 1999; Walker and Stirling, 2008). The use of fumigants and nematicides have been used to control root knot nematode parasitism; however, these chemical treatments are often ineffective,

expensive and damaging to the environment or not permitted (Walker and Stirling, 2008). In contrast, the use of rootstocks provides a viable and sustainable management approach that allows growers to maintain healthy productive vines in the presence of root knot nematodes.

In Australia, *M. javanica* appears to be the predominant species of root knot nematode identified in the grapevine growing regions of Sunraysia, Victoria (Sauer, 1962; Harris, 1984), as well as the Barossa Valley and Riverland of South Australia (Stirling, 1982). In the Hunter Valley of New South Wales, *M. javanica* and *M. arenaria* represent common species of root knot nematodes (McLeod and Steel, 1999). The commercially available grapevine rootstocks that display resistance to root knot nematode such as Dog Ridge, Ramsey, Freedom and Harmony, are highly vigorous and take up excess potassium, which reduces wine quality (Mpelasoka et al., 2003; Whiting, 2004). Therefore, an understanding of the genetic control of root knot nematode resistance is essential for breeding new rootstocks with resistance to these pests, as well as other horticulturally favourable traits. Genetic analysis from interspecies crosses indicates that root knot nematode resistance is conferred by a single dominant locus in *V. champinii* and 1613C (Lider, 1954). The inheritance of root knot nematode resistance was examined further in commercially available rootstocks, such as Dog Ridge, Ramsey, Freedom, Harmony, 1616C and 1613C, using a Design II mating analysis (Cousins and Walker, 2002). Results from this study indicate that a single dominant locus confers root knot nematode resistance in these rootstocks. At this time, it is unclear whether the resistance locus is identical in rootstocks of different parentage. Experimental evidence indicates that a recessive resistant locus is also present in grapevine based on inheritance of root knot nematode resistance in *V. rupestris* x *V. vinifera* (Lider 1954) and 1613C x 3309C (Cousins and Walker, 2002). Since the emergence of virulent root knot nematode pathotypes, which have broken resistance to Harmony, Freedom and Ramsey (Cain et al., 1984; Anwar et al., 2000; McKenry et al., 2001; Esmendjaud and Bouquet, 2009), recent breeding efforts for root knot nematode resistance have led to the production of five new rootstocks UCD-GRN1-5 in California (Ferris et al., 2012).

The goals for the research in this chapter were to (1) map the root knot nematode resistant locus in *Vitis cinerea*, (2) validate and characterise molecular markers flanking the resistance locus, (3) screen rootstock material targeted for release and (4) isolate a new

aggressive root knot nematode pathotype and begin screening rootstock material for resistance.

Materials and Methods

Plant Material

A *Vitis cinerea* accession (C2-50) was used as a female parent in three crosses for rootstock breeding. One hundred and twenty F_1 individuals from C2-50 x Riesling plus C2-50 and Riesling were screened for root knot nematode resistance (Table 1). Forty-two backcrossed (BC)-individuals (F35-4 [C2-50 x Pinot Meunier microvine] x Pinot Meunier picovine) plus F35-4 and Pinot Meunier picovine were also screened from root knot nematode resistance (Table 1). Forty-four rootstock selections from C2-50 x Schwarzmann were screened for root nematode resistance, plus C2-50, Schwarzmann and Riesling (Table 1). Lastly, twenty-one selected rootstocks were screened for root knot nematode resistance, which included 140 Ruggeri, 1103 Paulsen, K49-56, K5-02, K5-04, K5-20, C114, *Vitis amurensis* accession, LN33, 171-13L and 11 F_1 individuals derived from C2-50 x Cabernet Sauvignon. In total, 278 genotypes were screened for root knot nematode resistance (Table 1). For root knot nematode screening, at least three dormant cuttings for the material above was propagated and transplanted into 6.5 x 6.5 x 20 cm pots for nematode screening. In total, 933 propagated cuttings were screened for root knot nematode resistance during the duration of the project (Table 1).

Root knot nematode screening

The aggressive *M. javanica* 'pt 1103P' pathotype, which is able to effectively parasitise the moderately resistant 1103 Paulsen rootstock, was used in the root knot nematode screening assay (Smith et al., 2017). *M. javanica* 'pt. 1103P' was propagated on susceptible *Solanum lycopersicum* (tomato) cv California Red Cherry plants at 22-25^{o}C in a glasshouse. Eight weeks after inoculation, galls containing the *M. javanica* 'pt. 1103P' egg masses were dissected from the infected tomato roots and incubated in water at 30°C for 72 h to promote hatching. Approximately 1000 *M. javanica* 'pt. 1103P' juveniles were added to each potted cutting and incubated for eight weeks in the glasshouse at 22-25°C. Plants were classified as susceptible if the root knot nematodes were able to complete their life cycle by producing egg masses on the roots. For egg mass determination, the roots for each replicated cutting were washed gently in water to remove the soil and incubated in 0.01 g/L of erioglaucine disodium salt for 60 min to stain the egg masses. Egg masses were counted under a Daylight

Magnifying Lamp (Model number: A22020-01). A chi-square goodness of fit test was performed with the phenotype data using X^2=total of $(O-E)^2/E$ for resistance and susceptible phenotypes, with one degree of freedom.

An aggressive *M. arenaria* pathotype referred to as *M. arenaria* 'pt 1103Pa', was isolated from a vineyard in the Barossa Valley planted on the 1103 Paulsen rootstock. Tomato plants were grown with soil samples taken from the infected area of the vineyard and root knot nematode galls were produced on the plants eight weeks after inoculation. Genotype determination showed that all twenty females genotyped were *M. arenaria*. Egg masses produced by these females were hatched and a single tomato plant was inoculated with the juveniles. This root knot nematode genotype and purification cycle was performed again, and the subsequent *M. arenaria* 'pt 1103Pa' juveniles were used to inoculate the 42 F_1 C2-50 x Riesling plants (42 x 3 replicated plants). Screening for root knot nematode resistance is described above.

Genotyping-by-sequencing

DNA isolation for C2-50, Riesling and the F_1 individuals was performed at the Australian Genome Research Facility using the Nucleic Acid Extraction service, which utilises the NucleoSpin® 96 Plant II DNA extraction kit (http://www.mn-net.com). The Institute for Genomic Diversity at Cornell University provided the GBS service, which included the construction of the ApeKI libraries, Illumina HiSeq 2000/2500 DNA sequencing and SNP calling using a TASSEL-GBS pipeline, v3.0.166 (Glaubitz et al., 2014). Note: the library generated from a 96 well-plate containing the 90 F_1 individuals and two samples of Riesling and C2-50 was sequenced 2X in order to maximise the number of reads at each locus. For the SNP calling procedure, the 64 bp sequence tags were aligned to the 12X *V. vinifera* 'PN40024' reference genome. The called SNPs were filtered using VCFtools v.1.12b (Danecek et al., 2011). In this procedure, SNPs were filtered with an average depth of read coverage >10 and a minor allele frequency >0.2, 0% missing data and a genotype quality score >98%. Next, SNPs with an allele frequency between 40–60% were retained. After filtering, the SNP set was parsed into two data sets based on a pseudo-test cross mapping strategy (Grattapaglia and Sederoff, 1994). The C2-50 SNP set containing 3974 SNPs was obtained by retrieving SNPs that were heterozygous in C2-50 and homozygous in Riesling. The Riesling SNP set consisted of SNPs that were homozygous in C2-50 and heterozygous in Riesling. The Riesling SNP set contains 2973 SNPs.

Single SNP association analysis

Single SNP association analysis using a general linear model (GLM) was performed using TASSEL 5.0 (Bradbury et al., 2007) with the C2-50 and Riesling SNP sets. In this procedure, the results of the root knot nematode screening were converted to a 'trait' file in which the resistant and susceptible phenotypes were converted to a '0' or '1' numerical value, respectively. After transforming the SNP data sets to numerical values, principal component analysis was performed to reduce spurious associations due to population structure. The raw *p*-values identified in the single SNP association analysis were adjusted for false discovery rate according to the Bonferroni and Benjamini-Hochberg procedures using the R/multtest package (Pollard et al., 2005).

Linkage map construction

Linkage mapping was performed with R/OneMap (Margarido et al., 2007) using the Kosambi function. SNPs were ordered with a linkage LOD of 3.0 and a recombination frequency of 0.25. The C2-50 (3974 SNPs) and Riesling (2973 SNPs) SNP sets were reduced to 367 and 403 SNPs, respectively, by limiting the distance between markers to 2.0-5.0 cM and removing markers with a high mean recombination fraction and low mean LOD score using the 'rf.graph.table' function.

SNP validation

DNA was isolated from C2-50, Riesling, 65 C2-50 x Riesling F_1 individuals, Pinot Meunier microvine, Pinot Meunier picovine, F35-4, and 28 BC-individuals (F35-4 [C2-50 x Pinot Meunier microvine] x Pinot Meunier picovine) individuals using the NucleoSpin® 96 Plant II DNA extraction kit. Thirty SNPs that spanned the *M. javanica* 'pt. 1103P' resistance locus were validated using the SNP genotyping Sequenom MassARRAY iPLEX platform (Sequenom, San Diego, CA, USA) service at the Australian Genome Research Facility. The SNP genotypes determined by the GBS pipeline and the Sequenom MassARRAY platform were compared to validate each SNP.

Results

Phenotype analysis of root knot nematode resistance

Experimental results indicated that *V. cinerea* is resistant to *M. incognita* (Walker et al., 1994). In support of this observation, zero *M. javanica* 'pt. 1103P' egg masses were

observed on the roots of three propagated cuttings from C2-50, a *V. cinerea* accession in the CSIRO Rootstock Collection (Table 2). Therefore, C2-50 was chosen as the female parent for mapping a root knot nematode resistance locus in grapevine. As indicated by the mean egg masses and mean egg masses per root weight, *M. javanica* 'pt. 1103P' was able to effectively parasitise the roots of the Riesling demonstrating that this *V. vinifera* cultivar is susceptible to *M. javanica* 'pt. 1103P' (Table 2). Therefore, to map *M. javanica* 'pt. 1103P' resistance, a cross was made between C2-50, which produces only female flowers, and Riesling, which initiates hermaphrodite flowers.

Using a glasshouse based root knot nematode screening method (Smith et al., 2017), 120 F_1 individuals plus C2-50 and Riesling were screened for *M. javanica* 'pt. 1103P' resistance (Table 1). In this screening assay, at least three propagated cuttings per individual were screened. Results showed that *M. javanica* 'pt 1103P' was able to effectively parasitise the roots of Riesling and 52 out of the 120 F_1 individuals. In this experiment, gall and egg mass development occurred in all three replicates for Riesling and 49 susceptible genotypes. Three genotypes, which had egg mass development on 2 out of the 3 replicates, were rated as susceptible. For the replicates having zero egg masses, as well as individuals with <10 egg masses (data not shown), the screening was performed in winter months, a time in which the nematodes appear to be less parasitic. To further verify the genotype of these three susceptible individuals, additional replicated screens should be performed in the summer months when nematodes are highly parasitic. Overall, the average number of egg masses per root system for the 52 susceptible F_1 individuals was 42.1. For the remaining 68 F_1 individuals screened for *M. javanica* 'pt. 1103P' resistance, no egg masses or galls were detected on the roots in all three replicates.

A chi-square goodness of fit test was performed to determine whether the phenotypic ratio for *M. javanica* 'pt. 1103P' resistance segregates with a 1:1 ratio. Using one degree of freedom, the chi-square value was 2.13, which is less than the critical value of 3.84. In addition, the probability was >0.05 indicating that *M. javanica* 'pt. 1103P' resistance segregates with a 1:1 ratio. Therefore, the data support a model that *M. javanica* 'pt. 1103P' resistance, referred to as *MJR1*, is conferred by a single dominant locus in C2-50.

Genotyping-by-sequencing and SNP filtering

Genotyping-by-sequencing (GBS) was performed on 90 out of the 120 F_1 individuals, as well as C2-50 and Riesling parents. This was due to the fact that genotyping service offered at the University of Cornell performed GBS on 95 samples at a time. As a result, we were unable to obtain GBS data for the entire population. After next generation sequencing followed by SNP calling, a 1.3M SNP set was produced (Figure 1). Given that grapevine is highly heterozygous, SNPs were filtered with an average depth of sequence coverage greater than 10 and a genotype quality score greater than 98%, to reduce genotyping errors (Figure 1). After SNP filtering, the 1.3M SNP set was reduced to 18,124 SNPs (Figure 1). For single SNP association and linkage mapping, the 18,124 SNP set was further filtered by retaining SNPs with an allele frequency from 40-60% than parsing the SNP set for a pseudo-test cross mapping strategy (Grattapaglia and Sederoff, 1994). Subsequently, a SNP set of 3974 and 2973 was produced for C2-50 and Riesling, respectively (Figure 1). Note: SNPs heterozygous in C2-50 and homozygous in Riesling were retained in the C2-50 SNP set while the opposite set of SNPs was retained in the Riesling SNP set. The 1:1 phenotype ratio for *M. javanica* 'pt 1103P' resistance indicates that the C2-50 is heterozygous for the *MJR1* locus. Therefore, the expectation is that SNPs associated or linked to *MJR1* will be identified only in the C2-50 SNP set.

Single SNP association analysis

A single SNP association analysis was performed to map *MJR1* using the C2-50 (3974 SNPs) and Riesling (2973 SNPs) SNP sets. Single SNP association using the C2-50 SNP set identified 7 SNPs on chromosome 18 had high association based on *p*-values equal to 0 and R^2 equal to 1 (Table 3). In addition, most of the immediate set of flanking markers also displayed high association with *p*-values ranging from 4.26E-58 to 1.45E-27 and R^2 between 0.789 to 0.955 (Table 3). In contrast, single SNP association performed with the Riesling SNP set did not show any significant association, as the adjusted *p*-values were >0.05 for both Bonferroni and Benjamini-Hochberg (data not shown). In addition, the highest R^2 value was equal to 0.170 (data not shown). Taken together, results indicate that *MJR1* is located on chromosome 18 in C2-50.

Genetic mapping of the *MJR1* locus

The C2-50 and Riesling SNP sets were reduced to 367 and 403 SNPs, respectively, as described in the materials and methods and R/OneMap was used to curate and construct the genetic maps. For each SNP set, 19 linkage groups (LGs) were produced and the final size for the C2-50 and Riesling genetic maps were 1587.3 and 1706.4 cM, respectively (Table 4 and 5), which is similar in size to other *Vitis* genetic maps produced by next generation sequencing (Barba et al., 2014; Hyma et al., 2015; Cadle-Davidson et al., 2016; Yang et al., 2016). The map density or average distance between SNP markers for C2-50 and Riesling genetic maps was 4.3 and 4.2 cM, respectively.

In order to map the *MJR1* locus, the phenotype data for each F_1 individual was converted to a genotype. Given that the phenotype data closely matched a 1:1 ratio, it is highly likely that the genotype of the C2-50 was heterozygous for *MJR1*, while Riesling was homozygous recessive. Therefore, resistant and susceptible F_1 individuals were assigned either an *MJR1/MJR1* or *MJR1/MJR1* genotype, respectively. To map the *MJR1* locus, the *MJR1* marker was included in the reduced C2-50 (367) and Riesling (403) SNP sets and linkage analysis was performed using R/OneMap. Results from the genetic mapping showed that *MJR1* mapped to linkage group 18 (LG18) at 102.6 cM using the C2-50 367 SNP set (Figure 2; Table 6). In this analysis, *MJR1* was flanked by S18_30104122 and S18_33162606 at 98.1 and 105.9 cM, respectively. When the *MJR1* marker was included in the Riesling 403 SNP set, *M. javanica* 'pt 1103P' resistance was not mapped to any of the 19 linkage groups (data not shown).

SNP validation and genetic mapping of *MJR1*

The goal of mapping the *MJR1* locus was to identify a set of SNPs for predicting resistance to *M. javanica* 'pt. 1103P' for marker assisted selection. Therefore, 36 SNPs that mapped in close proximity to *MJR1* were selected for SNP genotyping using the Sequenom MassARRAY platform (Oeth et al., 2009). In this analysis, 6 SNPs could not be assessed including S18_30104122, which flanked MJR1 (Figure 2), due to primer design constraints or failed PCR-genotype assays (data not shown). After performing the SNP genotyping, 15 out of the remaining 30 SNPs genotyped were polymorphic and the genotypes matched with those predicted by TASSEL (Table 7). In addition, the major and minor allele frequencies identified by the Sequenom MassARRAY and TASSEL were equivalent. Of the 30 Sequenom MassARRAY genotyped SNPs, 11 SNPs were non-polymorphic and did not match the genotype predicted by TASSEL (Table 7). In addition, four polymorphic SNPs,

S18_31481177, S18_33162606, S18_33959722 and S19_34060245 verified by Sequenom MassARRAY did not match the genotype determined by GBS (Table 7). Taken together, the GBS pipeline accurately predicted the SNP genotype with a 50% success rate at the *MJR1* locus.

To validate the previous genetic mapping results, a 382 SNP set was created, which included the 15 accurately genotyped SNPs at the *MJR1* locus as well as the *MJR1* marker. In addition, S18_30104122 was removed from this SNP set, as the genotype of this marker could not be verified. Results from R/OneMap analysis showed that *MJR1* mapped to LG18 at position 100.3 cM (Figure 3; Table 8). The SNPs S18_31787750, S18_31886894, S18_32680428, S18_33070954 cosegregated with *MJR1* at position 100.3 cM. SNPs flanking *MJR1* at 99.2 (S18_30711041, S18_30722375 and S18_31160355) and 101.4 cM (S18_33954011) may serve as useful markers for marker-assisted selection (Figure 3; Table 8). In table 9, the validated R/OneMap data were combined with the association mapping data and results showed that the *MJR1* co-segregating SNPs at 100.3 all had adjusted *p*-values and R^2 values were equal to 0 and 1, respectively. The SNPs flanking *MJR1* at 99.2 and 101.4 cM also had high association as reflected by the R^2 and *p*-values. Overall, as the genetic distance increases for SNPs linked to *MJR1* there is a decline in association (Table 9). Taken together, combining results from the genetic mapping and SNP association after Sequenome MassARRAY validation correlated well and provide complementary methods for validating the mapping of *MJR1*.

Marker assisted breeding with SNPs tightly linked to the *MJR1* locus

To further evaluate markers linked to *MJR1*, C2-50 was crossed to the Pinot Meunier microvine and an F_1 individual, F35-4, was identified that was resistant to *M. javanica* 'pt 1103P'. A backcross (BC) was performed in which F35-4 was crossed to the Pinot Meunier picovine and 42 BC-individuals were established by embryo rescue. Three replicated plants for each of the 42 BC-individuals were propagated and screened for root knot nematode resistance using the glasshouse based nematode screening method (Smith et al., 2017). Results showed that F34-5 and 18 BC-individuals were resistant to *M. javanica* 'pt 1103P', as egg mass and gall development was not observed on any of the replicated roots. In contrast, the Pinot Meunier picovine and 24 out of the 42 BC-individuals were susceptible, as *M. javanica* 'pt 1103P' was able to effectively parasitise the roots in all three replicates.

The phenotype ratio (18 resistant:24 susceptible) for the 42 BC-individuals closely matched a 1:1 segregation, which was observed in the F_1 C2-50 x Riesling population. To verify this, a chi-square test was performed using one degree of freedom. Results showed that the chi-square value was 0.857, which is significantly less than the critical value of 3.84. In addition, the probability was >0.05 indicating that *MJR1* segregates with a 1:1 ratio. The data support a model that *MJR1* acts as a single dominant locus when backcrossed into the Pinot Meunier picovine. Therefore, the Pinot Meunier picovine will serve as a rapid breeding system to generate new rootstocks with *MJR1*.

To evaluate the fifteen validated markers that are linked and cosegregate with *MJR1*, DNA was extracted from 28 out of the 42 BC individuals and Sequenome MassARRAY analysis was performed using the 15 validated SNPs linked to *MJR1* (Table 1). In C2-50, F34-5 and all F_1 C2-50 x Riesling individuals that have *MJR1*, the genotype for S18_30711041 is GT, while in Riesling and the susceptible F1 C2-50 x Riesling individuals the genotype is GG (Table 3). Interestingly, the genotype for S18_30711041 was TT and GG for resistant and susceptible BC-individuals, respectively. At this time, it is uncertain as to why the genotype of S18_30711041 would change in the resistant BC-individuals. The genotypes for the remaining 14 validated markers in table 3 were identical with in the C2-50 x Riesling and BC-individuals, indicating that these 14 validated markers can be successfully used for breeding *MJR1* in the Pinot Meunier picovine.

***MJR1* confers resistance to *M. arenaria* 'pt 1103Pa'**

Soil surveys performed in the major grapevine growing regions of Australia indicate that *M. javanica* is the predominant species of root knot nematode (Sauer, 1962; Harris, 1984; Stirling, 1982). However, it is essential that the CSIRO Rootstock Breeding Program develops new rootstocks with a broad range of resistance to root knot nematode species. For example, we recently identified *M. arenaria* 'pt 1103Pa' from a vineyard in the Barossa Valley (see Materials and Methods). This *M. arenaria* pathotype can successfully parasitise 1103 Paulsen rootstock in the field and in potted glasshouse trials. To determine if *MJR1* mediates resistance to *M. arenaria* 'pt 1103Pa', three replicated plants were propagated for 42 F_1 C2-50 x Riesling individuals as well as C2-50 and Riesling plants, which were used as controls. Thirty-two out of the 42 F_1 C2-50 x Riesling plants were resistant to *M. javanica* 'pt 1103P' and the remaining 10 individuals were susceptible. After screening the 42 F_1 C2-50 x Riesling individuals, we found that all 32 plants resistant to *M. javanica* 'pt 1103P'

were also resistant to *M. arenaria* 'pt 1103Pa'. In addition, all 10 plants susceptible to *M. javanica* 'pt 1103P', were also susceptible to *M. arenaria* 'pt 1103Pa'. Therefore, these results indicate that *MJR1* confers resistance to two species of root knot nematode, *M. javanica* and *M. arenaria*. Future work is aimed at determining whether *MJR1* confers resistance to *M. incognita*.

Evaluation of the Family 86 rootstock breeding population for *M. javanica* 'pt 1103P' resistance

Family 86 is a rootstock breeding population derived from C2-50 x Schwarzmann. As shown above, C2-50 is resistant to *M. javanica* 'pt 1103P' (Table 1). Schwarzmann, which is derived from *V. riparia* x *V. rupestris*, is also resistant to *M. javanica* 'pt 1103P' (Smith et al., 2017). To identify F_1 C2-50 x Schwarzmann individuals with root knot nematode resistance, forty-four F_1 individuals were screened with *M. javanica* 'pt 1103' plus C2-50, Schwarzmann, and Riesling, which served as controls. Results showed that 32 F_1 individuals were resistant, while 12 were susceptible.

Results from screening F_1 C2-50 x Riesling individuals showed that C2-50 is heterozygous for *MJR1*. However, the heritability of root knot nematode resistance in Schwarzmann has yet to be characterised. If Schwarzmann is homozygous dominant for root knot nematode resistance than all F_1 C2-50 x Schwarzmann individuals would be resistant and segregate with a 1:0 ratio (1 resistant: 0 susceptible). However, results from the *M. javanica* 'pt 1103P' screening assay was closely matched a pattern equivalent to a 3:1 segregation ratio (3 resistant:1 susceptible), indicating that Schwarzmann is heterozygous dominant for root knot nematode resistance. Using one degree of freedom, a chi-square test was performed and results showed that the chi-square value was 0.121, which is less than the critical value of 3.84. In addition, the probability was >0.05 indicating that Schwarzmann is heterozygous dominant for root knot nematode resistance, which is referred as *MJR2*.

Durable resistance is achieved when two different resistance loci are combined in a single individual. Based on the heritability of *MJR1* and *MJR2* from C2-50 and Schwarzmann, respectively, approximately 11 F1 individuals would have both *MJR1* and *MJR2*. Using the *M. javanica* 'pt 1103P' screening assay, it is not possible to identify F1 individuals having *MJR1* and *MJR2*. Genetic mapping of *MJR2* and characterisation of linked molecular markers would provide the necessary tools together with the SNPs linked to *MJR1* to screen

and identify F1 individuals harboring *MJR1* and *MJR2*. Future work is aimed at genetic mapping of *MJR2* in 101-14, which has the same pedigree as Schwarzmann, and the *M. javanica* ‘pt 1103P’ resistance locus in Dog Ridge.

Discussion

In this report, we showed that a single dominant locus, called *MJR1*, confers resistance to *M. javanica* ‘pt 1103P’ and *M. arenaria* ‘pt 1103Pa’. Genotyping by sequencing was used to identify polymorphic markers, SNPs, which were used to map *MJR1* to chromosome 18 via single SNP association and linkage mapping. We also provide evidence Schwarzmann is dominant heterozygous for *M. javanica* ‘pt 1103P’ resistance. Genetic studies indicate that resistance to *M. incognita* is conferred by a single dominant locus in the *V. champinii* rootstocks Harmony, Freedom, Dog Ridge and Ramsey (Cousins and Walker, 2002). At this time, it is not clear as to whether the location and identity of root knot nematode resistance locus and gene(s), respectively, is similar in C2-50, Schwarzmann and the *V. champinii* derived rootstocks.

Genetic and molecular studies have resulted in the identification and functional characterisation of root knot nematode resistance genes in *S. peruvianum, S. arcanum* and *P. cerasifera* (Milligan et al., 1998; Jablonska et al., 2007; Claverie et al., 2011). These root knot nematode genes encode classic resistant (R)-proteins containing a nucleotide binding (NB) and leucine-rich repeat domains (LRR). Candidate *R*-genes containing the NB and LRR domains have been identified in the *Vitis vinifera* genome (Di Gaspero and Cipriani 2002, 2003; Donald et al., 2002; Di Gaspero et al., 2007). An integrated genetic map derived from two grapevine crosses was used to map 82 *R*-genes and results showed that clusters of *R*-genes are located on chromosome 18 (Di Gaspero et al., 2007), which may overlap with the region corresponding to *MJR1*. While *M. javanica* ‘pt. 1103P’ resistance is conferred by C2-50, 16 *R*-genes, annotated as TMV resistance genes and 5 disease resistance genes implicated in plant immunity are located between S18_30711041 and S18_33954011 on chromosome 18 of *V. vinifera* (data not shown). Interestingly, a recent study identified two related *R*-genes, resistance to *Uncinula necator* (*MrRUN1*) and resistance to *Plasmopara viticola* (*MrRPV1*), on chromosome 12 in *M. rotundifolia*, a wild grapevine species related to *V. vinifera* (Feechan et al., 2013). Results showed that a high

level of synteny was observed between the *MrRUN1/MrRPV1* locus in *M. rotundifolia* and the corresponding region on chromosome 12 in *V. vinifera*. Furthermore, a high level of sequence similarity between *MrRUN1/MrRPV1* and the *V. vinifera R*-genes in this syntenic region of chromosome 12 indicated that this group of *R*-genes from *V. vinifera* and *M. rotundfolia* are evolutionarily related. Given that *R*-like and plant immunity genes are located between S18_30711041 and S18_33954011 on chromosome 18 of *V. vinifera* (data not shown), it may be possible that the *M. javanica* 'pt. 1103P' resistance is conferred by a related *R*-gene(s) or plant immunity gene(s) at the *MJR1* locus in C2-50.

Recent studies have utilised a GBS approach to generate genetic maps for identifying favourable horticultural traits including powdery mildew resistance/susceptibility, flower sex and fruit quality traits in grapevine (Wang et al., 2012; Barba et al., 2013; Hyma et al., 2015; Cadle-Davidson et al., 2016; Yang et al., 2016). In our work, next generation sequencing followed by SNP calling/filtering further demonstrates the feasibility of using a GBS approach for SNP discovery, linkage map construction and mapping at least one Mendelian trait. However, validation of SNPs using Sequenom MassARRAY analysis indicated that the GBS pipeline results do not easily transfer with only 50% of the SNPs producing useful markers at the *MJR1* locus. Given that the SNPs were filtered with an average depth of read >10, it is less probable that sequencing errors could account for the 50% errors in SNPs genotypes. In contrary, called SNPs were filtered with a SNP quality score >95%, suggesting that errors in aligning sequence tags (64 bp in length) to the 12X *V. vinifera* 'PN40024' reference genome occurred. The SNP calling errors are likely due to sequence repeats or paralogous duplicate regions in the grapevine reference genome.

Single SNP association analysis is used to map traits of interest in populations of unrelated individuals (Hamblin et al., 2011). However, a recent study, which utilised single SNP association together with interval mapping, identified a QTL for powdery mildew susceptibility in a grapevine F_1 mapping population (Barba et al., 2014). Results showed that most SNPs with significant LOD values were highly associated with powdery mildew susceptibility. For *M. javanica* 'pt 1103P' resistance, SNPs that co-segregated and were tightly linked to *MJR1* also displayed significant association with nematode resistance. As indicated by Barba et al., 2014, single SNP association can easily be performed with a greater number of markers compared to standard genetic mapping software. Therefore,

single SNP association may serve as a useful tool to narrow down a set of markers for genetic mapping.

Conclusions

In summary, traditional breeding approaches to develop rootstocks with resistance to plant parasitic nematodes and other soil borne pests is costly, time consuming and dependent upon labor-intensive work including plant propagation and nematode screening. However, employing a marker assisted breeding approach with molecular markers that predict nematode resistance would be an efficient and cost-effective approach. Moreover, marker assisted selection will allow for stacking multiple root knot nematode resistant loci into a single genetic background for producing new rootstocks with durable resistance. In this research, we have identified two sets of SNPs that flank the *MJR1* locus on LG18 at positions 99.2 and 101.4 cM, which spans 2.3 cM region, as well as four markers that co-segregate with *MJR1* resistance. Based on the alignment of the sequence tags to the 12X *V. vinifera* 'PN40024' reference genome, the estimated distance between S18_30711041 and S18_33954011 is approximately 3.24 Mb. Together, the genetic mapping of *X. index* resistance, *XiR1* (Xu et al., 2008; Hwang et al., 2010), and *MJR1* provide breeders with the molecular tools for pyramiding plant parasitic resistance loci for rootstock development.

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Table 1: Root knot nematode screening

Population:	Root knot nematode species	Genotypes	Total Plant Screened
C2-50 x Riesling	*M. javanica* 'pt 1103P'	122	485
C2-50 x Riesling	*M. arenaria* 'pt 1103Pa'	44	132
F35-4 x Pinot Meunier picovine	*M. javanica* 'pt 1103P'	44	132
C2-50 x Schwarzmann	*M. javanica* 'pt 1103P'	47	141
Other rootstock material	*M. javanica* 'pt 1103P'	21	43
		Total = 278	Total = 933

Note: Each genotype was screened at least three times to verify the phenotype. The number of genotypes per population includes the two parents, which were screened and used as controls.

Table 2: *M. javanica* 'pt. 1103P' resistance in the C2-50 and Riesling

Genotype	**EM**	**DR-wt**	**EM/DR-wt**
C2-50	0	2.89 ±0.94	0
Riesling	20.0 ±15.6	2.90 ±0.75	5.58 ±2.04

EM = mean egg masses
DR-wt = mean dry root weight
EM/DR-wt = mean egg masses per dry root weight (g)

Table 3: Single SNP association (GLM) for *M. javanica* 'pt1103P' resistance.

SNP ID	Raw *p*-value	Adj. *p*-value Bonferroni	Adj. *p*-value Ben-Hoch	R^2
S18_30104122	1.18E-34	4.69E-31	9.57E-33	0.825
S18_30104225	5.32E-40	2.11E-36	5.56E-38	0.868
S18_30226628	2.54E-47	1.01E-43	3.26E-45	0.910
S18_30235933	3.66E-31	1.45E-27	2.85E-29	0.789
S18_30236024	2.54E-47	1.01E-43	3.26E-45	0.910
S18_30381428	2.36E-60	9.38E-57	4.26E-58	0.955
S18_30515641	2.54E-47	1.01E-43	3.26E-45	0.910
S18_30711041	2.36E-60	9.38E-57	4.26E-58	0.955
S18_30722375	2.36E-60	9.38E-57	4.26E-58	0.955
S18_30764625	2.36E-60	9.38E-57	4.26E-58	0.955
S18_31010973	5.80E-35	2.30E-31	4.80E-33	0.828
S18_31059094	2.36E-60	9.38E-57	4.26E-58	0.955
S18_31158466	0.034	1	0.251	0.051
S18_31158467	0.034	1	0.251	0.051
S18_31160355	2.36E-60	9.38E-57	4.26E-58	0.955
S18_31164496	2.36E-60	9.38E-57	4.26E-58	0.955
S18_31196588	2.36E-60	9.38E-57	4.26E-58	0.955
S18_31481177	2.36E-60	9.38E-57	4.26E-58	0.955
S18_31547619	0.608	1	0.922	0.003
S18_31624788	0.450	1	0.911	0.007
S18_31787750	0	0	0	1
S18_31822250	7.49E-48	2.98E-44	1.24E-45	0.913
S18_31886894	0	0	0	1
S18_32027399	1.77E-60	7.03E-57	4.26E-58	0.955
S18_32680428	0	0	0	1
S18_33070954	0	0	0	1
S18_33070972	0	0	0	1
S18_33070983	0	0	0	1
S18_33070986	0	0	0	1
S18_33162605	2.36E-60	9.38E-57	4.26E-58	0.955
S18_33162606	5.32E-40	2.11E-36	5.56E-38	0.868
S18_33276771	2.36E-60	9.38E-57	4.26E-58	0.955
S18_33388900	5.80E-35	2.30E-31	4.80E-33	0.828
S18_33757536	0.977	1	0.978	9.4103E-06
S18_33876484	2.54E-47	1.01E-43	3.26E-45	0.910
S18_33876485	2.54E-47	1.01E-43	3.26E-45	0.910
S18_33954011	2.36E-60	9.38E-57	4.26E-58	0.955
S18_33959722	5.32E-40	2.11E-36	5.56E-38	0.868
S18_34060245	5.80E-35	2.30E-31	4.80E-33	0.828
S18_34140592	1.31E-47	5.21E-44	2.08E-45	0.912

Raw *p*-values obtained from TASSEL 5.0 and adjusted (Adj.) were shown. The 3974 SNP set was used in the association analysis. Benjamini-Hochberg (Ben-Hoch)

Table 4: C2-50 Genetic Map using 367 SNPs

LG Number	Size (cM)
LG1	98.2
LG2	75
LG3	67
LG4	89.1
LG5	79.5
LG6	81.5
LG7	86.3
LG8	93.1
LG9	117.1
LG10	72.5
LG11	111.6
LG12	54.7
LG13	95.9
LG14	108.4
LG15	55.2
LG16	83.7
LG17	56.9
LG18	109.3
LG19	52.3
Total Size	1587.3

Table 5: Riesling Genetic Map using 403 SNPs

LG Number	Size (cM)
LG1	138.6
LG2	111.9
LG3	93.7
LG4	105
LG5	102.5
LG6	131.5
LG7	82.6
LG8	74.1
LG9	66.0
LG10	90.7
LG11	17.8
LG12	88.0
LG13	106.2
LG14	115.9
LG15	76.9
LG16	69.3
LG17	51.6
LG18	103.9
LG19	80.2
Total Size	1706.4

Table 6: The position of *MJR1* on LG18

SNP ID	cM
S18_325393	0.0
S18_516915	7.8
S18_961949	13.4
S18_2352399	16.8
S18_3422830	22.3
S18_4042023	26.8
S18_4349352	29.0
S18_4722224	31.2
S18_5173458	34.6
S18_5131100	37.9
S18_7610729	41.3
S18_7880648	43.5
S18_9345851	46.8
S18_10387891	50.2
S18_11175529	56.9
S18_11730974	61.3
S18_11730982	63.5
S18_12707428	68.0
S18_12709644	71.3
S18_13446851	75.8
S18_18671664	82.5
S18_22504012	88.1
S18_27884817	95.9
S18_30104122	98.1
MJR1_Marker	102.6
S18_33162606	105.9
S18_34276271	109.3

Table 7: Validation of 30 SNPs at the *MJR1* locus

		Sequenom MassARRAY			TASSEL		
SNP ID	Chr	Genotype	MF	MAF	Genotype	MF	MAF
S18_26580875	18	GG/GA	0.72	0.28	GG/GA	0.72	0.28
S18_26558715	18	GG/GT	0.73	0.27	GG/GT	0.73	0.27
S18_27884817	18	CC/CT	0.73	0.27	CC/CT	0.73	0.27
S18_28372660	18	AA/AG	0.73	0.27	AA/AG	0.73	0.27
S18_30104225	18	CC/CG	0.72	0.28	CC/CG	0.72	0.28
S18_30226628	18	AA/AG	0.72	0.28	AA/AG	0.72	0.28
S18_30236024	18	GG/GA	0.72	0.28	GG/GA	0.72	0.28
S18_30711041	18	GG/GT	0.71	0.29	GG/GT	0.71	0.29
S18_30722375	18	GG/GA	0.71	0.29	GG/GA	0.71	0.29
S18_31160355	18	AA/AG	0.71	0.29	AA/AG	0.71	0.29
S18_31787750	18	GG/GA	0.72	0.28	GG/GA	0.72	0.28
S18_31886894	18	AA/AT	0.72	0.28	AA/AT	0.72	0.28
S18_32680428	18	AA/AG	0.72	0.28	AA/GA	0.72	0.28
S18_33070954	18	GG/GA	0.72	0.28	GG/GA	0.72	0.28
S18_33954011	18	AA/AG	0.71	0.29	AA/AG	0.71	0.29
S18_30381428	18	TT	1	0	TT/TC	0.71	0.29
S18_30515641	18	TT	1	0	TT/TA	0.71	0.29
S18_30764625	18	CC	1	0	CC/CT	0.71	0.29
S18_31059094	18	TT	1	0	TT/TG	0.71	0.29
S18_31158467	18	TT	1	0	TT/TA	0.78	0.22
S18_31164496	18	TT	1	0	TT/TC	0.71	0.29
S18_31196588	18	AA	1	0	AA/AT	0.71	0.29
S18_31547619	18	GG	1	0	GG/GA	0.75	0.25
S18_33070986	18	CC	1	0	CC/CT	0.72	0.28
S18_33276771	18	CC	1	0	CC/CT	0.71	0.29
S18_33876484	18	CC	1	0	CC/CT	0.71	0.29
S18_31481177	18	GG/GT	0.79	0.21	GG/GT	0.79	0.21
S18_33162606	18	CC/CA	0.79	0.21	CC/CA	0.79	0.21
S18_33959722	18	CC/CT	0.79	0.21	CC/CT	0.79	0.21
S18_34060245	18	AA/AG	0.78	0.22	AA/AG	0.78	0.22

SNPs validated by Sequenom MassARRAY for 65 of the F_1 individuals and compared with TASSEL GBS data. Chr = Chromosome; MF=Major Allele Frequency; MAF=Minor Allele Frequency

Table 8: The position of *MJR1* on validate LG18

SNP ID	cM
S18_325393	0.0
S18_516915	7.8
S18_961949	13.4
S18_2352399	16.8
S18_3422830	22.3
S18_4042023	26.8
S18_4349352	29.0
S18_4722224	31.2
S18_5173458	34.6
S18_5131100	37.9
S18_7610729	41.3
S18_7880648	43.5
S18_9345851	46.8
S18_10387891	50.2
S18_11175529	56.9
S18_11730974	61.3
S18_11730982	63.5
S18_12707428	68.0
S18_12709644	71.3
S18_13446851	75.8
S18_18671664	82.5
S18_22504012	88.1
S18_26580875	92.5
S18_26558715	93.6
S18_27884817	95.9
S18_28372660	95.9
S18_30104225	97.0
S18_30236024	98.1
S18_30226628	98.1
S18_30722375	99.2
S18_31160355	99.2
S18_30711041	99.2
MJR1_Marker	100.3
S18_31886894	100.3
S18_33070954	100.3
S18_32680428	100.3
S18_31787750	100.3
S18_33954011	101.4
S18_34276271	107.0

Table 9: Integration of genetic mapping with single SNP association

SNP ID	cM	Adj. p-value Bonferroni	Adj. p-value Ben-Hoch	R^2
S18_22504012	88.1	8.39E-14	8.56E-16	0.565
S18_26580875	92.5	3.16E-21	4.79E-23	0.706
S18_26558715	93.6	7.83E-24	1.28E-25	0.743
S18_27884817	95.9	2.30E-31	4.80E-33	0.828
S18_28372660	95.9	2.30E-31	4.80E-33	0.828
S18_30104225	97.0	2.11E-36	5.56E-38	0.868
S18_30226628	98.1	1.01E-43	3.26E-45	0.910
S18_30236024	98.1	1.01E-43	3.26E-45	0.910
S18_30711041	99.2	9.38E-57	4.26E-58	0.955
S18_30722375	99.2	9.38E-57	4.26E-58	0.955
S18_31160355	99.2	9.38E-57	4.26E-58	0.955
MJR1	100.3	NA	NA	NA
S18_31787750	100.3	0	0	1
S18_31886894	100.3	0	0	1
S18_32680428	100.3	0	0	1
S18_33070954	100.3	0	0	1
S18_33954011	101.4	9.38E-57	4.26E-58	0.955
S18_34276271	107.0	7.83E-24	1.28E-25	0.743

The order and position of validated SNPs in cM on LG18 based on genetic mapping using R/onemap. Adjusted (Adj) *p*-values for single SNP association are shown next to cM for comparison.
Ben-Hoch, Benjamini-Hochberg; NA, Not Applicable

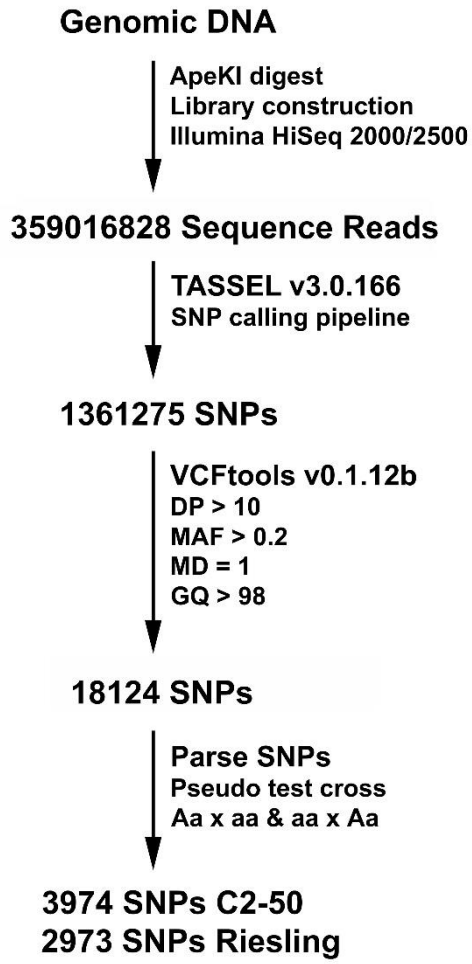


Fig. 1 Flow chart for GBS and filtering of C2-50 and Riesling SNP sets.
Genomic DNA isolated from the parents and F1 progeny genotypes were sequenced using Illumina HiSeq. SNP calling was performed using Tassel v3.0.166 and a 1.3M SNP set was generated. The called SNPs were filtered using VCFtools, which reduced the SNP set to 18K. SNPs parsed using pseudo test cross into 3974 and 2973 SNP sets for C2-50 and Riesling, respectively.

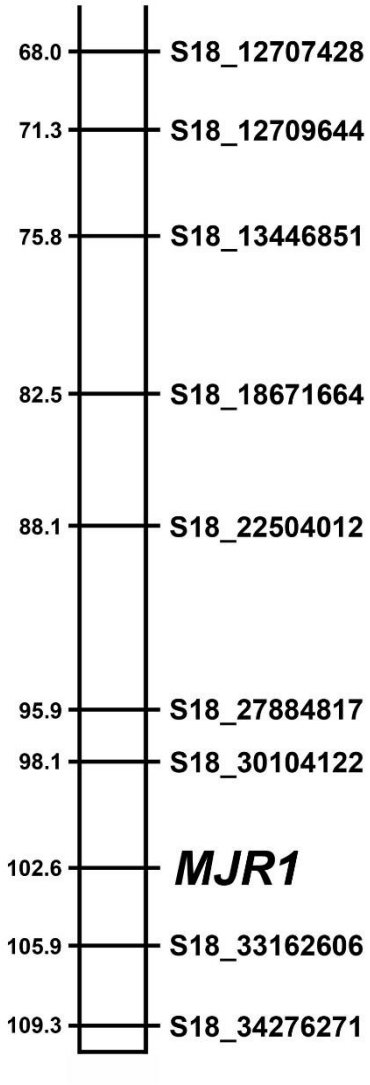


Fig. 2 *MJR1* maps to LG18 at 110.5 cM in C2-50.
The region containing the *MJR1* locus is displayed in this figure. Supplemental table 1 contains the full list of SNPs and genetic distance for LG18. The image was derived using the 367 SNP set plus the *MJR1* marker followed by genetic mapping using R/OneMap. SNPs designated on the right side of chromosome. Genetic distance in cM is displayed on the left side of the chromosome.

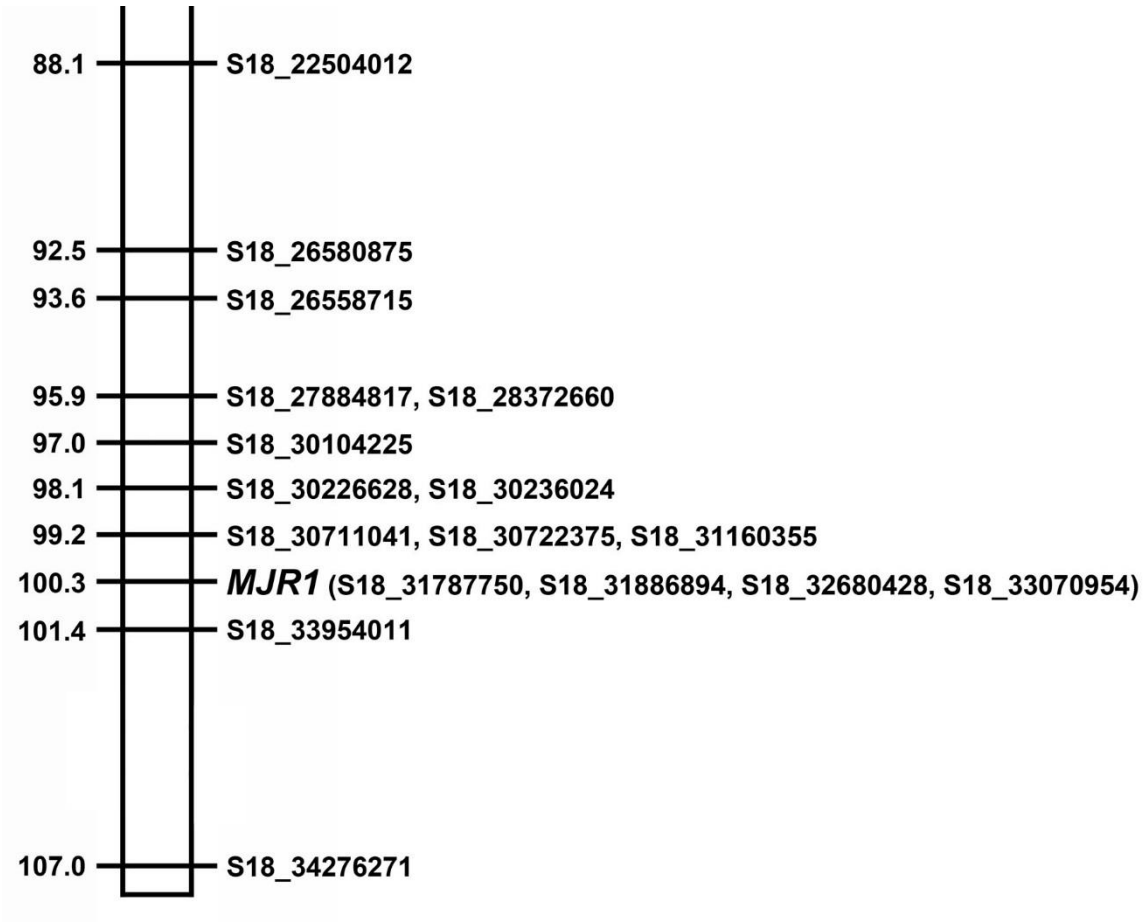


Fig. 3 Validation of SNPs and genetic mapping of the *MJR1* locus.
SNPs at the *MJR1* locus were validated using the Sequenom MassARRAY. Non-polymorphic GBS-predicted SNPs and incorrectly genotypes were removed before mapping *MJR1* using the 382 SNP set. SNP ID is shown on the right and distance in cM on displayed on the left side of the chromosome.

2.3.2. Phylloxera resistance in grapevine: screening, genetics and molecular marker development

(This study has been prepared and submitted as a research paper to a scientific journal)

Introduction

Grape phylloxera (*Daktulosphaira vitifolia* Fitch) is an insect native to specific regions of North America and *Vitis vinifera* cultivars used in wine, table and dried grape production are highly susceptible to this pest (Forneck and Huber, 2009; Powell 2012). Grape phylloxera genetic strains that feed primarily on either roots or leaves are referred as radicicoles or gallicoaes, respectively, and in some cases strains will feed on both roots and leaves (Powell 2012; Powell et al., 2013). Grape phylloxera feeding induces gall formation on leaves and roots. Galls formed on young and older lignified roots are referred as nodosities and tuberosities, respectively. Nodosity formation typically occurs on susceptible *Vitis* species, while tuberosity development occurs primarily on the roots *V. viniferea* and hybrid vines with *V. vinifera* parentage. Feeding damage by radicicoles on *V. vinifera* reduces the roots' ability to uptake water and nutrient resulting in yield losses (Powell et al., 2013). Moreover, wounding caused by feeding, as well as the cracking of tuberosities, provide an entry point for soil borne fungal pathogens, which results in vine death. (Omer et al., 1995; Granett et al., 1998). It has been estimated that phylloxera infestation on *V. vinifera* roots can lead to vine death in 4-7 years when exposed to aggressive phylloxera strains or biotypes (Powell et al., 2013). While the life cycle of grape phylloxera was originally classified as cyclic parthenogenesis, which alternates between sexual and asexual forms (Granett et al., 2001), recent studies in North America, Europe and Australia indicate that phylloxera reproduction occurs asexually (Corrie et al., 2003; Forneck and Huber, 2009).

In the mid 1800s, the accidental introduction of phylloxera from North America to Europe nearly destroyed the European wine industry (Forneck and Huber, 2009; Powell 2012). However, the eventual use of North American *Vitis* species that evolved resistance to grape phylloxera were utilised as rootstocks to re-establish wine grape production in Europe. In addition to Europe, grape phylloxera spread to other wine growing regions of the world including South Africa, Middle East, Asia and Australasia in the mid to late 1800s (Powell 2012). The use of North American *Vitis* species, which evolved with grape phylloxera in its native habitat, provides key genetic material to breed new rootstocks with grape phylloxera resistance.

The utilisation of rootstocks having a broad spectrum and high level of resistance to grape phylloxera biotypes is an essential component for maintaining production in the presence of this soil-borne insect pest. Initial rootstock breeding for grape phylloxera resistance produced rootstocks, which supported a low level of phylloxera feeding and reproduction with minimal damage to the root system. For example, AxR#1, a *V. vinifera* Aramon x *V. rupestris* Ganzin hybrid was widely used in California (Granett et al., 1985). In addition, *V. berlandieri* x *V. riparia* rootstocks, including Teleki 5C, SO4, 5BB Kober and 420A, also support phylloxera feeding and reproduction from specific biotypes in California, Europe and Australia (De Benedictis et al., 1996; Kocsis et al., 1999; Powell 2012; Powell et al., 2013). Wide spread usage of AxR#1 in California and *V. berlandieri* x *V. riparia* rootstocks in Europe resulted in the emergence of grape phylloxera populations better adapted to feeding and reproducing on these rootstocks (Granett et al., 1985; De Benedictis et al., 1996; Kocsis et al., 1999). In California, failure of the AxR#1 rootstock cost the wine industry $750 million to $1.25 billion (Sullivan 1996). Therefore, selection pressure resulting from wide spread usage of partially resistant rootstocks with a minimum spectrum of protection will likely lead to the emergence of less endemic grape phylloxera biotypes and the eventual breakdown of the rootstock.

Heritability studies indicate that *V. vinifera* and *V. rupestris* contain susceptibility loci to grape phylloxera, which function to promote radicicole formation (Boubals 1966; Roush et al., 2007). A recent study utilised the grape phylloxera biotype B population to examine the inheritance of nodosity and tuberosity (Roush et al., 2007). In this analysis, a full-sibling cross was performed between a biotype A resistant and susceptible F_1 *V. vinifera* Aramon x *V. rupestris* Ganzin individual and the resultant F_2 population was screened for resistance to biotype B. Results from this analysis indicate that nodosity formation is controlled by at least two loci as this trait had a phenotype ratio of 1:7. While the heritability of tuberosity development is unclear, the mechanism controlling nodosity and tuberosity formation appears to be discrete (Roush et al., 2007).

Evaluation of grape phylloxera resistance in F_1 hybrids from *V. vinifera* x *M. rotundifola* crosses indicates that a single dominant locus confers phylloxera resistance (Bouquet, 1983). The resistance mechanism involves a hypersensitive response reaction that prevents the grape phylloxera from establishing a feeding site. However, when two resistant F_1 individuals were backcrossed to *V. vinifera*, only 19% of the individuals had complete

resistance. As a result, Bouquet, 1983, concluded that grape phylloxera resistance in the *M. rotundifolia* is mediated by a semi-dominant locus, which is controlled by three genetic modifiers (Bouquet, 1983). In another study, analysis of grape phylloxera resistance in F_1 *V. vinifera* x *M. rotundifolia* individuals indicates that resistance is controlled by more than one dominant locus (Davidis and Olmo, 1964). Therefore, grape phylloxera resistance in *M. rotundifolia* appears to involve multiple loci.

Using a design II mating scheme, grape phylloxera resistance was examined in seven rootstocks with different levels of resistance or susceptibility and results indicate that resistance is controlled by at least two dominant loci (Ramming et al., 2010). In addition, resistance appears to be controlled by multiple dominant loci in *V. berlandieri* and *V. cinerea* (Boubals, 1966; Zhang et al., 2009). Genetic studies showed that a major phylloxera resistant QTL, *Resistance Daktulosphaira vitifoliae 1* (*RDV1*), is located on chromosome 13 in the Borner rootstock (*V. riparia* x *V. cinerea*) (Zhang et al., 2009). The *RDV1* locus appears to have originated from *V. cinerea*, as markers linked to this resistant locus were derived from this North American *Vitis* species.

The goal of the CSIRO Rootstock Breeding team is to breed new rootstocks with durable resistance to both phylloxera and root knot nematode. In order to breed durable phylloxera and nematode resistant rootstocks, different resistant loci will need to be stacked into a single plant, which will require molecular markers linked to each of the resistance loci. The *MELOIDOGYNE JAVANICA RESISTANT 1* Locus (*MJR1*) was recently mapped to chromosome 18 in a *V. cinerea* C2-50 and molecular markers linked to this trait have been identified (Smith et al., 2017, submitted). In this report, we describe the mapping of a phylloxera resistant locus in *V. cinerea* C2-50 and the identification of linked molecular markers.

Materials and Methods

Phylloxera Stock Cultures

The G1 and G4 grape phylloxera genotypes were single sourced from roots of ungrafted *V. vinifera* vines from a commercial vineyard located in the Maroondah and North East Victoria Phylloxera Infested Zones (PIZ), respectively. Prior to the trial infestation G1 and G4 were maintained and multiplied on *V. vinifera* cv. Shiraz excised root pieces under controlled conditions in growth cabinets (25±3°C; 12 hours) followed recommended

excised root bioassay procedures (Kingston et al., 2007; Forneck et al., 2016). Prior to trial commencement, and at trial completion, phylloxera was sub-sampled and genotyped at DEDJTR, Bundoora using six microsatellite markers (Umina et al., 2007). All phylloxera stock cultures were maintained under strict quarantine conditions in a laboratory at Agriculture Victoria, Rutherglen located in the North East PIZ.

Planting Material

The grape phylloxera screening trial utilised six-week old potted plants propagated from dormant cuttings. The genotypes screened were: (1) 133 F_1 *V. cinerea* (C2-50) x Riesling individuals, (2) germplasm material, which included *V. aestivalis*, *M. rotundifolia* and *V. acerifolia* accessions, (3) 24 F_1 *M. rotundifolia* x *V. vinifera* individuals and (4) 33 F_1 *V. cinerea* (C2-50) x Pinot Meunier (microvine) individuals. Each genotype was screened in triplicate. All the material from above was screened with G1 and 76 out of 133 F_1 *V. cinerea* (C2-50) x Riesling individuals were also screened with G4. All plants were potted in 20% Perlite (to improve soil aeration) and watered via drip irrigation for at least six weeks, in a controlled-temperature glasshouse (Figure 1), to enable good root development prior to treatment application (i.e. phylloxera inoculation).

Phylloxera infestation

To infest the rootstock breeding material, each vine was removed from its pot and the roots cleaned of potting mix. Next, twenty phylloxera eggs were placed on moistened filter paper strips. The roots of the vines were exposed, and a single, lignified root piece was selected (~2.75 cm in diameter) and the filter paper was carefully wrapped around the root, avoiding damage during handling. This method of enclosing the phylloxera around an infested root piece is similar to the root enclosure in planta bioassay method described by Korosi *et al.* (2007). This is one of the methods recommended as a standardised potted plant bioassay protocol to allow comparative phenological observations induced by different phylloxera biotypes (Forneck *et al.* 2016). The infested vine was placed in a sterile 8 cm diameter pot with and addition of fresh sterile soil mixture added where required (80% potting mix, 20% perlite). Tanglefoot was placed around the rim of each pot to avoid cross contamination of phylloxera between vines. For evaluating resistance and susceptibility to grape phylloxera, the number of nodosities and insects were scored on the roots for each replicate. Next, the average number of nodosities and insects was determined. Genotypes with an average score of nodosities and insects equal to zero were classified as resistant, while plants with >0 nodosities and insects were classified as susceptible. All phylloxera infested grapevine

material was maintained under strict quarantine conditions in a secure glasshouse facility at Agriculture Victoria, Rutherglen located in the North East PIZ.

Post infestation growth conditions

At trial commencement, all vines were fertilised with 3.5 g Osmocote^{TM} and 500 mL Thrive (8 g of Thrive^{TM} mixed with 4.5 L water) per potted vine. Each vine was drip irrigated for two minutes daily. To prevent the vines from going into dormancy, artificial growth lights were automatically turned on each day from 6am to 8pm and 1am to 2am during the trial. During the trials, Gemini Tinytag Ultra^{TM} dataloggers (Hastings Data Loggers, Port Macquarie, New South Wales) monitored the temperature and relative humidity in the glasshouse. The temperature settings were minimum 20^{o}C and maximum 24^{o}C from 8am to 9pm, min 18^{o}C and maximum 22^{o}C from 9pm-8am.

Trial design

A randomised complete plot design was established to examine the interaction between G1 and G4 grape phylloxera and the various rootstock genotypes. Three (depending on availability) replicate vines were used per treatment in a block design to account for any variation in the environment conditions.

Genetic and association mapping of the grape phylloxera resistance

The *V. cinerea* C2-50 and Riesling SNP sets generated by genotyping-by-sequencing for localising the *MJR1* locus (Smith et al., 2017, submitted) was used for mapping phylloxera resistance. Note: The C2-50 SNP set contained SNPs that were heterozygous in *V. cinerea* C2-50 and homozygous in Riesling while the opposite set of SNPs were retained in the Riesling SNP set. Single SNP association was performed in TASSEL and linkage mapping was performed using R/OneMap as described in Smith et al., 2017, submitted. SNPs spanning the phylloxera resistant locus were validated using the Sequnome MassArray at the Australian Genome Research Facility, Adelaide, South Australia.

Results

Identification of rootstock breeding material with resistance to G1 grape phylloxera

Experimental evidence indicates that *V. cinerea*, *V. aestivalis* and *M. rotundifolia* accessions are sources of phylloxera resistance (Boubals 1966; Grzegorczyk and Walker 1998; Zhang et al., 2009). Therefore, we screened *V. cinerea* C2-50, *M. rotundifolia* and *V. aestivalis* accessions, as well as DRX55 ([*M. rotundifolia* x *V. vinifera*] x open pollinated) and 171-L

(*V. aestivalis* x *V. vinifera*), for resistance to the G1 grape phylloxera genotype. As a control, Riesling and Shiraz were included in this screen, as these *V. vinifera* cultivars are highly susceptible to grape phylloxera. Results showed that *V. cinerea* C2-50, *M. rotundifolia* and *V. aestivalis* accessions, as well as DRX55, were resistant to the G1 grape phylloxera genotype as nodosity formation and insect development did not occur on the roots of these vines (Table 1). However, nodosity formation and insect development were prevalent on the roots of 171-13L (*V. aestivalis x V. vinifera*), Riesling and Shiraz (Table 1).

Segregation of grape phylloxera resistance in *V. cinerea* C2-50

The heritability of phylloxera resistance in *V. cinerea* C2-50 was examined by screening 133 F_1 C2-50 x Riesling individuals with the G1 grape phylloxera genotype. Plants with nodosity formation and insect development on roots were classified as susceptible, as these two parameters indicate that the G1 grape phylloxera can effectively parasitise (feed and reproduce) the plants. The roots of vines devoid of nodosity formation and insect development were classified as resistant, as the G1 grape phylloxera was unable to establish a feeding site and reproduce. Results showed 68 *V. cinerea* C2-50 x Riesling F_1 individuals were resistant, as nodosity formation and insect development did not occur on the roots of these plants (Table 2). In contrast, the remaining 65 F_1 individuals had nodosity development and significant number of insects was found on the roots (Table 2). The average number of nodosity and insects varied among the susceptible F_1 individuals (data not shown). The heritability of G4 grape phylloxera resistance was also examined by screening 72 out of the 133 F_1 *V. cinerea* C2-50 x Riesling individuals for resistance and susceptibility. Results showed that 34 were resistant due to the absence of insects and nodosity formation (Table 2). However, 38 individuals were susceptible as nodosity formation and insect development were apparent (Table 2). It should be pointed out that all 34 F_1 individuals resistant to G4 grape phylloxera were also resistant to G1. In addition, the F_1 plants susceptible for G4 grape phylloxera were effectively parasitised by G1 as well. Therefore, the mode of G1 and G4 grape phylloxera resistance appears to be mediated by the same mechanism.

A chi-square goodness of fit test was performed to determine whether the phenotypic ratio for G1 and G4 grape phylloxera resistance segregates with a 1:1 ratio. Using one degree of freedom, the chi-square value for G1 and G4 was 0.067 and 0.22, respectively, and these values are less than the critical value of 3.84 (Table 2). In addition, the probability for each of the values was >0.05 indicating that G1 and G4 grape phylloxera resistance segregates

with a 1:1 ratio. Therefore, the data support a model that G1 and G4 resistance, referred to as *RESISTANCE DAKTULOSPHAIRA VITIFOLIAE 2* (*RDV2),* is conferred by a single locus in *V. cinerea* C2-50, which can be explained by two hypotheses. In the first hypothesis, *V. cinerea* C2-50 is heterozygous dominant for *RDV2* (*RDV2/rdv2*) and Riesling is homozygous recessive (*rdv2/rdv2*). Alternatively, the second hypothesis predicts that resistance is conferred by a recessive allele (*rdv2*) in which *V. cinerea* C2-50 is homozygous recessive (*rdv2/rdv2*) and Riesling is heterozygous (*RDV2/rdv2*) for the recessive allele.

Single SNP association analysis

A single SNP association analysis was performed to map *RDV2* using the C2-50 (3974 SNPs) and Riesling (2973 SNPs) SNP sets. Single SNP association using the C2-50 SNP set identified 10 SNPs on chromosome 14, which had high association based on *p*-values equal to 0 and R^2 equal to 1 (Table 3). In addition, most of the immediate set of flanking markers also displayed high association with *p*-values ranging from 2.53E-58 to 1.44E-19 and R^2 between 0.679 to 0.956 (Table 3). In contrast, single SNP association performed with the Riesling SNP set did not show any significant association, as the adjusted *p*-values were >0.05 for both Bonferroni and Benjamini-Hochberg (data not shown). In addition, the highest R^2 value was equal to 0.106 (data not shown). Taken together, results indicate that *RDV2* is located on chromosome 14 in C2-50. Further, given that association was only detected with SNPs in the C2-50 SNP set supports the hypothesis that G1 grape phylloxera resistance is controlled by a single dominant locus that is heterozygous (*RDV2/rdv2*) in *V. cinerea* C2-50.

Genetic mapping of the *RDV2* locus

The location of the *RDV2* locus was determined using the C2-50 and Riesling genetic maps, which consisted of 367 and 403 SNPs, respectively (Smith et al., 2017, submitted). In order to map the *RDV2* locus, the phenotype data for each F_1 individual was converted to a genotype. Given that the phenotype data closely matched a 1:1 ratio, it is highly likely that a single locus in *V. cinerea* C2-50 confers G1 and G4 grape phylloxera resistance. Further, in the single SNP association analysis, only SNPs in the C2-50 SNP set (heterozygous in C2-50 and homozygous in Riesling) associated with G1 phylloxera resistance. Together, these results showed that *V. cinerea* C2-50 is heterozygous for *RDV2/rdv2*, while Riesling was homozygous recessive, *rdv2/rdv2*. Therefore, resistant and susceptible F_1 individuals were assigned either an *RDV2/rdv2* or *rdv2/rdv2* genotype, respectively. To map the *RDV2* locus, the *RDV2* marker was included in the curated C2-50 and Riesling SNP sets and

linkage analysis was performed using R/OneMap. Results from the genetic mapping showed that *RDV2* mapped to linkage group 14 (LG14) at 26.8 cM using the C2-50 367 SNP set (Table 4). In this analysis, *RDV2* was flanked by S14_4196799 and S1410108325 at 22.3 and 38.1 cM, respectively. When the *RDV2* marker was included in the Riesling 403 SNP set, G1 grape phylloxera resistance was not mapped to any of the 19 linkage groups (data not shown).

SNP validation and genetic mapping of *RDV2*

The TASSEL GBS pipeline was used to identify SNPs in the F_1 *V. cinerea* C2-50 x Riesling individuals (Smith et al., 2017, submitted). Because the accuracy rate of predicting SNPs at the *MJR1* locus was 50% (Smith et al., 2017, submitted), we validated 48 SNPs spanning the *RDV2* locus using the Sequenom MassARRAY platform (Oeth et al., 2009). In this analysis, the Sequenom MassARRAY identified 26 SNPs genotyped, which were polymorphic and the genotypes matched with those predicted by TASSEL. In addition, the major and minor allele frequencies were nearly identical with those predicted by TASSEL (Table 5). Twenty SNPs genotyped by Sequenome MassARRAY were non-polymorphic and failed to match the genotypes predicted by TASSEL (Table 5). Two polymorphic SNPs, S14_79250044 and S14_7925062 did not match with TASSEL (Table 5). Lastly, thirteen additional genotyping errors were identified in eight F_1 individuals (Table 6). Results from this validation indicate that TASSEL GBS pipeline accurately predicted SNPs with a 54% success rate at the *RDV2* locus.

To validate the previous genetic mapping results, a 394 SNP set was created, which included the 26 accurately genotyped SNPs at the *RDV2* locus, as well as the *RDV2* marker. This SNP set was curated to correct the thirteen-genotyping errors in the eight F1 individuals (Table 6). Results from R/OneMap analysis showed that *RDV2* mapped to LG14 at position 16.7 cM (Table 7). The ten SNPs, S14_491219, S14_5274160, S14_5737727, S14_5771919, S14_5804788, S14_5994738, S14_6008125, S14_6071298, S14_6071669, S14_6175917 and S14_6596440, cosegregated with *RDV2* at position 16.7 cM. The cosegregating SNPs, as well as the SNPs flanking *RDV2* at 15.6 (S14_4329518 and S14_4403666) and 18.9 cM (S14_6803756) will be useful markers for marker-assisted selection (Table 7). The validated R/OneMap data were combined with the association mapping data and results showed that the *RDV2* co-segregating SNPs at 16.7 all had adjusted *p*-values and R^2 values were equal to 0 and 1, respectively (Table 7). The SNPs flanking *RDV2* at 15.6 and 18.9 cM also had high association as reflected by the R^2 and *p*-

values (Table 7). In addition, a decline in SNP association decreased as the genetic distance increased for SNPs linked to *RDV2* (Table 7). Taken together, the combined results from the linkage mapping and single SNP association after Sequenome MassARRAY validation provides complementary methods for mapping of *RDV2* and identifying SNPs for marker assisted selection.

Discussion

Experimental studies aimed at understanding the genetic control of grape phylloxera resistance indicate that resistance is controlled by multiple loci in *V. berlandieri*, *V. cinerea*, *V. rupestris* and *M. rotundifolia* (Boubals 1966; Davidis and Olmo, 1964; Bouquet, 1983; Ramming, 2010). Consistent with these studies, we showed that *V. cinerea* C2-50, DRX55 and a *M. rotundifolia* accession were resistant to the G1 phylloxera genotype. In addition, we also showed that a *V. aestivalis* accession also provides resistance to G1 grape phylloxera. In the Börner rootstock ([*V. riparia* x *V. cinerea* Arnold) x *V. vinifera*), it is speculated that the partial resistant mechanism in *V. riparia* has been combined with the complete resistance originated from *V. cinerea*. As a result, multiple grape phylloxera resistance loci originating from these two *Vitis* species function to mediate grape phylloxera resistance in Börner. Genetic analysis of phylloxera resistance in *V. cinerea* C2-50 showed that resistance is controlled by a single locus. The single SNP association and linkage mapping results support the hypothesis that G1 and G4 phylloxera resistance in *V. cinerea* C2-50 is heterozygous dominant for *RDV2* (*RDV2/rdv2*).

Grape phylloxera resistance in the Börner rootstock is mediated in part by the *RDV1* locus located on chromosome 13 (Zhang et al., 2009). The *RDV1* locus is derived from *V. cinerea* Arnold, as the alleles of the *Gf13_1* and *Gf13_9* SSR markers are derived from this accession and not *V. riparia*. The number and location of the partially resistant loci originating from *V. riparia* have yet to be identified in Börner. In contrast to *RDV1*, both single SNP association and linkage mapping localised *RDV2* to chromosome 14. Given that *RDV1* and *RDV2* localise to different chromosomes indicates that *V. cinerea* have at least two grape phylloxera resistant loci. Moreover, stacking *RDV1* and *RDV2* into a single genetic background, as a means to produce rootstocks durable resistance to grape phylloxera, can be achieved using the markers linked to these two resistant loci.

Grape phylloxera biotype emergence is major problem when management practices rely solely on rootstocks with partial resistance and/or a limited spectrum of resistance. A range

of phylloxera biotypes have been described (Forneck et al., 2016). For example, while AXR#1 provided adequate levels of resistant to biotype A (Lider 1958), the emergence of biotype B led to the eventual breakdown of phylloxera resistance (Granett et al., 1985), which cost the California viticulture industry up to $1.25 billion (Sullivan, 1996). Börner appears to have a broad spectrum of high resistance to a number of biotypes of phylloxera (Zhang et al., 2009). In Australia, variability of phylloxera genetic lineages that differ in their ability to parasitise the roots of rootstocks and *V. vinifera* within specific grape phylloxera populations presents a major challenge in breeding rootstocks with a broad spectrum resistance. Results from our study show that *RDV2* mediates resistance to G1 and G4 grape phylloxera, which are two 'superclones' that are widespread in Australia (Umina et al., 2007). Based on genotype determination using microsatellite markers, G1 and G4 grape phylloxera appear to be genetically similar and are likely are derived from a common lineage (Corrie et al., 2002; Umina et al., 2007). Therefore, it is not surprising that *RDV2* provides resistance to both G1 and G4 grape phylloxera. However, given that 83 endemic grape phylloxera genotypes have been identified in Australia (Umina et al., 2007), future work is aimed to accessing resistance to other grape phylloxera genotypes with different clonal lineages found in specific regions of Australia.

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Table 1: Evaluation of rootstock breeding material for G1 grape phylloxera resistance

Variety/Accession	Pedigree	Nodosities	Insects	Rating
V. cinerea C2-50	*V. cinerea* accession	0	0	R
DRX55	(*V. vinifera* x *M. rotundifolia*) open pollinated	0	0	R
M. rotundifolia	*M. rotundifolia* x *M. rotundifolia* acession	0	0	R
V. aestivalis	*V. aestivalis* accession	0	0	R
171-13L	*V. aestivalis* x *V. vinifera*	6	20	S
Riesling	*V. vinifera* variety	18	208	S
Shiraz	*V. vinifera* variety	33	947	S

R, resistant; S, susceptible

Table 2: Segregation of G1 and G4 grape phylloxera resistance

Grape Phylloxera Biotype	R	S	Proposed R:S ratio	Calculated X^2
G1	68	65	1:1	0.0677
G4	34	38	1:1	0.2222

R, resistant; S, susceptible

Table 3: Single SNP association for *RDV2*

SNP ID	raw p-value	Adj. p-value Bonferroni	Adj. p-value Ben-Hoch	R2
S14_3596942	2.69E-40	1.07E-36	3.56E-38	0.87
S14_3596980	3.62E-23	1.44E-19	1.50E-21	0.68
S14_3891954	6.21E-28	2.47E-24	3.92E-26	0.75
S14_4065142	2.69E-40	1.07E-36	3.56E-38	0.87
S14_4196799	8.58E-41	3.41E-37	2.44E-38	0.87
S14_4228116	0.17	1.00	0.52	0.02
S14_4228151	2.37E-25	9.42E-22	1.22E-23	0.71
S14_4228160	0.17	1.00	0.52	0.02
S14_4228161	0.17	1.00	0.52	0.02
S14_4228162	0.17	1.00	0.52	0.02
S14_4290008	2.61E-28	1.04E-24	1.79E-26	0.76
S14_4921219	0	0	0	1.00
S14_4924133	7.78E-36	3.09E-32	9.37E-34	0.84
S14_5274160	8.29E-61	3.29E-57	2.53E-58	0.96
S14_5274186	8.29E-61	3.29E-57	2.53E-58	0.96
S14_5737618	7.78E-36	3.09E-32	9.37E-34	0.84
S14_5737727	0	0	0	1.00
S14_5771919	0	0	0	1.00
S14_5804788	0	0	0	1.00
S14_5841752	7.78E-36	3.09E-32	9.37E-34	0.84
S14_6003431	5.23E-32	2.08E-28	4.72E-30	0.80
S14_6003437	5.23E-32	2.08E-28	4.72E-30	0.80
S14_6003441	0	0	0	1.00
S14_6008125	0	0	0	1.00
S14_6071298	0	0	0	1.00
S14_6071669	0	0	0	1.00
S14_6079907	0	0	0	1.00
S14_6175917	8.29E-61	3.29E-57	2.53E-58	0.96
S14_6596440	0	0	0	1.00
S14_6712024	5.23E-32	2.08E-28	4.72E-30	0.80
S14_7452936	9.12E-26	3.62E-22	5.25E-24	0.72
S14_7482081	1.31E-23	5.21E-20	6.05E-22	0.69
S14_7588554	9.12E-26	3.62E-22	5.25E-24	0.72
S14_7588575	2.52E-40	1.00E-36	3.56E-38	0.87
S14_7623091	2.61E-28	1.04E-24	1.79E-26	0.76
S14_7684469	5.05E-35	2.01E-31	4.89E-33	0.83
S14_7744603	9.12E-26	3.62E-22	5.25E-24	0.72
S14_7776601	9.12E-26	3.62E-22	5.25E-24	0.72
S14_7829543	1.31E-23	5.21E-20	6.05E-22	0.69
S14_7831615	9.12E-26	3.62E-22	5.25E-24	0.72

Raw *p*-values obtained from TASSEL 5.0 and adjusted (Adj.) were shown. The 3974 SNP set was used in the association analysis. Benjamini-Hochberg (Ben-Hoch)

Table 4: Linkage mapping of *RDV2* to linkage group 14

SNP ID	cM
S14_26389	0
S14_395496	3.3
S14_1537587	6.7
S14_997752	13.4
S14_413942	15.6
S14_4196799	22.3
*RDV2*_Marker	26.8
S14_10108325	38.1
S14_21743238	43.6
S14_23880342	49.2
S14_24208940	52.6
S14_25072265	54.8
S14_25236204	61.5
S14_25421489	63.7
S14_26419865	71.6
S14_25535909	78.3
S14_26235789	80.5
S14_26788064	83.8
S14_26955068	88.3
S14_27418336	91.6
S14_27233192	96.1
S14_27779977	100.5
S14_28533026	106.1
S14_29638581	112.8

Table 5: Validation of 48 SNPs at the *RDV2* locus

		Sequenom MassARRAY			TASSEL		
SNP ID	Chr	Genotype	MF	MAF	Genotype	MF	MAF
S14_2062712	14	GG/GT	0.72	0.28	GG/GT	0.72	0.28
S14_2846470	14	CC/TC	0.72	0.28	CC/TC	0.72	0.28
S14_3066185	14	AA/GA	0.72	0.28	AA/GA	0.72	0.28
S14_3222720	14	TT/TA	0.72	0.28	TT/TA	0.72	0.28
S14_3296164	14	CC/TC	0.72	0.28	CC/TC	0.72	0.28
S14_3596942	14	CC/CT	0.72	0.28	CC/CT	0.72	0.28
S14_4065142	14	TT/GT	0.72	0.28	TT/GT	0.72	0.28
S14_4196799	14	AA/AG	0.72	0.28	AA/AG	0.72	0.28
S14_4329518	14	GG/AG	0.73	0.27	GG/AG	0.74	0.26
S14_4403666	14	GG/GA	0.73	0.27	GG/GA	0.74	0.26
S14_4921219	14	CC/CT	0.74	0.26	CC/CT	0.74	0.26
S14_5274160	14	TT/CT	0.74	0.26	TT/CT	0.74	0.26
S14_5737727	14	AA/TA	0.74	0.26	AA/TA	0.74	0.26
S14_5771919	14	AA/CA	0.74	0.26	AA/CA	0.74	0.26
S14_5804788	14	AA/GA	0.74	0.26	AA/GA	0.74	0.26
S14_5994738	14	TT/CT	0.74	0.26	TT/CT	0.74	0.26
S14_6008125	14	GG/CG	0.74	0.26	GG/CG	0.74	0.26
S14_6071298	14	GG/AG	0.74	0.26	GG/AG	0.74	0.26
S14_6071669	14	CC/CA	0.74	0.26	CC/CA	0.74	0.26
S14_6175917	14	TT/CT	0.74	0.26	TT/CT	0.74	0.26
S14_6596440	14	TT/TA	0.74	0.26	TT/TA	0.74	0.26
S14_6803756	14	GG/GT	0.74	0.26	GG/GT	0.74	0.26
S14_7684469	14	AA/AG	0.74	0.26	AA/AG	0.75	0.25
S14_8894287	14	GG/GT	0.75	0.25	GG/GT	0.76	0.24
S14_9154944	14	TT/GT	0.76	0.24	TT/GT	0.76	0.24
S14_9705369	14	TT/CT	0.76	0.24	TT/CT	0.77	0.23
S14_3076105	14	TT	1	0	TT/TC	76	24
S14_3084705	14	GG	1	0	GG/GT	77	23
S14_3216815	14	CC	1	0	CC/CT	76	24
S14_3256262	14	GG	1	0	GG/GC	76	24
S14_3256274	14	AA	1	0	AA/AT	76	24
S14_3321418	14	CC	1	0	CC/CT	77	23
S14_3596980	14	TT	1	0	TT/TA	76	24
S14_3891954	14	CC	1	0	CC/CT	77	23
S14_4228151	14	TT	1	0	TT/TA	76	24
S14_6003431	14	CC	1	0	CC/CG	74	26
S14_7623091	14	GG	1	0	GG/GA	75	25
S14_7744603	14	AA	1	0	AA/AC	74	26
S14_7776601	14	GG	1	0	GG/GA	74	26
S14_7829543	14	AA	1	0	AA/AG	73	27
S14_7831615	14	CC	1	0	CC/CT	74	26
S14_7939992	14	CC	1	0	CC/CT	72	28

S14_8196757	14	AA	1	0	AA/AG	72	28
S14_8660835	14	GG	1	0	GG/GC	73	27
S14_9036371	14	GG	1	0	GG/GA	75	25
S14_9154939	14	GG	1	0	GG/GA	75	25
S14_7925044	14	AA/GG	NA	NA	GG/GA	76	24
S14_7925062	14	AA/CC	NA	NA	AA/AC	76	24

SNPs validated by Sequenom MassARRAY for 58 of the F_1 individuals and compared with TASSEL GBS data. Chr = Chromosome; MF=Major Allele Frequency; MAF=Minor Allele Frequency

Table 6: Genotyping errors in F_1 individuals

F_1 Individual ID	**SNP ID**	**Sequenome MassARRAY**	**TASSEL**
k2b_15_17_8_6c	S14_4403666	GA	GG
k2b_16_04_4_6c	S14_4329518	GA	GG
k2b_16_06_6_6c	S14_9705369	CT	TT
k2b_16_08_6_6c	S14_9705369	CT	TT
k2b_16_11_5_6c	S14_9705369	CT	TT
k2b_16_13_2_6c	S14_2846470	CT	CC
	S14_5274160	CT	TT
	S14_6175917	CT	TT
	S14_9705369	CT	TT
k2b_16_13_7_6c	S14_7684469	AG	AA
k2b_16_15_1_6c	S14_4196799	AA	GA
	S14_7684469	AA	GA
	S14_9154944	TT	GT

Table 7: Linkage mapping and single SNP association with validated SNPs

SNP_ID	cM	raw p-value	Adj. p-value Bonferroni	Adj. p-value Ben-Hoch	R2
S14_2062712	13.4	2.69E-40	1.24E-38	5.62E-40	0.870
S14_2846470	13.4	2.69E-40	1.24E-38	5.62E-40	0.870
S14_3066185	13.4	2.69E-40	1.24E-38	5.62E-40	0.870
S14_3222720	13.4	2.69E-40	1.24E-38	5.62E-40	0.870
S14_3296164	13.4	2.69E-40	1.24E-38	5.62E-40	0.870
S14_3596942	13.4	2.69E-40	1.24E-38	5.62E-40	0.870
S14_4065142	13.4	2.69E-40	1.24E-38	5.62E-40	0.870
S14_4196799	14.5	4.50E-48	2.07E-46	1.48E-47	0.914
S14_4329518	15.6	8.85E-61	4.07E-59	3.13E-60	0.956
S14_4403666	15.6	8.85E-61	4.07E-59	3.13E-60	0.956
*RDV2*_Marker	16.7	NA	NA	NA	NA
S14_4921219	16.7	0	0	0	1.000
S14_5274160	16.7	0	0	0	1.000
S14_5737727	16.7	0	0	0	1.000
S14_5771919	16.7	0	0	0	1.000
S14_5804788	16.7	0	0	0	1.000
S14_5994738	16.7	0	0	0	1.000
S14_6008125	16.7	0	0	0	1.000
S14_6071298	16.7	0	0	0	1.000
S14_6071669	16.7	0	0	0	1.000
S14_6175917	16.7	0	0	0	1.000
S14_6596440	16.7	0	0	0	1.000
S14_6803756	18.9	1.07E-47	4.92E-46	3.28E-47	0.912
S14_7684469	21.2	5.05E-35	2.32E-33	1.01E-34	0.828
S14_8894287	22.3	4.53E-31	2.08E-29	8.68E-31	0.788
S14_9154944	23.4	6.21E-28	2.86E-26	1.10E-27	0.750
S14_9705369	23.4	6.21E-28	2.86E-26	1.10E-27	0.750

SNPs validated by Sequenom MassARRAY for 58 of the F_1 individuals and compared with TASSEL GBS data. Chr = Chromosome; MF=Major Allele Frequency; MAF=Minor Allele Frequency

2.3.3. Potassium uptake: screening, distribution across genotypes and association mapping

Potassium uptake data from the glasshouse screening of the Family 34 test population (*V. cinerea* C2-50 x Riesling) under limited K and excess K supply have been provided above (see 2.3.2.3). There were significant differences between rootstock genotypes under both the low and high K treatments in petioles. As K uptake under these conditions was not significantly correlated, the data support a model for two mechanisms of K uptake, i.e. an active role under limited K supply and a passive role under high K supply (Ruhl 1989). SNP sets generated by genotyping-by-sequencing from 90 Family 34 individuals (described above for root knot nematode marker development, 2.3.1) were used to identify markers that associate with K uptake. Despite the highly significant differences of genotype in petiole K from both glasshouse treatments, none of the markers in the SNP sets associated with K uptake when single SNP association was performed. These results indicate that K uptake is a complex trait controlled by numerous loci in Family 34. Further, the near normal distributions of petiole K across the population (Figure 2.3.2.6) support this view. In this study, petioles were collected from the first mature leaves from the shoot tip. It is possible that an association could be found if samples were collected from for younger, rapidly developing leaves. This approach to refine the glasshouse screening warrants further investigation.

2.3.4. Screening of other traits and association mapping

Association mapping of key viticultural traits was performed using SNP sets generated from the genotyping-by-sequencing for 90 Family 34 individuals. The viticultural traits assessed were measured in the field for single vine seedlings (leaf loss under drought, pruning weight, trunk diameter, root architecture), the replicated nursery trial (pruning weight and end of season canopy condition with well-watered and deficit plants) and in the Plant Accelerator facility (relative and absolute growth rates). Results from single SNP association identified a single locus that predicts root diameter. Unfortunately, no SNP association was detected for the remaining viticultural traits indicating that these traits are complex and controlled by numerous loci. The association of SNPs with root diameter at a single locus indicates that it is possible to develop genetic markers that predict root diameter. However, additional individuals would need to be phenotyped, single SNP association was performed with 65 out of the 90 individuals. The association of SNPs linked to root diameter is very exciting as this trait strongly correlated with vine vigour traits above (see 2.3.2) and because, to the best of the authors knowledge, this is a unique scientific finding.

2.4 New trials with rootstocks for final stage of evaluation

One of the recognised limits to rootstock adoption discussed in 2.1 is the lack of experience and knowledge with rootstocks across regions and varieties. It is also recognised that the cost of funding rootstock trials covering the diverse array of varieties and regional environments for detailed evaluation by research providers is prohibitive. Hence, the aim of this project was to foster industry input and interaction with regional groups and interested wine companies by providing material of grafted vines for establishment of trials involving 'near to release' selections and standard comparator rootstock varieties in different regions grafted with a range of varieties for local evaluation. Expected benefits from this approach are:

- Enhanced industry interaction with regard to rootstock development and adoption in different regions
- Provision of experience, knowledge and confidence to industry regarding the local performance of rootstocks, in particular the 'near to release' CSIRO selections to facilitate early adoption upon release
- Enhanced knowledge, in the longer term, on the Genotype (including rootstock scion combination), Environment and Management interactions (GxExM). Information generated in this process will be a useful resource, not only for development of extension messages for regional use but also for the wider national audience. It could also be incorporated into extension materials including updates of the Wine Australia Rootstock Selector Tool.
- Identification of key regional issues regarding rootstock use, adoption and requirements to underpin future rootstock breeding and development.

In this project, rootstock trials involving the 'near to release' selections and standard comparator rootstocks, have been established at six regional sites in an attempt to cover a range of climates, varieties, soil types, pest and abiotic stresses. They include:

- Two Pinot Noir established in Mornington, in two commercial vineyards with very different soil types. These trials were planted in spring 2015 and will produce the first crops in the 2018 harvest season.
- A Shiraz trial planted in Padthaway
- A Barbera trial planted in December 2016 in the Adelaide Hills
- A Cabernet Sauvignon trial which will be planted in January 2018 in in the Yarra Valley
- A Malbec trial which will be planted in Langhorne Creek in January 2018.

7. Outcomes and conclusions

The project has met its objectives and delivered on the planned outputs and performance targets. The outputs are likely to provide significant benefits to both grapegrowers, winemakers and the environment. Evaluation of existing field trials, established in the previous projects has identified three new rootstock selections (C114, C113 and C20) for release and adoption by industry. These new rootstocks maintain high levels of productivity in replant situations, have shown tolerance of drought and salt and demonstrate high water use efficiency and are resistant to selected root knot nematode and phylloxera isolates. They produce wines with acceptable fruit composition. The future release of a further suite of rootstocks, still under evaluation, should continue to overcome the deficiencies of existing rootstocks and provide similar benefits to industry.

C114 is a very high vigour, high yielding, salt and drought tolerant alternative to Ramsey which has issues with high K uptake, high pH and red wine quality and is not phylloxera resistant. C114 has also maintained high yields with deficit irrigation across a number of dried grape trials in related studies. It is likely to be used for white wine production and be widely adopted across both the dried grape and table grape industries. C114 may be useful for red wine production as a risk management tool against drought and salinity, where water for irrigation is limited and because of its delayed ripening, to extend the season.

C113 is a high vigour and high yielding alternative not only to Ramsey but also to 140 Ruggeri and 1103 Paulsen which have both performed poorly in replant situations in this study in the hot climate region. In Padthaway it displayed more moderate vigour and provides an alternative to the salt and drought sensitive 101-14 widely used in the district. Furthermore, wines produced from C113 at Padthaway had enhanced spectral properties compared to own rooted vines. C113 has also performed well in dried grape trials, including management with deficit irrigation.

C20 is as a moderate vigour rootstock with potential as an alternative to 1103 Paulsen and 140 Ruggeri which have both performed poorly in replant situations in the hot climate region. In Padthaway it provides an alternative to the salt and drought sensitive 101-14 widely used in the district. Wines produced from C20 in Padthaway have high colour density and ionised anthocyanins. Across all rootstocks and in both regions it had high Ravaz index values, an

indicator of good vine balance as it was able to adequately mature the fruit with high water use and carbon assimilation efficiency.

In undertaking the field trial evaluations, significant rootstock x scion interactions and rootstock x region interactions were found. This indicates that a suite of rootstocks is required to meet industry requirements and that no single rootstock will be suited to all varieties and regions. For example, in the hot region studies, fruitfulness and yield were higher for Cabernet Sauvignon grafted on rootstocks with moderate vigour whereas yields of Chardonnay were highest with high vigour rootstocks. Similarly for Shiraz, yield losses with deficit irrigation were minimal for high vigour rootstocks because fruitfulness was higher. The establishment of trials with advanced rootstock selections, in collaboration with regional groups and interested growers undertaken as part of this project, provide further information on adaptability to region and specific varietal information to enhance knowledge and foster adoption.

In the longer term from more efficient rootstock breeding, based on the development of rapid screening techniques including the use of molecular markers will enable development of new rootstocks in short time frames with durable resistance to root knot nematode and phylloxera, that are water use efficient, resistant to drought and other abiotic stresses that meet with optimum specifications for growth and fruit quality traits. This will not only reduce the time to develop new rootstocks with desired key traits, but reduce the number of selections included in field trials and allow testing across a wider range of regions and varieties. This project has demonstrated that simple trait measurement, particularly in the nursery situation, can be linked to grafted vine growth characteristics and fruit composition in grafted field trials. In this case simple traits measured in the nursery for well-watered and deficit plants that could be linked to conferred vigour, yield and its components and fruit composition included measurement of growth traits (shoot length, pruning weight, trunk diameter), physiological traits (conductance, leaf temperature, transpiration efficiency by carbon isotope discrimination), canopy condition and leaf loss (from both drought and precocity perspective) and plant mineral element status, in particular K. Furthermore, once lifted from the nursery, root architecture could be determined (i.e. root type, diameter, number and angle) as these traits were linked to vine growth, drought tolerance, fruit composition and nutrient status.

While the three new selections possess resistance to certain root knot nematode species and phylloxera genotypes, there is a pressing need to develop rootstocks with more durable resistance to a broad-spectrum of root knot nematode and phylloxera genotypes. As a first step in achieving this aim we have identified new sources of resistance and developed molecular markers that will ultimately be used to combine these new resistance traits with existing resistance loci in new rootstock breeding lines. In this project, we successfully mapped the *Vitis cinerea* C2-50 root knot nematode resistance locus, *MJR1*, which provides complete resistance to two major species of root knot nematode in Australia, *Meloidogyne javanica* and *M. arenaria*. The *V. cinerea* C2-50 phylloxera resistant locus, *RDV2*, was also mapped and molecular markers were identified. The *RDV2* locus provides good resistance to the two major phylloxera genotypes in Australia: G1 and G4.

The project, across all the evaluation trials has demonstrated large impacts of rootstock genotype on vine growth characteristics with subsequent effects on fruit and wine composition. In particular there appeared to be strong genetic control of conferred vigour (pruning weight) which was generally associated with negative effects on fruit composition (high pH, malate and K and in some instances with reduced berry anthocyanins and wine colour). In the case of the detailed study with Shiraz, the relationships between rootstock genotype and pruning weight were stable across well-watered and deficit treatments in a hot climate and the milder Padthaway site. With the exception of malate, which was highly linked to pruning weight, all other growth and fruit composition parameters appeared to be more strongly impacted by environmental conditions across sites than by rootstock. Of the other growth characteristics measured, rootstocks with large berries and large bunches generally had poor fruit composition (high pH, malate, K and in some cases reduced berry anthocyanins and wine colour). In general, relationships between yield across rootstocks and berry composition were low. The results of the Shiraz study, across the sites in two regions showed that it was possible to identify new rootstock genotypes with acceptable productivity, which maintained yield under deficit irrigation that have enhanced fruit and wine composition compared to widely adopted commercial rootstocks. Further assessment of the most promising 'next generation rootstock' genotypes is required.

Over the course of the project there has been significant interaction with industry with presentations and updates presented to industry groups, wine companies and growers supported by tasting of wines produced from new rootstock selections and commercial varieties. As part of the release strategy, mother vine plantings of new selections have been

established to ensure that adoption is not limited by the supply of propagation material. The evaluation of regional sites trials covering a range of environments and varieties will provide ‘local’ information for interested parties and is seen as a mechanism to foster adoption of new rootstocks.

8. Recommendations

- Three new rootstock selections have been identified for release to industry (C113, C114 and C20).
    - It is recommended that PBR protection be sought for these selections. This will involve completion of data collection and analysis from the DUS comparator trial established for this purpose.
    - It is recommended that mother vine plantings, currently established by licensed nurseries be expanded to ensure sufficient material is available to meet industry demand.
    - It is recommended that the performance of these selections continues to be monitored at the CSIRO Irymple site to ensure their performance is maintained with mechanical hedging, which is the standard district practice.
    - It is recommended that the performance of these selections at regional sites is monitored in collaboration with regional groups and participating growers to provide specific varietal and site information for use in extension materials to support and enhance adoption.
- It is recommended that the Shiraz and Pinot Gris trials in Padthaway involving the salt tolerant, moderate vigour C7 selection continue to be evaluated to assess longer term performance under moderate saline conditions before a final decision to release for industry use is made.
- It is recommended that the performance of the best 'next generation rootstocks' grafted with Shiraz, identified for winemaking in 2017 from studies conducted in both the hot climate and in Padthaway continue to be monitored to identify the most promising types with potential for release in the future. In particular, it will be important to ensure their performance is maintained with mechanical hedging, which is the standard district practice at the hot climate site.
- The study has demonstrated that simple trait measurement, particularly in the nursery situation, can be linked to grafted vine performance in field trials. Hence it is recommended that for complex traits, where molecular markers are not available, that a simple screening procedure be implemented using replicated nursery studies to minimise the number of plants to be evaluated in field trials. This approach should involve:
    - a bench grafting propagation step prior to establishment, to screen for rootability (Smith and Clingeleffer 2013) and graftability

- establishment in a replicated high density nursery trial under both well-watered and deficit irrigation conditions
- measurement of growth traits (shoot length, pruning weight, trunk diameter), physiological traits (conductance, leaf temperature, transpiration efficiency by carbon isotope discrimination), canopy condition and leaf loss (from both drought and precocity perspective) and plant mineral element status, including N
- assessment of root architecture traits when the vines are lifted (type, diameter, number and angle)
- data analysis to identify the most promising 'fit for purpose' genotypes for evaluation in field trials which would be established with the material from the nursery.

- It is also recommended that further research be undertaken to develop rapid screening techniques with small plants in the glasshouse to further improve screening efficiency and minimise numbers established in the nursery. These could include growth rates and drought tolerance (as has been demonstrated in the Plant Accelerator studies with F34), mineral element status (e.g. K and Ca), trunk diameter and root architecture. In this regard, early assessment of useful traits including trunk diameter and root architecture (root diameter, number, type and angle) would be very useful because of the links to pruning weight, drought tolerance and K uptake. In previous studies (Smith and Clingeleffer 2011), trunk diameter of grafted vines has been linked to wood density, an indicator of xylem vessel size. More detailed studies should be undertaken to assess trunk diameter, root architecture and anatomy of the small plants to verify linkages with the traits measured in the field, to enhance the understanding of the physiological mechanisms involved and their genetic control.
- It is recommended that further research be undertaken to understand the underpinning physiology leading to the relationships with conferred vigour and other growth characteristics and fruit composition across rootstock genotypes. Such studies should involve transport of sugars and mineral elements into the berry, ion balance within the berry and the role played by organic acids. Specifically, the negative relationships between Ca and yield and bunch number warrant investigation from a fruitfulness perspective.
- Breeding rootstocks with durable resistance to root knot nematode and phylloxera is highly recommended. Mapping MJR1 and RDV2 and developing molecular markers linked to these resistance loci is the first step for breeding durable resistance. The recommendation is to map and develop molecular markers for resistance from other

species and rootstock breeding material. Once this achieved, the resistant loci can be stacked and field trials can be performed to evaluate the new material.

- From this project, there is a clear genetic component for potassium uptake e.g. as assessed in the F34 population. However, we were not successful in mapping a trait for potassium uptake indicating that numerous genes are likely control potassium uptake in F34. To map and develop molecular markers for potassium uptake, it is recommended to perform a SNP association analysis in the CSIRO species and rootstock germplasm, as this approach would have a higher success rate.
- A strong link between nitrogen and vigour was established in earlier studies (Clingeleffer 2000) and supported by berry YAN measurements in this project. It is recommended that this link be researched by examining nitrate transporters (expression, localisation and abundance) to correlate the function, expression and localisation of these genes/proteins with vigour. Having this understanding could be leveraged and utilised as a tool for optimising vigour management in the vineyard. Recent studies linking N uptake by rootstocks of varying vigour with gene regulation support this view (Cochetel et. 2017 and Medici et al. 2017).

9. Appendix 1: Communication

The project aimed to strengthen interaction with industry on rootstock development and evaluation to enhance adoption of appropriate rootstocks for Australian conditions. CSIRO has been actively engaged with industry to improve communication on the benefits of rootstocks and provide updates on the past and current research. In this regard, workshops were organised by CSIRO at both the 15th (13/07/2013) and the 16th Australian Wine Industry Technical Conference (24/07/2016). Overviews of the rootstock breeding and development project, accompanied by tastings of the small-scale wine lots from 'near to release' selections and standard were provided. Furthermore, presentations accompanied by tastings have been provided to various industry, wine company and grower groups. These included:

- The Mornington Peninsula technical group meeting May 2014
- Industry meeting in Stanthorpe in May 2014
- Regional officers supported by the WA extension program at the Waite campus, November 2014
- The 'Varieties and Rootstocks Regional Workshop', AGWA Extension and Adoption Program, SA Central (07/05/2015)
- The AWRI sponsored, 'Next Generation Planting Material Workshop' in Sunbury, Victoria (14/05/2015)
- the McLaren Vale Technical Viticultural Conference (13/09/2016)
- the Barossa Valley Vine Improvement meeting (07/12/2016)
- The Murray Valley Wine Grape Growers Council (09/12/2016)
- Yarra Valley growers meeting (04/08/2017)

Furthermore, over the course of the project, presentations and updates, accompanied by tastings of wines from advanced selections, have been provided to the Wine Australia Rootstock Reference Group which includes members from industry, Wine Australia and CSIRO (25/11/2013 and 25/11/2015) and a meeting with Wine Australia (30/07/2015).

CSIRO personnel also provided input into the development of the Wine Australia web-based 'Rootstock Selector Tool'. Experience on the performance of standard commercial rootstocks in the project trials and the CSIRO low/moderate vigour rootstocks was included in the development of this tool.

A presentation was also given and a manuscript produced for the GiESCO meeting in Argentina in November 2017 (Clingeleffer, P.R., Morales, N and Smith, H. Simple trait measurements across rootstock genotypes indicates performance as field grown, grafted vines. In proceedings 20th GiESCO International Meeting, Mendoza, Argentina, 5 -17 November 2017. 240-245).

The following research publication was published in 2017.

Smith, B.P., Morales, N.B., Thomas, M.R., Smith, H.M. and Clingeleffer, P.R. (2017) Screening of grapevines for resistance to the root knot nematode *Meloidogyne javanica*. Australian Journal Grape and Wine Research. **23**, 125-131.

Two manuscripts have been produced for submission to scientific journals to date which have been included in the results section above. They include:

1. Root knot nematode resistance in grapevine: screening, genetics and molecular markers development
2. Phylloxera resistance in grapevine: screening, genetics and molecular marker development

10. Appendix 2: Intellectual Property

Background intellectual property (BIP) involved CSIRO breeding lines and selections of rootstocks at various stages of development. They included seeds/seedlings of unique species/rootstock material, seeds/seedlings of segregating populations (including cinerea x vinifera and Ruggeri x K51-40), and unique multi-species breeding lines established as ungrafted seedling material, and multiplied plantings of approximately 10 advanced selections. These were provided for use in the project, but they (and any derived new rootstocks) will not be licensed to the Commonwealth (DAFF) beyond the project on a perpetual, royalty-free basis for further use and adaptation or for any commercialisation. Any further use, adaptation or commercialisation of such IP will be determined by CSIRO and Wine Australia. Formal contracts between the former GWRDC and CSIRO were tailored to manage the BIP.

Third party BIP may include biological materials or grapevine germplasm made available to CSIRO for research purposes under confidential Material Transfer Agreements and other third party contracts. Use of such materials in the project will be managed in accordance with third party IP conditions. Formal contracts between the former GWRDC and CSIRO have been tailored to manage the third party IP.

Outcomes generated from the project were:
- Improved rootstocks derived from background intellectual property.
- Data sets, genetic maps, functional genes, and DNA markers for breeding.
- Journal papers.
- Reports and other communication material.

Beneficial information disclosing breeding markers and genes will be disseminated to Australian industry via scientific publications or other means as appropriate (after assessment for potential protection). Any new plant variety rights or other IP including the grapevine varieties derived from background intellectual property will remain under the management and control of CSIRO and Wine Australia. The PBR will be assigned to CSIRO. Only copyright material such as reports will be licensed to the Commonwealth on a royalty-free basis for Commonwealth use.

11. Appendix 3: References

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12. Appendix 4: Staff

CSIRO

Peter Clingeleffer	- project leader
Dr Brady Smith (deceased)	- project development
Dr Harley Smith	- senior research scientist
Dr Mark Thomas	- senior research scientist
Dr Rob Walker	- senior research scientist
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DEDJTR

Kevin Powell and team	- phylloxera screening
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AWRI

John Gledhill, Michael Coode and team	- small-scale winemaking

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