ROOTSTOCK BREEDING AND DEVELOPMENT FOR AUSTRALIAN WINE GRAPES



FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **CSP 05/03**

Principal Investigators: **Mr. Peter Clingeleffer and Dr. Brady Smith**

Research Organisation: **CSIRO**

Date: **04/10/2011**

Title: Rootstock breeding and development for Australian wine grapes

Author details: Peter Clingeleffer and Brady Smith
CSIRO Plant Industry
PO Box 350, Glen Osmond SA 5064, Australia

peter.clingeleffer@csiro.au
brady.smith@csiro.au

Date published: 4/10/20011

Copyright statement

© 2011 CSIRO. To the extent permitted by law, all rights are reserved and no part of this publication covered by copyright may be reproduced or copied in any form or by any means except with the written permission of CSIRO Plant Industry.

CSIRO Plant Industry advises that the information contained in this publication comprises general statements based on scientific research. The reader is advised and needs to be aware that such information may be incomplete or unable to be used in any specific situation. No reliance or actions must therefore be made on that information without seeking prior expert professional, scientific and technical advice.

To the extent permitted by law, CSIRO (including its employees and consultants) excludes all liability to any person for any consequences, including but not limited to all losses, damages, costs, expenses and any other compensation, arising directly or indirectly from using this publication (in part or in whole) and any information or material contained in it.

Disclaimer

CSIRO Plant Industry advises that the information contained in this publication comprises general statements based on scientific research. The reader is advised and needs to be aware that such information may be incomplete or unable to be used in any specific situation. No reliance or actions must therefore be made on that information without seeking prior expert professional, scientific and technical advice.

To the extent permitted by law, CSIRO (including its employees and consultants) excludes all liability to any person for any consequences, including but not limited to all losses, damages, costs, expenses and any other compensation, arising directly or indirectly from using this publication (in part or in whole) and any information or material contained in it.

ISBN or ISSN if required

Table of Contents

1. Abstract 1

2. Executive summary 2

3. Background 5

4. Project Aims and Performance targets 8

5. Method 9

5.1 Phylloxera screening 9
5.2 Root-knot nematode screening 10
5.2.1 Commercial rootstocks in Australia 12
5.2.2 Potential new rootstocks from CSIRO breeding program 12
5.2.3 Genetic mapping population 12
5.3 Potassium uptake screening 12
5.4 Salt exclusion screening 15
5.5 Evaluation of rootstock nursery 15
5.5.1 Vigour 16
5.5.2 Drought tolerance 16
5.5.3 Nutrient uptake 17
5.6 scion rootstock compatibility 17
5.7 Characterization of root architecture and drought response of grapevine germplasm 17
5.8 Inheritance of rooting ability 19
5.9 Field trials of new rootstock selections 23
5.9.1 New CSIRO low – medium vigour rootstocks 23
5.9.2 Evaluation of next generation of rootstocks 24
5.9.3 Selection of potential new rootstocks from CSIRO breeding program 26

6. Results/Discussion 29

6.1 Phylloxera screening 30
6.2 Root-knot nematode screening 30
6.2.1 Commercial rootstocks in Australia 30
6.2.2 Genetic mapping population 34
6.2.3 Potential new rootstocks from CSIRO breeding program 34
6.3 Potassium uptake screening 36
6.4 Salt exclusion screening 37
6.5 Evaluation of rootstock nursery 38
6.5.1 Vigour 38
6.5.2 Drought tolerance 41
6.5.3 Nutrient uptake 42
6.6 scion rootstock compatibility 43
6.7 Characterization of root architecture and drought response of grapevine germplasm 45
6.8 Inheritance of rooting ability 49
6.9 Field trials of new rootstock selections 51
6.9.1 New CSIRO low – medium vigour rootstocks 51
6.9.2 Tolerance of soil borne pests 53
6.9.3 Progress with industry evaluation 54

6.9.4 Material availability 57
6.9.5 Evaluation of next generation of rootstocks 57
6.9.6 Selection of potential new rootstocks from CSIRO breeding program 67

7. Outcome/Conclusion 75

8. Recommendations 79

Appendix 1: Communication 83

Appendix 2: Intellectual property 86

Appendix 3: References 87

Appendix 4: Staff 92

Appendix 5: Budget reconciliation 93

Appendix 6: Performance of the CSIRO low-medium vigour rootstocks 94

Table of Figures

Figure 5.3.1 Flood tanks used for potassium uptake assay. 14
Figure 6.3.1 Correlation of ungrafted vine petiole potassium and grafted vine fruit pH. 37
Figure 6.4.1 Chloride exclusion of grapevines in glasshouse assay. 38
Figure 6.5.1 Mean scion vigour of rootstock families in a nursery 39
Figure 6.5.2 The correlation between pruning wood weights of ungrafted rootstock and grafted nursery vines 40
Figure 6.5.3 The correlation between pruning wood weights of grafted nursery vines and grafted field trial vine 40
Figure 6.5.4 δC13 of nursery vines.. 41
Figure 6.5.5 Correlation between petiole calcium content and vine vigour.. 42
Figure 6.6.1 Correlation between fruit anthocyanin and vine vigour traits.. 44
Figure 6.6.2 Correlation of scion wood density and vine vigour traits. 45
Figure 6.7.1 Correlation between trunk diameter and mean root diameter.. 48
Figure 6.7.2 The mean root diameter of 3 families of grapevines. 49
Figure 6.9.1 Mothervine planting of new CSIRO rootstocks 57
Figure 6.9.2 Chardonnay fruit traits Sunraysia (2011). 61
Figure 6.9.3 Cabernet Sauvignon fruit traits Sunraysia (2011) 61
Figure 6.9.4 Shiraz fruit traits Sunraysia (2011).. 62
Figure 6.9.5 Average berry weight Sunraysia (2011).. 62
Figure 6.9.6 Average bunch number per vine Sunraysia (2011). 63
Figure 6.9.7 Average fruit yield Sunraysia (2011). 64
Figure 6.9.8 The 3 year mean for grape juice pH Sunraysia (2009-2011). 64
Figure 6.9.9 The 3 year mean yield per vine Sunraysia (2009-2011).. 65
Figure 6.9.10 The 3 year mean harvest fruit soluble solids Sunraysia (2009-2011) 65
Figure 6.9.11 The 3 year mean Ravaz index Sunraysia (2009-2011) 67
Figure 6.9.12 The yearly dormant vine pruning weight for rootstock families. 72
Figure 6.9.13 The effect of limited irrigation on ungrafted and grafted grapevine rootstock families.. 73
Figure 6.9.14 Potassium uptake of rootstock families. 74

1.Abstract

Adoption of rootstocks suited to the Australian environment will be critical to the long-term sustainability of the Australian wine industry to offer protection against Australian bio-types of soil borne pests, reduce the impacts of high potassium soils, limited water supply, salinity, and enhance wine quality attributes. Accelerated techniques to screen rootstock hybrids for key traits were developed enabling their inheritance to be determined and minimise the number of selections to be progressed to field trials. Five new rootstock selections with phylloxera and nematode resistance, good field performance and acceptable wine quality attributes have been identified for further development and potential release. A further 55 selections selected for good propagation characteristics, appropriate vigour, low potassium and drought tolerance have been grafted with Shiraz and planted in a replicated field trial. A further 160 genotypes from the screening studies have been retained as ungrafted plants for future breeding and evaluation purposes. The 3 low-medium vigour CSIRO rootstock varieties have been established in commercial vineyards in Griffith, Sunraysia, Padthaway, and other regional sites. Ongoing assessment of these rootstocks has shown significant potential to facilitate production of wines with lower alcohol contents.

2.Executive summary

Adoption of rootstocks suited to the Australian environment will be critical to the long-term sustainability of the Australian wine industry with regards to risk aversion, as they offer protection against Australian bio-types of soil borne pests, limited water supply and salinity, and enhance wine quality attributes, including minimising the effects attributed to high potassium soils. The main purpose of the rootstock breeding program was to maintain the development of new rootstocks with characteristics suited to the Australian environment and industry needs. It aimed to screen new rootstock hybrid families against key selection criteria and investigate the inheritance of key traits to underpin development of future breeding strategies and identify new selections for further assessment as grafted plants. The field performance, wine quality attributes, root-knot nematode and phylloxera resistance of promising rootstock selections grafted with key wine varieties (Chardonnay, Cabernet Sauvignon and Shiraz) were also assessed to identify selections for further development and potential release to industry. Studies were also undertaken to investigate rootstock effects on vine growth and productivity, mineral discrimination, water use efficiency and drought tolerance to underpin the development of accelerated screening techniques. Industry adoption of the 3 CSIRO low-medium vigour rootstocks, Merbein 5489Ⓡ, Merbein 5512Ⓡ and Merbein 6262Ⓡ was facilitated in different regions to enable assessment of Genotype x Environment interactions with vine performance, fruit composition and wine quality attributes. The phylloxera screening studies were undertaken in collaboration with Dr Kevin Powell, Department of Primary Industries PI Victoria, Rutherglen.

From an industry perspective, the studies have shown that 30 of 69 genotypes available as rootstocks in Australia, including the widely planted 1103 Paulsen rootstock, allow reproduction of an aggressive strain of the root-knot nematode *Meloidogyne javanica* (Trueb) in glasshouse assays. In regard to bio-security, it will be important to establish how widespread the aggressive strain of *M. javanica* is in viticultural regions, to minimise further spread and provide growers with information for rootstock selection. Five new rootstocks with phylloxera and root-knot nematode resistance have been identified for further development based on their field performance and wine quality attributes when grafted with Chardonnay, Cabernet Sauvignon and Shiraz, which may offer growers with alternative rootstocks to meet future challenges.

Ongoing studies with the low-medium vigour CSIRO rootstocks, Merbein 5489Ф, Merbein 5512Ф and Merbein 6262Ф have shown that rootstocks are a powerful tool to manipulate berry composition (i.e. break the nexus between 'sugar ripeness' and 'flavour ripeness') and produce acceptable and preferred red wines at lower levels of alcohol than can be achieved with standard rootstocks such as Ramsey and 1103 Paulsen (Clingeleffer et al. 2011). Initial studies with the three rootstocks prior to their release had shown that they had good root-knot nematode tolerance (Dry 2007) against *Meloidogyne incognita* and two strains of *M. javanica*. When tested against the aggressive strain of *M. javanica*, Merbein 5512Ф and Merbein 6262Ф were shown to be tolerant, wheras Merbein 5489Ф was susceptible. This finding further highlighting the issue of rootstock choice for different locations. The project has also facilitated industry adoption of the three CSIRO rootstocks which were granted Plant Breeders Rights in 2009, and made available initially through a nursery with rapid propagation and green grafting facilities to overcome the issue of limited mothervine material. This was followed by the licensing of a nursery in South Australia, and one in Western Australia, and more recently a nursery in Sunraysia. Mothervine plantings have been established in Victoria and South Australia.

In the current study, 55 genotypes have been selected with good propagation characteristics, appropriate vigour and drought tolerance. Plants of these genotypes, grafted with Shiraz, have been included in a replicated trial to assess performance under well watered and deficit irrigation treatments and will produce the first crop in 2013. A further subset of these genotypes grafted with Shiraz has also been planted at Padthaway, South Australia to assess genetic influences that rootstocks have on fruit quality traits and grafted vine performance under limited irrigation. It is likely that a number of drought tolerant rootstocks will be identified in these trials for further development, once the trials are completed and phylloxera and root-knot nematode tolerance assessed. Furthermore, 160 genotypes with a diverse range of *Vitis* species as parents have been retained for future breeding and evaluation purposes as they were the best performers in the various screening studies involving selection for drought tolerance, low potassium uptake, salt exclusion, and propagation characteristics.

The development of efficient, high throughput, rapid screening techniques to improve rootstock breeding efficiencies and minimise the number of selections established in field trials has been an important component of this project. Multi-trait glasshouse screening techniques using the same plants have been developed and applied for root-knot nematode tolerance, potassium uptake, and chloride exclusion. The appropriateness of the potassium uptake screen has been verified using well established field trials with grafted plants. Furthermore, the evaluation of a large number of genotypes in a nursery situation has provided a very useful approach to assess vine vigour, plant mineral status, and drought tolerance without the establishment of long term field trials.

The project has also generated significant new knowledge to underpin future strategies for efficient rootstock breeding through targeted parental selection based on the known inheritance of key traits, which may also be augmented by the use of molecular markers to rapidly assist selection at early seedling stage. In this regard, the studies have shown that the breeding parent *Vitis cinerea* is an excellent source of root-knot nematode resistance, and is potentially also a source of phylloxera resistance. Additionally, drought tolerance and vine vigour has been linked to aspects of root architecture. The inheritance of adventitious root formation on dormant lignified canes (rootability) has also been determined and breeding values assigned to identify future parents to be used in new breeding to optimise performance for this trait. The results indicate potential to develop genetic markers for some key traits such as phylloxera, root-knot nematode resistance, and rootability, which would complement the rapid screening techniques and reduce numbers for screening as grafted plants in the nursery (i.e. for vigour and drought tolerance) and ultimately for planting in field trials.

3.Background

To remain internationally competitive in targeted export markets, the Australian wine industry has recognised a need to improve vineyard productivity while maintaining quality and reducing inputs. There was a rapid increase in wine grape plantings in the order of 100, 000 ha in the 1990's. A significant proportion of these plantings occurred using own-rooted vines, particularly under drip irrigation in sandy soils in the warmer inland production regions. New plantings in these areas are at great risk from nematode infestation as has occurred with devastating consequences for table grape production in the Northern Territory. Future redevelopment of such sites to varieties in demand by wine companies will require rootstocks. 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.

Root-knot nematode tolerant rootstocks were first assessed in the warm irrigated regions in the early 1960's (Johnston et al. 1992, Sauer 1972). Prior to the rapid development of wine grape plantings there was a steady adoption of rootstocks, in particular the high vigour, root-knot nematode tolerant types in the warmer irrigated regions. For example in 1996 more than 40% of Chardonnay, 25% of Cabernet Sauvignon, Semillon and Shiraz grapes were produced from grafted vines (source Australian Bureau of Statistics). A previous benefit:cost study showed substantial benefits from adoption of root-knot nematode tolerant rootstocks in the warm irrigated regions which are the major suppliers of fruit targeted for export wine styles (Johnston et al. 1992).

Adoption of rootstocks will be critical to the long-term sustainability of the industry particularly with regards to risk aversion (ie. protection from nematodes, phylloxera, salinity and other edaphic stresses), more responsible use of natural resources (ie. soil, nutrients and water), reduced use of nutrients and thus minimising run-off and eutrophication. Rootstocks also have the potential for vigour control and this may influence water use efficiency, enhance juice composition and wine quality attributes and facilitate adoption of low input management systems. There is a considerable body of evidence, both from commercial experiences and research results 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. This has produced a general negative attitude by industry to rootstocks, which together with their higher cost has discouraged adoption.

Studies across a range of scion varieties have demonstrated that rootstocks not only affect vine vigour but also modify scion berry and juice composition (eg. mineral and organic acid composition, pH and colour), which have flow-on effects on wine quality (Cirami et al. 1984, Clingeleffer 1996, Hale & Brien 1978, Ruhl 1989, Ruhl et al. 1988, Walker et al. 1998). Indeed, negative wine attributes associated with high vigour rootstocks such as Ramsey and Dogridge have been associated with high potassium uptake, high pH and malate levels and reduced colour in berries and poor spectral properties in red wine. In a previous CSIRO project supported by GWRDC (CSP 99/02), three new low-to-medium vigour, phylloxera and root-knot nematode tolerant rootstocks were identified for industry release with Plant Breeding Rights (PBR) protection. Compared to performance on Ramsey, 1103 Paulsen and 140 Ruggeri, these new rootstocks when grafted to Shiraz maintained adequate yields, produced enhanced quality fruit and wine that was associated with low potassium uptake, improved organic acid composition, and improved spectral properties as recently reported (Clingeleffer et al. 2011, Walker & Clingeleffer 2009). There is evidence that these rootstock selections have improved water use efficiency (Walker et al. 2005), which was linked to reduced canopy growth and lower transpiration, yet maintained an ability to ripen the fruit. Furthermore, requirements for less addition of tartaric acid for pH adjustment resulting from improved organic acid composition should reduce the cost of winemaking. These rootstocks were released to allow a coordinated commercial assessment in conjunction with wine companies and growers across a wide range of Australian environments as part of this project.

The previous project, which ended in December 2005, led to the selection of a further twenty six selections from existing hybrid populations. These new rootstock selections met key selection criteria (ie. good rooting and grafting ability, low potassium accumulation in their own foliage, low to medium ungrafted vine vigour and high root-knot nematode tolerance). In this project (CSP 05/03) they were evaluated under field conditions when grafted with key scions (Chardonnay, Cabernet Sauvignon and Shiraz), screened for resistance against additional strains of root-knot nematode, and their phylloxera tolerance assessed.

Furthermore, new *Vitis* inter- and intra-specific crosses were also generated in the previous project and established in the field as ungrafted plants with the aim to understand the underlying mechanisms and inheritance of key rootstock traits and develop accelerated screening techniques for water use efficiency, drought tolerance and scion/rootstock compatibility, potassium uptake, chloride exclusion, and root-knot nematode resistance screening.

This project focussed on four areas of research.

1. Screen new hybrid families to deliver genetic information for future breeding and identify new selections for further development
2. Assess the field performance of 26 advanced selections grafted with key wine varieties, i.e. Chardonnay, Cabernet Sauvignon and Shiraz
3. Develop more efficient screening procedures for water use efficiency, drought tolerance and scion/rootstock compatibility and identify new genotypes for further development
4. Facilitate industry adoption of the 3 low-medium vigour rootstocks released by CSIRO to enable their evaluation in different regions and assess Genotype x Environment interactions.

4.Project Aims and Performance targets

The project had four main components with the following objectives:

- To screen new rootstock hybrid families against key selection criteria, and thus investigate the inheritance of such traits which can be applied to future breeding activities, and also identify new selections for further assessment as grafted plants.

- To evaluate the field performance of promising rootstock selections when grafted with key wine varieties and their effects on fruit composition and wine quality.

- To investigate rootstock effects on vine growth and productivity, carbohydrate partitioning, mineral discrimination, water use efficiency and drought tolerance, and develop accelerated screening techniques for water use efficiency, drought tolerance and scion/rootstock compatibility.

- Facilitate industry adoption of the 3 CSIRO low-medium vigour rootstocks in different regions to enable assessment of Genotype x Environment interactions

5.Method

5.1 PHYLLOXERA SCREENING

A rapid screening technique has been developed by (Granett et al. 1987)to test rootstock resistance to phylloxera in controlled laboratory conditions. A modified version (Kingston et al. 2007) of this bioassay method was conducted at the Department of Primary Industries (DPI) Victoria under the supervision of Dr. Kevin Powell. Grapevine rootstocks were tested against two phylloxera clones, G4 and G30 in initial assays and clones G1 and G4 in subsequent trials.

Root Material

Lignified root pieces (10-15mm diameter) excised from field grown whole grapevines were used for inoculation with phylloxera for the laboratory bioassay (Kingston et al. 2007) . All rootstocks used were grown in an experimental vineyard at CSIRO Plant Industry, Merbein, Victoria. For the trial, the root pieces were transferred from Merbein, together with excised roots of control *Vitis vinifera* L. 'Sultana', to quarantine laboratory facilities at the DPI, Rutherglen, Victoria.

All excised roots were dipped in Ridomil™ fungicide at the rate of 7.3g/3L water to deter fungal attack and then rinsed three times in ultra pure water to ensure no residual fungicide remained on the root pieces (Kingston et al. 2007). Treatment and control roots were replicated 5-10 times with two 60-70mm long root pieces from each rootstock being placed in a Petri dish (140x25mm) lined with filter paper. One end of each root piece was wrapped in moistened cotton wool to ensure root survival during the trial period.

Insect Material

The three root-galling phylloxera clones used in all trials were single sourced from three commercial vineyards in Victoria. G4 phylloxera was sourced from the King Valley and G30 from Rutherglen, and the G1 strain was isolated from the Yarra Valley. Strains were kept under controlled laboratory conditions (23°C ± 2°C) at the DPI Rutherglen, Victoria, Australia, until the commencement of each trial.

Phylloxera clones were tested using six nuclear microsatellite DNA markers (Umina et al. 2007) at the commencement and completion of the trials to ensure no cross contamination during the trial.

Bioassay Method

Trials were set up to compare the relative resistance of grapevines against the two phylloxera strains. Experimental material was tested for resistance against a control genotype of known susceptibility to phylloxera (*V. vinifera* 'Sultana').

Each rootstock and control root piece was infested with ten phylloxera eggs of G1, G4 or G30 strain. After the infestation, the Petri dishes were sealed with Vitifilm™ and kept under controlled conditions in a constant temperature room of 23°C ± 2°C.

Phylloxera were counted two weeks after bioassays were set up, and where necessary, reinfested with another ten eggs if infestation had not taken place. After any reinfestation, bioassays were left for another two weeks before the final count of phylloxera was taken.

Measurements of population growth, egg number, crawler number and adult number were recorded. Only the statistical analysis of the total phylloxera survival is presented.

Statistical Analysis

Data were analysed using Genstat 5.4.2 (Lawes Agricultural Trust, Rothamsted, Harpenden, U.K.). The total number of surviving phylloxera was log10 transformed for ANOVA. Least significant differences (l.s.d.) were used to calculate significant differences at $p<0.05$.

5.2 ROOT-KNOT NEMATODE SCREENING

Root-knot nematodes (*Meloidogyne* spp.) are an endo-parasitic pest of grapevine which can cause severe damage to roots and reduce vine productivity (Nicol et al. 1999, Walker & Stirling 2008). *Meloidogyne javanica* (Treub) has been identified as the major root-knot

nematode species in Australian viticultural regions (McLeod & Steel 1999, Sauer 1962, Stirling 1976, Stirling & Cirami 1984). Glasshouse based screening is a rapid method for assessing the susceptibility through quantification of the number of juvenile females that can infest the roots and reproduce (Cousins & Walker 2001).

Glasshouse based screening for resistance to root-knot nematodes was conducted according to Cousins and Walker (2001). A virulent strain of *M. javanica* was isolated from a vineyard in McLaren Vale, South Australia. Monoculture of nematodes was established by inoculation of tomato plants with single egg masses in sand culture. Sub culture of egg masses collected from the single tomato plants was conducted to ensure fidelity of the strain of nematode isolated. Egg masses from the sub-cultured tomato plants were isolated for molecular fingerprinting. Utilizing previous reported species, specific primer combinations (McLeod & Steel 1999, Zijlstra et al. 2000) and novel oligos (data not shown), root-knot nematode species were indentified with use of DNA from standard strains of *M. incognita* (VW6), *M. javanica* (VW4) and *M. hapla* (VW9) (Valerie Williamson, Department of Nematology, University of California Davis). The sub-cultured tomato vines were moved to hydroponic culture to collect J2 juveniles. Nematodes were collected every other day and used to inoculate tomato seedlings in sterilized sand. After 6-8 weeks, tomatoes were transferred to hydroponic culture to provide J2 juveniles for inoculation of grapevine cuttings. Grapevine cuttings were transplanted to round black plastic pots (102 mm x 98mm, volume =0.6 L, Garden City Plastics) planted in a modified Cornell potting soil (2:2:1 coco peat:vermiculite:Waikerie sand) approximately 6-10 weeks after rootstrike. Three replicated randomized blocks were contained in a single glasshouse, and inoculated in sequential order according to placement on the bench. Grapevine cuttings were inoculated with approximately 1500 juvenile nematodes over the course of approximately 4 weeks. Vines were scored for egg mass development 6 weeks after inoculation. The soil was gently rinsed away and roots were soaked in eosin Y (0.25g/L) for one hour to stain the egg masses of reproductive females. Susceptible (*Vitis vinifera* ‘Ruby Cabernet’, ‘Riesling’, and ‘5BB Kober’) and resistant (Ramsey, Schwarzmann, Freedom) controls were included in each trial. Root systems of all vines were dried at 60°C overnight and weighed.

5.2.1 *Commercial rootstocks in Australia*

We identified 69 grapevine rootstocks in Australia which have been developed for commercial use. Glasshouse screening for susceptibility to the McLaren Vale strain of *M. javanica* was conducted in 2010-2011. Inoculation of 3 replications of each genotype was carried out as described in section 5.2.

5.2.2 *Potential new rootstocks from CSIRO breeding program*

We have screened 182 grapevine genotypes for root-knot nematode fecundity in the glasshouse. This group consists of germplasm for breeding new rootstocks as well as rootstocks which are currently being evaluated for other viticultural characteristics as described in sections below. Screening was conducted in the same manner detailed in section 5.2.

5.2.3 *Genetic mapping population*

A major component of our rootstock breeding programs involves introgression of disease resistant traits from *Vitis cinerea* '71'. The development of molecular markers associated with root-knot nematode resistance would increase the efficiency of breeding efforts by allowing selection of seedlings based on DNA fingerprinting. The cross of a root-knot nematode resistant female *V. cinerea* vine with the susceptible genotype *V. vinifera* 'Riesling' has resulted in 190 progeny. We have completed glasshouse screening of 80 of these progeny through the methods outlined in section 5.2.

5.3 POTASSIUM UPTAKE SCREENING

The uptake of potassium by grapevines is of particular importance to the Australian wine industry due to the relatively high levels of this ion that can be found in the soils of many viticultural areas. Screening for potassium uptake was initially conducted by determining the K^+ content of ungrafted vines at anthesis (Ruhl 1989). The petiole content of potassium of ungrafted rootstocks in the CSIRO germplasm collection was found to have a strong correlation with the juice pH of scion fruit grafted to these rootstocks in 3 field trials in a single year (Ruhl 1989). It was suggested by Ruhl (1990) that ungrafted vines in a glasshouse could be grown in hydroponic solution with a known level of K^+ in the media to observe the level of accumulation in the petioles. In order to test the efficacy of this screening method, a pilot study was undertaken. Petioles were collected from a set of

rootstocks for which long term fruit pH data of scions was available. These rootstocks were included in a field trial grafted to Shiraz and initiated in 1989 at a CSIRO site in Sunraysia. The petioles of the ungrafted vines were collected at anthesis from the CSIRO germplasm vineyard in Merbein, Victoria, in 1988 and 2009 for Inductively Coupled Plasma (ICP) analysis to determine potassium content. These rootstock genotypes were also present in a grapevine nursery which was established in 2006 and chip budded with Shiraz scions in 2007. Petioles from these nursery vines were collected at anthesis in 2009.

Petiole collection

Petioles from field grown vines were collected opposite from the primary inflorescence at anthesis.

The petioles of 3 commercial rootstocks and 7 experimental rootstocks were collected from vines grown in a glasshouse. Cuttings of mature vines were rooted in a mist propagation bed in a nutrient devoid medium (1:1 vermiculite to perlite). Approximately 6 weeks after rooting, cuttings were transplanted to a sterilized nutrient free soil mix (9:1 coco peat to coarse sand) and placed in a flood tray (Figure 5.3.1). Trays were flooded with reverse osmosis water twice a day for 2 hours for 14 days. Vines were then pruned to two internodes above the soil line with a single leaf. After the initial two weeks in the flood tray, a nutrient solution was provided for 6 weeks by flooding the roots twice a day for 2 hours. A standard Hoagland hydroponic solution was used with supplemented levels of potassium. Three replicates of each genotype were exposed to potassium at levels of 1.5mM or 15mM. Nutrient solution was changed weekly. After six weeks, up to 6 basal petioles were collected from each vine for mineral analysis.

Petioles from field grown vines were collected opposite the primary inflorescence at anthesis. Samples were washed in detergent and rinsed in Millipore water with detergent and HCl (approximately 2 drops ‘Morning fresh’ and 2 drops 1M HCl/5 L water) and then double rinsed in Millipore water. Samples were dried at 60°C for 72 hours and then ground with a ball mill. Approximately 0.10g of dried sample was combined with 2ml of 15.5mM HNO_3 for overnight digestion. A final volume of 20ml was achieved by addition of nano-pure water prior to ICP analysis (Spectroflame ICP, Spectro Analytical Instruments, Kleve, Germany).

Six petioles from each of the replicates of glasshouse grown plants were collected and kept separate. Petioles were washed and ground as describe above. Between 0.01g and 0.05g of dried material was digested with 1ml of 15.5mM HNO_3. Nano-pure water was added after overnight digestion to reach a final volume of 10ml. Potassium levels were determined through ICP analysis.

Regression analysis of grape juice pH and petiole potassium content was conducted with Sigma plot 11.0, Systat Software Inc., San Jose, CA.

Figure 5.3.1 Flood tanks used for potassium uptake assay. Three replicates of each vine were randomised in two flood tanks. Tanks were flooded for 1 hour twice a day with a Hoagland solution supplemented with either 1.5mM K^+ or 15mM K^+ for six weeks. Petioles were collected after six weeks, dried and assessed for potassium content.



5.4 SALT EXCLUSION SCREENING

The ability of grapevines to exclude chloride from lamina of ungrafted vines was examined in the glasshouse at CSIRO facilities at Urrbrae, South Australia. Vines were initially examined for potassium accumulation (Figure 5.3.2) in flood tanks. Upon completion of potassium screening, vines were pruned back to a single node on a non-lignified shoot with one leaf retained. Flood tanks were leached for 3 weeks with reverse osmosis water, and pruned again to a single node with one leaf retained.

After the leaching period, a nutrient solution was provided for 6 weeks by flooding the roots twice a day for 2 hours. A standard Hoagland hydroponic solution was used with supplemented levels of chloride salts. The low salt treatment had Hoagland solution supplemented with 5mM NaCl, 2.5mM $MgCl_2$ and 2.5 mM $CaCl_2$ for a final concentration of 10mM Cl^-. The high salt Hoagland solution was supplemented with 20mM NaCl, 15mM $MgCl_2$ and 15 mM $CaCl_2$ for a final concentration of 50mM Cl^-. Three replicates of each genotype were exposed to Cl^- at levels of 10mM or 50mM. Nutrient solution was changed weekly. After six weeks, laminae from the bottom half of the vine were collected.

Laminae were washed in detergent and rinsed in Millipore water with detergent and HCl (approximately 2 drops 'Morning fresh' and 2 drops 1M HCl/5 L water) and then double rinsed in Millipore water. Samples were dried at 60°C for 72 hours and then ground with a ball mill. Chloride analysis was conducted with a Sherwood Chloride Analyser. Model 926 Chloride Analyser, Sherwood Scientific Limited, Cambridge UK

5.5 EVALUATION OF ROOTSTOCK NURSERY

A rootstock nursery consisting of 20 replicates of 310 genotypes was established in the CSIRO site in Merbein, Victoria in 2006. The 310 genotypes represented 11 families (Table 5.5.1). Vines were planted as tandem pairs of genotypes at 10cm spacing in 10 blocks in a complete block randomized manner. The plot consisted of 12 rows with 622 vines per row with 2 meter spacing between rows. Rootlings were chip budded with Shiraz scions in spring 2007.

The nursery was dug up at the conclusion of the 2008-2009 season with grafted vines planted in two separate field trials (Padthaway, South Australia – September 2009 and Sunraysia,– October 2010).

Additionally, two blocks of graftlings were established with 60 rootstocks that were utilized in a long term field trial established at a CSIRO vineyard in Sunraysia in 1989. Both blocks were applied a well watered irrigation treatment.

Table 5.5.1 Rootstock families evaluated in the nursery trial at Merbein, Victoria. A total of 310 genotypes from 11 families were grafted with Shiraz scion and evaluated for 3 years in a rootstock nursery. The most promising genotypes for commercial application were planted as replicated field trials.

Family	Seed Parent	Pollen Parent
MC1	*Vitis cinerea* var. *helleri* [(Engelm.) ex. M. O. Moore]* '100'	*Vitis cinerea* var. *helleri* '12'
MC3	*Vitis cinerea* var. *helleri* '100'	*Vitis cinerea* (Engelm.)
MC61	*Vitis cinerea* '71'	*Vitis xchampinii* (Planch.) x *V. rupestris* (Scheele)
MC62	*Vitis cinerea* '71'	*Vitis xchampinii* x *V. riparia* (Michx)
MC63	*Vitis cinerea* '71'	*Vitis cinerea* var. *helleri* '12'
MC64	*Vitis cinerea* '71'	*Vitis cinerea* var. *helleri* '111'
MC65	*Vitis cinerea* '71'	*Vitis cinerea*
MC67	*Vitis cinerea* '71'	*Vitis xchampinii* x *Vitis* spp.
MC68	*Vitis cinerea* '71'	*Vitis rupestris*
MC86	*Vitis cinerea* '71'	*Vitis rupestris* x *Vitis riparia*
MC88	*Vitis cinerea* '71'	*Vitis acerifolia* (Raf.)

* synonymous with *Vitis berlandieri* (Planch.)

5.5.1 *Vigour*

Each vine was assessed for vigour by measurement of the total shoot length of dormant vines in 2007 and 2008. In 2008, the dormant grafted vines were cut to two bud spurs and pruning weight was recorded.

5.5.2 *Drought tolerance*

Drought tolerance was estimated by the reduction in scion vigour of vines receiving the low irrigation treatment relative to the well watered treatment. The nursery was watered with overhead sprinklers in the 2006-07 season and converted to in-line drip irrigation for the 2007-08 season. All blocks received the same irrigation regime in the 2006-2007 season, which equated to approximately 1.3L/vine, twice a week. Two irrigation treatments were applied across the 10 blocks in the nursery in the 2007-08 and 2008-09 seasons. In the 2007-

08 season, blocks receiving the treatment of 'sufficient irrigation' received 0.49L/vine three times per week, blocks with reduced irrigation treatment received 0.49L/vine once per week. In the 2008-09 season, the sufficient irrigation treatment was increased to 0.98L/vine three times a week, while the reduced irrigation was kept at 0.49L/vine once per week. The dormant cane pruning weight of the Shiraz scion was measured after the 2007-08 and 2008-09 seasons. In the 2008-09 season, soil moisture content was automatically logged daily at 10am with a Campbell Loggers PC200W. Sensors were placed at the centre of each block.

5.5.3 *Nutrient uptake*

The nutrient uptake of young grafted vines planted in a nursery was evaluated. Petioles were collected at anthesis from grafted scions for two groups of vines. One group consisted of 19 genotypes which were also included in the long term trial in Sunraysia, Victoria. The other group consisted of 145 genotypes representing 10 families under evaluation for potential commercial application as rootstocks. Petioles were collected in November, 2009. Petioles were processed and nutrient content was determined as described in section 5.3.

5.6 SCION ROOTSTOCK COMPATIBILITY

The relative wood density of the field trial from 1989 was measured with a pilodyn on 3-5 replicates of the 60 rootstocks above and below the graft union. The pilodyn is a handheld tool that fires a metal rod into wood and measures the penetrance in millimetres. The penetrance of the rod is a function of wood density.

5.7 CHARACTERIZATION OF ROOT ARCHITECTURE AND DROUGHT RESPONSE OF GRAPEVINE GERMPLASM

The root morphology and habits of 603 grapevine genotypes, representing 14 rootstock families, was observed upon removal of mature vines from a germplasm block at the CSIRO site in Merbein, Victoria (Table 5.7.1). Vines were established in 2001-2003, and removed in August 2011.

All vines were measured upon removal for total root number at the root crown, overall root angle (orientation of >90% of total roots), root branching (amount of branching of >90% of total roots), and root thickness (relative to trunk diameter of majority of roots). Vines which

had >90% of roots with a final orientation of vertical were given a score of "1", and those with >90% roots with a final orientation of horizontal a score of "3", all other vines were given a score of "2" and considered intermediate. In a similar manner, vines with >90% of the roots which were fine and multi-branched were scored "1", and those with >90% large roots with no or little branching given a score of "3", with all others being graded "2".

More detailed inspection of roots was conducted for 3 families (MC34, MC86, and MC88) derived from a single seed parent (*Vitis cinerea* '71'). All vines (*n*=213) were measured for trunk diameter above root zone, and the 6 largest roots were measured for diameter and final root angle relative to the trunk.

The performance of 310 of the ungrafted grapevine germplasm genotypes was compared to grafted vines in a rootstock nursery.

Drought was imposed from 2008 to 2011, however natural rainfall in the 2010-2011 season resulted in sufficient ground water for drought recovery to be observed by return of pruning weights to above pre-drought levels. Annual rainfall in seasons 2008-2009, 2009-2010, and 2010-2011 was 229 mm, 289 mm and 924 mm, respectively. Dormant canes were collected each winter from 2008-2011 and the pruning weights recorded.

Table 5.7.1 Rootstock families examined for root architecture. A single vine replicate block of ungrafted mothervines consisting of 14 families was examined for root traits upon removal in 2011.

Family	Seed Parent	Pollen Parent
MC1	*Vitis cinerea* var. *helleri* '100'	*Vitis cinerea* var. *helleri* '12'
MC2	*Vitis cinerea* var. *helleri* '100'	*Vitis cinerea* var. *helleri* '111'
MC3	*Vitis cinerea* var. *helleri* '100'	*Vitis cinerea*
MC34	*Vitis cinerea* '71'	*Vitis vinifera* 'Riesling"
MC42	*Vitis cinerea* '71'	*Vitis vinifera* 'Cabernet Sauvignon'
MC61	*Vitis cinerea* '71'	*Vitis xchampinii* x *V. rupestris*
MC62	*Vitis cinerea* '71'	*Vitis xchampinii* x *V. riparia*
MC63	*Vitis cinerea* '71'	*Vitis cinerea* var. *helleri* '12'
MC64	*Vitis cinerea* '71'	*Vitis cinerea* var. *helleri* '111'
MC65	*Vitis cinerea* '71'	*Vitis cinerea*
MC67	*Vitis cinerea* '71'	*Vitis xchampinii* x *Vitis* spp.
MC68	*Vitis cinerea* '71'	*Vitis rupestris*
MC86	*Vitis cinerea* '71'	*Vitis rupestris* x *Vitis riparia*
MC88	*Vitis cinerea* '71'	*Vitis acerifolia*

5.8 INHERITANCE OF ROOTING ABILITY

Propagation of dormant grape canes

Dormant lignified grapevine canes were collected in June and July as 4 node cuttings from mature vines growing in the CSIRO germplasm collection in Merbein, Victoria. Source material was from mature vines at least 3 years old. In total, 535 individuals were assessed for rooting of dormant canes. This represented 25 populations derived from the controlled mating of 26 parent vines (Table 5.8.1). Rooting ability of dormant canes for progeny populations which did not utilize *Vitis cinerea* '71' or *Vitis cinerea* var. *helleri* '100' as the seed parent was assessed in two consecutive years (1999 and 2000). Progeny populations with *V. cinerea* '71' or *V. cinerea* var. *helleri* '100' as the seed parent were examined for rootstrike of dormant canes in 2006 and 2010. All cuttings were soaked in water for hydration, placed in polyethylene bags, loosely sealed and maintained at 4°C for a minimum of 6 weeks. The basal bud of the material examined in 1999 and 2000 was removed after cold storage treatment and vines were planted in 20L crates containing 15kg washed sterilized sand. Crates were placed on a sand bed with bottom heat of 25°C for 6-8 weeks and hand watered daily. The number of vines which developed roots was calculated for each

genotype each year, with the mean for the two years used for analysis. Each genotype was replicated as twenty cuttings in 1999, and twenty-five cuttings in 2000.

The progeny populations of *V. cinerea* '71' and *V. cinerea* var. *helleri* '100' were sorted into 5 bundles of 4 cuttings in 2006 and single bundles of 10 cuttings in 2010. Material was removed from cold storage and prepared for rooting in August in 2006 and November-December in 2010. In 2006 dormant canes were packed horizontally in moistened sterile vermiculite in styrofoam boxes. Each replicate was placed in separate boxes assorted randomly. Internal temperature of callusing boxes was 23-24°C in 2006 and 26-28°C in 2010. The progeny populations of *V. cinerea* '71' and *V. cinerea* var. *helleri* '100' were prepared in a similar manner in 2010, except that callusing/rooting media was 1:1 mixture of vermiculite and perlite. Vines were scored for roots after 4-6 weeks, dependent on the extent of callus and shoot development. In all years, controls of easy to root material were included in the trials, and any non viable cuttings (having brown cambium at time of scoring for rooting) were discarded.

Table 5.8.1 Heritability of rooting ability. The rate of rootstrike of dormant lignified cuttings of 535 individuals was assessed in two years to determine the inheritance of this trait. Genotypes with higher rates of rootstrike were noted for commercial applications and germplasm for breeding.

Seed parent	Pollen parent	Number of progeny examined
Dogridge	Schwarzmann	16
K 51-32	*Vitis cinerea* var. *helleri* '12'	5
K 51-32	SO4	21
K 51-40	1103 Paulsen	11
K 51-40	140 Ruggeri	11
Kober 5BB	1103 Paulsen	6
Kober 5BB	J 17-69	15
Kober 5BB	K 49-56	35
Vitis cinerea var. *helleri* '100'	Merbein 6262	18
Vitis cinerea var. *helleri* '100'	*Vitis cinerea* var. *helleri* '12'	23
Vitis cinerea '71'	J 17-69	9
Vitis cinerea '71'	K 49-56	29
Vitis cinerea '71'	Merbein 6262	16
Vitis cinerea '71'	*Vitis cinerea* var. *helleri* '12'	48
Vitis cinerea '71'	*Vitis cinerea* var. *helleri* '111'	15
Vitis cinerea '71'	Ramsey x 1103 Paulsen hybrid	14
Vitis cinerea '71'	Schwarzmann	52
Vitis cinerea '71'	*V. acerifolia*	58
Vitis cinerea '71'	St. George	12
Ramsey	1103 Paulsen	10
Ramsey	Schwarzmann	51
Vitis cinerea 'B58'	*Vitis cinerea* 'B194-1'	17
Vitis cinerea 'B55'	*Vitis cinerea* 'B194-1'	9
Resseguier #1	Mazade	17
Resseguier #1	7651	17

Estimation of narrow sense heritability and breeding value

The estimates of narrow sense heritability (h^2) of rooting was calculated using a generalised linear mixed model (GLMM). Statistical analysis for h^2 and associated variances was

conducted with ASReml-R (VSN International Ltd.). A logistic generalised linear mixed model (GLMM) has been fitted to the data, of the form:

$$\log_e\left(\frac{\pi}{1-\pi}\right) = \text{constant} + \text{Year} + \text{Genotype} + \text{Block}$$

Where π = probability that a propagated vine becomes rooted; Year = effect of year that vines were propagated (fixed); Genotype = random Genotype effect, linked through a pedigree; and Block = random block effect. Not all vines were propagated in all years, with groups being propagated in 1999, 2000, 2005, and 2010. The term block referred to the original block where the mothervine was planted. Pedigree files for ASReml-R were constructed with inclusion of all known parents and grandparents, with progenitor with unknown rooting coded "NA" (Gilmour et al. 2009).

The set of genotype effects was assumed to be normally distributed, $N(\mathbf{0}, \sigma_A^2\mathbf{A})$ where σ_A^2 is the additive genetic variance, and **A** is the numerator relationship matrix corresponding to the defined pedigree structure. Block effects are assumed to be mutually independent, $N(0, \sigma_B^2)$. Binary data were treated in accordance to Falconer and Mackay (1996), where additive genetic variance applies on an underlying liability (unobserved) scale. The narrow sense heritability on this underlying scale is $h^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_B^2 + \pi^2/3}$, where the term $(\pi^2/3)$ corresponds to the (residual) variance for a standard logistic distribution (Gilmour et al. 2009). An approximate standard error for h^2 was calculated using a delta-method approach (Gilmour et al. 2009).

Best Linear Unbiased Predictions (BLUPs) of the random Genotype effects were obtained after fitting the model. These correspond to estimated breeding values (EBVs) for each grapevine. As full pedigree information was available to all populations, every individual was fitted with an EBV. Estimated breeding values were calculated on a log odds scale.

Estimation of pedigree and parent effect on adventitious rooting from dormant canes

The estimation of the pedigree and parent effects was conducted with a REML model with GenStat 13 (VNS International). The square root of arcsine of percent rooting was calculated to transform the data into to a normal distribution for variance analysis. Pedigree effects were estimated with the response variate set as the transformed rooting percentage, fixed for

seed and pollen parents, with the genotype assigned as the random model. Pedigree effects were back transformed on the original scale. Parent effects were calculated from the same data set with the fixed model set for either seed parent or pollen parent. Parent effects were back transformed on the original scale.

5.9 FIELD TRIALS OF NEW ROOTSTOCK SELECTIONS

We have established 8 field trials for the evaluation of rootstocks at different stages of selection for commercial release.

5.9.1 *New CSIRO low – medium vigour rootstocks*

Three low-medium vigour rootstocks, developed from the CSIRO grapevine rootstock breeding and evaluation program (Clingeleffer et al. 2011) have been released for larger scale industry evaluation. The purpose of wider industry evaluation was to assess their suitability in regional environments with varying abiotic stresses (i.e. temperature, water and salt) and with varying levels of soil-borne pest pressures (e.g. strains of root-knot nematodes and phylloxera). The new rootstocks, grafted with Shiraz, have been embedded in new commercial plantings by companies in a number of regions. This material was developed through rapid propagation and green grafting under glasshouse conditions by a commercial nursery, due to the limited supply of propagation material prior to establishment of mothervine plantings. The performance of the new rootstocks in these commercial sites has been monitored to assess their adaptability in the different regions. The first commercial planting, grafted with Shiraz and located near Griffith, New South Wales, produced a significant crop in season 2010, with two further plantings in Pomona, New South Wales (Sunraysia region) and Padthaway, South Australia, both bearing in 2011.

Sunrayasia

The CSIRO Sunraysia site was first established in 1989. It involved 55 rootstock genotypes from the breeding program and 5 standard rootstocks (i.e. Ramsey, Dog Ridge, Freedom, 1103 Paulsen and 140 Ruggeri. The three low to medium vigour rootstocks were selected based on their performance at this site. Their ongoing performance has been monitored as part of this project.

Griffith

The Griffith site involves a comparison of Merbein 5489Φ, Merbein 5512Φ and own roots.

Pomona

The field trial planted at Pomona, New South Wales, involves a comparison of Merbein 5489 Φ, Merbein 5512Φ , Merbein 6262Φ and 140 Ruggeri.

Padthaway

The three new CSIRO rootstocks (Merbein 5489Φ, Merbein 5512Φ , Merbein 6262Φ) were included in two separate blocks at a commercial site in Padthaway, South Australia. The three rootstocks were planted in a commercial vineyard as whole row replicates intermixed with rows of standard rootstocks (140 Ruggeri, 101-14 Mgt, Ramsey, 1103 Paulsen, and 110 Richter). Yield of a portion of the trial was measured in 2011. Additionally, the 3 Merbein rootstocks were included in an experimental rootstock trial described in section 5.9.3., which will come into bearing in the 2011-2012 season

5.9.2 *Evaluation of next generation of rootstocks*

The next generation of rootstocks being developed consists of 26 experimental rootstocks that were selected for low potassium uptake of ungrafted vines, high rate of rootstrike and grafting success, and moderate to high vigour of ungrafted vines. The evaluation and further selection of this material is being carried out through field trials and glasshouse screening methods.

CSIRO Sunraysia trial

A rootstock trial consisting of 26 experimental rootstocks and 3 commercial rootstocks (1103 Paulsen, 140 Ruggeri, and Ramsey) was established in 2005. Each rootstock was grafted as 5 vine replicates with scions of *V. vinifera* ‘Shiraz’ (clone PT23), ‘Cabernet Sauvignon’ (clone 22-4), and ‘Chardonnay’ (clone I10V5). Each rootstock and scion combination was planted in a randomized complete block arrangement in adjoining blocks. Vines came into bearing in 2008 and it was then observed that the grafting contractor had erroneously grafted Viognier

in place of Shiraz. This was rectified in the following season by regrafting with Shiraz (clone PT23) scions. Fruit traits were measured at harvest in 2009, 2010 and 2011 for Cabernet Sauvignon and Chardonnay and 2010 for Shiraz. The berry weight was determined by 100 random berry samples per vine which was crushed to determine pH and acid levels. Total yield for each vine was recorded. Sugar levels at harvest were measured with a refractometer. The weight of the dormant vine cane prunings were recorded from 2009-2011. Small scale wine was made from a subset of Cabernet Sauvignon and Chardonnay vines in 2009, 2010, and 2011. In 2011, wine was made from grapes from all three scion varieties grafted on 4 genotypes which have been selected for potential commercial release and the 3 commercial rootstocks.

Padthaway trial

The 19 best performing genotypes from the CSIRO Sunraysia trial were selected for a rootstock trial in Padthaway, South Australia. The experimental rootstocks and 4 commercial rootstocks (101-14 Mgt, 140 Ruggeri, 1103 Paulsen, and Ramsey) were grafted with Shiraz (clone PT23). The trial was established in 2009 as 3 single vine replicates arranged in a complete block randomised manner. Vines will come into bearing in the 2011-2012 season.

Root-knot nematode screening

Screening for resistance against the McLaren Vale strain of *M. javanica* for the 26 rootstocks, and whenever possible, corresponding parents, was evaluated in the glasshouse as detailed in section 5.2.

Phylloxera screening

The phylloxera tolerance of 10 of the 26 genotypes was examined by Kevin Powell at DPI Victoria according to methods described in section 5.1.

Salt exclusion

Five genotypes (C03, C20, C29, C113, and C114), selected from the 26 experimental rootstocks for further commercial evaluation were screened for salt exclusion as detailed in

section 5.4. In addition, 7 commercial rootstock (110 Richter, 140 Ruggeri, 1103 Paulsen, 1202 Couderc, Dogridge, Schwarzmann, and St. George) and 3 *V. vinifera* (Chardonnay, Shiraz, Ruby Cabernet) were included for comparison.

Nutrient uptake

The potassium uptake and transport of the 5 rootstocks selected from the 26 original genotypes was examined along with appropriate controls in a glasshouse assay outlined in section 5.3.

Propagation

The propagation and grafting attributes of all 26 genotypes was initially examined while establishing the trial in 2005. In order to asses the commercial application of the 5 genotypes selected for potential release, two nurseries are trialling these rootstocks for propagation characteristics under a Testing Agreement.

5.9.3 *Selection of potential new rootstocks from CSIRO breeding program*

As a result of screening assays, selections from a group of 310 experimental rootstocks were conducted. There were a total of 11 families in this group, of which 9 shared the same seed parent (*V. cinerea* '71').

The 310 genotypes which were grafted with Shiraz (clone PT23) scions for evaluation in the nursery have been evaluated for the traits of rootstrike, vigour potential, and response to reduced irrigation. As a result of performance in the nursery for these traits, 159 of the genotypes were further evaluated for potassium uptake. Screening for root-knot nematode resistance was conducted on families MC 86 and MC 88 and 11 additional promising genotypes.

CSIRO Sunraysia trial

A rootstock trial was established in November, 2010 to evaluate the field performance of 55 of the most promising drought tolerant rootstocks from the 310 genotypes under investigation. In addition to the 55 rootstock genotypes grafted with Shiraz (clone PT23), 6 commercial rootstocks (140 Ruggeri, 1103 Paulsen, Merbein 5489Φ, Merbein 5512Φ and

Merbein 6262Ⓟ and Schwarzmann) and own rooted Shiraz were included. Vines were arranged as single vine replicates in randomised complete block design in 4 adjoining blocks. Seventeen genotypes were selected on the basis of high rate of rootstrike, vigour potential, potassium uptake and performance in the rootstock nursery under low irrigation treatment. The remaining 37 genotypes were selected from a single family (MC86) to evaluate the genetic component of drought tolerance. Two of the four blocks will be irrigated at 100% of evapotranspiration (ET), with the other two blocks receiving 35% ET. Glasshouse based root-knot nematode screening was completed in August 2011 as described in section 5.2. The anthesis potassium content of petioles of ungrafted mothervines was determined in 2009.

Padthaway trial

A 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 (MC86) grafted to Shiraz (clone PT23), along with 7 commercial rootstocks (101-14 Mgt, 140 Ruggeri, 1103 Paulsen, Dogridge, Merbein 5489Ⓟ, Merbein 5512Ⓟ and Merbein 6262Ⓟ) and own rooted Shiraz. Vines were arranged as 5 adjoining blocks of single vine replicates in a randomized complete block design. This family was selected on the basis of normal distribution pattern of rootstrike and vigour potential, root-knot nematode resistance in both parents, and average response in vigour reduction to reduced irrigation in a nursery. Nutrient status of ungrafted vines was assessed from petioles of ungrafted mothervines collected at anthesis in 2009.

Nematode resistance

The parents used to generate the 11 families were screened for root-knot nematode resistance in the manner described in section 5.2 in 2010. Two families (MC86 and MC88) were screened in entirety for root-knot nematode resistance in 2010-2011.

Phylloxera resistance

The phylloxera tolerance the seed parent of 268 of the 310 genotypes was examined by Dr. Kevin Powell according to the protocol in section 5.1. The seed parent *V. cinerea* '71' was examined for its root response against inoculation with G1 and G4 strains of phylloxera.

Salt exclusion

As part of the pilot study on salt exclusion screening in the glasshouse as detailed in section 5.4, two progeny of family MC86 and the pollen parent (Schwarzmann) were examined for salt accumulation in the laminae.

Vigour potential

The vigour potential of all 310 genotypes was examined as ungrafted vines in 2008-2011 by measurement of the dormant vine cane pruning weight. Also, the vigour potential of grafted vines in the nursery was measured by collection of dormant scion canes in 2009.

Drought tolerance

The carbon isotope discrimination (δ13C) was measured for Shiraz scions grafted on single vine replicates in 4 of the nursery blocks. The grafted vines in the nursery were examined for δ13C content at the conclusion of the 2006-2007 season, after receiving full irrigation for the season. Laminae and petioles were collected from the 10 most basal nodes of each scion and stored at 3°C until processing. Samples were washed in Millipore water with detergent and HCl (approximately 2 drops 'Morning fresh' and 2 drops 1M HCl/5 L water) and then double rinsed in Millipore water. Leaves and petioles were dried at 60°C for 72 hours. Laminae and petioles were ground as entire samples for 15 seconds using a bench top ring mill. The δ13C was determined by radio mass spectrometry with use of a working standard of carbon isotope composition relative to Pee Dee Belemnite. Carbon isotope discrimination analysis and calculations were conducted by CSIRO Plant Industry, Black Mountain facilities in Canberra, ACT. The mean value of δ13C for each genotype was calculated across the 4 blocks. A One Way Analysis of Variance (ANOVA) was conducted using Sigma plot 11.0 (Systat Software Inc., San Jose, CA) to test for significant difference in δ13C across families ($P<0.001$). All pairwise comparisons for significant differences between mean values of the 11 families were conducted with a Bonferroni t-test ($P<0.05$). Normality of data was examined with a Shapiro-Wilk test.

The response to reduced irrigation in the nursery was measured after 2 years across 6 blocks. Three of the blocks received full irrigation in seasons 2007-2008 and 2008-2009, and three

blocks received low irrigation in this time period as described in section 5.5.2. The vines were all trained to a single shoot, with lateral shoots removed at regular intervals throughout the 2008-2009 season. Final pruning weight of Shiraz scions was collected from dormant vines in 2009. Sigma Plot 11 was used for statistical analysis of treatment effects.

Propagation

The rootstrike of all 310 genotypes was observed in 2006 and 2010. In 2006, dormant canes were sorted into 5 bundles of 4 cuttings and single bundles of 10 cuttings in 2010. Vines were handled and scored as detailed in section 5.4. The Estimated Breeding Value (EBV) of all genotypes was calculated with a GLMM model detailed in section 5.8.

6.Results/Discussion

6.1 PHYLLOXERA SCREENING

The relatively poor survival and development of phylloxera on roots of experimental vines compared to susceptible controls indicates a high degree of tolerance. The new Merbein rootstocks, Merbein 5489®, Merbein 5512®, and Merbein 6262® were all found to have high tolerance to the G4 and G30 strains of phylloxera (Korosi et al. 2011). The next generation of rootstock under testing (C03, C20, C29, C113, and C114) were found to have moderate to high resistance against the G1 and G4 strains of phylloxera. The highest relative resistance was found for C03, C20, and C29. The remaining genotypes displayed moderate resistance relative to the aforementioned rootstocks and the susceptible control *V. vinifera* 'Sultana'.

The selection of rootstocks with phylloxera resistance remains a priority in breeding programs (Galet 1998). Rootstocks with *V. vinifera* as a large component of the pedigree is generally considered a risk, as breakdown in resistance is known to occur (Granett et al. 1996).Whilst the laboratory bioassay for screening can quickly identify genotypes susceptible to phylloxera, the long term resistance requires lengthy field trials (Granett et al. 1987).

6.2 ROOT-KNOT NEMATODE SCREENING

McKenry and Anwar (2006) consider grape resistance to root-knot nematode as the disruption of the life cycle of the nematode, and tolerance is viewed as the presence of the pest not being detrimental to growth of the vine compared to a non-infected control. They determined plants to be resistant if less than 0.2 nematodes were present in the soil per gram of root in a micro plot field trial. Cousins and Walker (2001) qualified resistance as being less than 2 egg masses in their potted seedling trial. McKenry and Anwar (2006) found that the rootstocks K 51-32, 99 Richter, and 3309 Couderc supported *M. javanica* reproduction at a level to warrant their classification of susceptible. However, all three of these rootstocks exhibited a gain in vegetative growth after 18 months, thereby receiving an overall rating of tolerant. Use of rootstock which allows for nematodes to freely reproduce, but do not negatively impact vegetative growth, may be a mechanism for selection of more virulent strains of the nematode and have a detrimental effect on subsequent replanting due to the large population of nematodes that can accumulate.

Resistance in our glasshouse screening was classified as no egg masses on all three replicates of potted vines in the glasshouse trial. Tolerant genotypes had less than one average egg mass per average gram of dry root weight across the three replicates. Vines were considered susceptible to *M. javanica* when more than one mean egg mass was present per mean gram of dry weight of roots.

6.2.1*Commercial rootstocks in Australia*

The glasshouse screening of 69 rootstock varieties and breeding germplasm for susceptibility to *M. javanica* root-knot nematodes resulted in 41 genotypes with no egg masses found after 6 weeks, 27 genotypes with a mean of 1 or more egg masses (per g vine dry root weight), and 8 with an average egg masses total 0>1 (per g vine dry root weight)(Table 6.2.1). These results generally agree with previous reports of resistance to *Meloidogyne incognita* (Cousins & Walker 2002, Dry 2007, Lider 1954, Snyder 1939). Notable exceptions (susceptible to *M. javanica*) were Kober 5BB, 1103 Paulsen, J 17-69, and SO4, which have all been reported to have resistance to *M. incognita* in trials in North America and Europe.

Identification of germplasm which is resistant to the McLaren Vale strain of root-knot nematode is important for both growers and future rootstock breeding. Grapevines which display resistance to this virulent strain of *M. javanica* will need to be tested for resistance against other species and strains found in Australian soils.

The composition of root-knot nematodes varies across Australian viticultural regions, (Harris 1984, McLeod & Gendy 1996, Quader et al. 2001) with conflicting reports of the identification of *Meloidogyne* species (Hugall et al. 1994). However, it is apparent that *M. javanica* is present in all areas where root-knot nematodes have been noted.

The level of resistance of a given rootstock has been shown to be specific to the species of root-knot nematode, the strain, as well as temperature dependent (Anwar & McKenry 2002, Boubals 1992, Cain et al. 1984, Chitambar & Raski 1984, Nicol et al. 1999, Walker 1997, Walker 2009). Testing for grapevines with resistance to root-knot nematodes in California was initially focused on *M. incognita* which was determined to be the prevalent RKN species. Grape genotypes which are resistant to *M. incognita* have been well characterized

(Bloodworth et al. 1980, Cousins & Lauver 2003, Cousins et al. 2003, McKenry & Kretsch 1995, Nicol et al. 1999, Snyder 1939, Walker et al. 1994), with resistance proposed to be a single dominant gene in most *Vitis* species (Cousins & Walker 2002, Lider 1954, Lowe & Walker 2006). Subsequent studies have investigated the resistance to virulent strain of *Meloidogyne arenaria* [(Neal, 1889) Chitwood, 1949] (Cain et al. 1984, McKenry & Kretsch 1995) which was shown to be effective at reproducing on rootstock known to be resistant to *M. incognita* (Anwar & McKenry 2002, Cousins et al. 2003, Ferris et al. 1982). The occurrence of multiple species of root-knot nematodes occurs at sites in North America, South America, Europe, South Africa and Australia (Aballay et al. 2009, Ferris & McKenry 1974, Loubser 1988, Stirling & Cirami 1984, Terlidou 1974, Walker & Stirling 2008).

Cultivars and breeding germplasm derived from *Muscadinia rotundifolia* [(Michx.) Small], *V. acerifolia*, *Vitis aestivalis* (Michx.), *V. cinerea*, *V. cinerea* var. *helleri*, *Vitis cordifolia* (Michx.), and *V. xchampinii* were identified to have resistance to *M. javanica*. Previous reports indicate that these species are also a source of resistance to *M. incognita* (Cousins et al. 2007, Cousins et al. 2003, Lider 1954, Snyder 1939, Walker et al. 1994). However, the observations that some cultivars derived from these species are resistant to one species of *Meloidogyne* and susceptible to another, indicates that this trait is controlled by different genes or alleles, or alternatively, there have been errors in the identification of these rootstocks. *M. rotundifolia* is the only grapevine species that has resistance to *M. arenaria* pathotypes ‘Harmony’ and ‘Freedom’, *M. incognita*, and *M. javanica* (Bloodworth et al. 1980, Cain et al. 1984, Firoozabady & Olmo 1982, Lider 1954).

Table 6.2.1 Grapevine rootstocks examined for *Meloidogyne javanica* resistance. Grapevines were examined in a glasshouse assay for ability of root-knot nematode reproduction. Resistant type completely prevented reproduction, while tolerant types allowed for only 1or less mean egg masses (per gram root dry weight) to form on 3 replicates, while susceptible types had greater than 1 mean egg masses (per gram root dry weight) on 3 replicates.

Resistant	Susceptible	Tolerant
101-14 Mgt	1103 Paulsen	1616 Couderc
107-11 Millardet	1202 Couderc	Cosmo 10
110 Ruggeri	160-19 Couderc	Merbein 6262
125 AA	31 Richter	Merbein 5512
140 Ru	5BB Kober	Teleki 5A
1613 Couderc	775 Paulsen	*V. acerifolia*
171-13 L	IAC 313	*V. riparia* 'Gloire'
188-04 Castel	Ill 554-5 seedling	
3306 Couderc	J 17-19	
34 EM	J 17-69	
41 B	K 48-15	
420 A Mgt	K 48-69	
57 Richter	Merbein 5489	
62-66 Couderc	Michurinets	
99 Richter	SO4	
AxR#1	SORI 92-14	
AxR#9	*V. rupestris* ‘St. George’	
Cosmo 2	Teleki 5C	
Dogridge	Teleki 8B	
Fercal	Teleki A	
Freedom	Teleki B male	
Harmony	*V. riparia* 'Grande glabre'	
J 17-48	*V. rupestris* 'Metallica'	
K 48-29b		
K 48-35		
K 48-43		
K 48-45		
K 5-02		
K 5-04		
K 5-07		
K 51-11		
K 51-32		
K 51-40		
K 5-20		
LN 33		
DRX 55		
Ramsey		
Schwarzmann		
Teleki C		

6.2.2 *Genetic mapping population*

The seed parent *V. cinerea* '71' of a test cross with *V. vinifera* 'Riesling' was found to be completely resistant to *M. javanica* McLaren Vale strain. The pollen parent Riesling was found to be highly susceptible to this strain of root-knot nematode. A total of 80 progeny were examined in the glasshouse, with total resistance (no egg masses on three reps) segregating to susceptible types 42:38. A chi square goodness to fit test of the hypothesis that resistance segregates as a single dominant gene in a 1:1 manner resulted in a x^2=0.20 with 1 degree of freedom. Therefore the null hypothesis was not rejected, and it is proposed that resistance is controlled by a single dominant gene. Further development of this test population may be useful to develop molecular markers associated with resistance to root-knot nematodes for marker assisted breeding of grapevine rootstocks.

6.2.3 *Potential new rootstocks from CSIRO breeding program*

Twenty-three of the twenty-six potential new rootstocks, and when possible parent vines, were screened in the glasshouse for resistance to the McLaren Vale strain of *M. javanica*. Three genotypes were not examined, as they had poor establishment in field trials and were therefore dropped from consideration for commercial development. Fourteen of the genotypes exhibited resistance, while the remaining nine were determined to be susceptible (Table 6.2.2). There were no resistant genotypes found from the cross of susceptible parents, and three resistant genotypes were derived from both parents being resistant. Therefore, it is possible that some individual may possess more than a single gene for resistance or multiple alleles of a resistance gene.

Table 6.2.2 Summary of perfomance of potential new rootstocks from CSIRO breeding program. A group of 26 genotypes were assessed for potential application as commercial rootstocks. Table presents a summary of the root-knot nematode (RKN) resistance, results of phylloxera tolerance screening, rootstrike of dormant lignified canes, the estimated breeding value (EBV), the potassium content of ungrafted mothervines at anthesis, and comments on commercial potential.

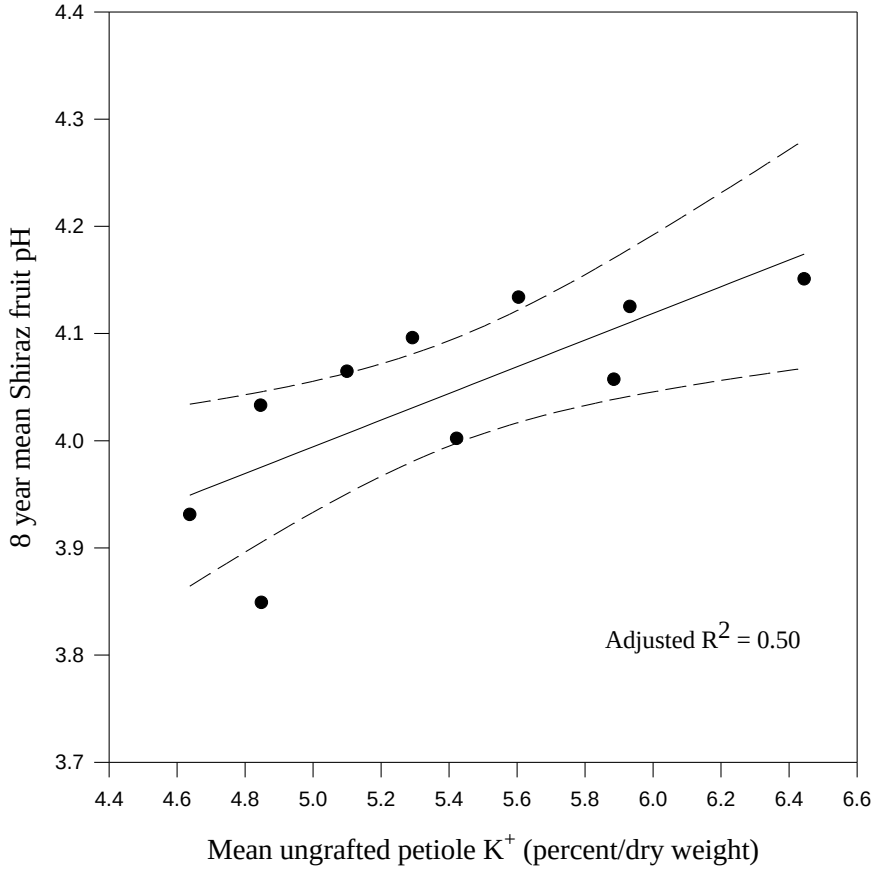
Root-stock	RKN egg masses/dried root weight (g)	Phylloxera tolerance	Rate of root strike	EBV	$\%K^+$/ petiole dry weight	Comments
C03	0.00	High	-	-	3.11	High Boron exclusion
C04	2.42	Not tested	95%	-	2.10	Root-knot nematode susceptible
C12	4.53	Not tested	73%	1.07	2.85	Root-knot nematode susceptible
C13	2.44	Not tested	94%	2.53	1.63	Root knot nematode susceptible
C16	0.60	Not tested	77%	1.41	2.41	Root-knot nematode susceptible
C20	0.00	High	90%	-0.32	1.20	Moderate vigour
C23	0.00	Not tested	52%	0.01	3.53	Poor drought tolerance, poor rootstrike
C26	7.81	High	93%	2.52	3.57	Root-knot nematode susceptible
C28	1.28	Not tested	93%	-0.07	2.67	Root-knot nematode susceptible
C29	0.00	High	92%	2.37	2.65	Low -moderate vigour
C30	Not tested	Not tested	-	-	-	Poor planting establishment
C31	0.00	Not tested	36%	-0.63	3.75	Poor root strike, poor planting establishment
C34	Not tested	Not tested	96%	0.32	-	Poor drought tolerance
C42	0.00	Not tested	92%	2.37	2.55	Poor drought tolerance
C44	7.90	High	84%	1.88	2.06	Root-knot nematode susceptible
C49	8.29	Not tested	92%	-	2.13	Root-knot nematode susceptible
C54	0.95	Moderate - High	33%	-0.03	3.17	Poor rooting, root-knot nematode susceptible
C55	0.00	Not tested	39%	-0.44	2.02	Poor root strike
C61	Not tested	High	96%	-	2.79	Poor planting establishment
C83	0.00	High	92%	-0.19	2.97	Poor planting establishment
C93	0.00	High	96%	-0.05	2.77	Poor planting establishment
C112	0.00	High	92%	-	3.58	Moderate- high vigour
C113	0.00	High	100%	-	3.58	Moderate- high vigour
C114	0.00	Moderate - High	100%	-	3.52	Moderate- high vigour
C341	0.00	High	80%	-	3.42	Moderate root strike
C841	0.00	High	91%	2.37	3.67	Poor graft compatibility

6.3 POTASSIUM UPTAKE SCREENING

As part of the nutrient uptake screening, vines were assessed for potassium accumulation in petioles. The percent of potassium per dry weight of petioles was measured in field grown vines and glasshouse raised vines. Previous work by CSIRO has shown there to be a strong correlation with the level of potassium in petioles at anthesis and the fruit pH at harvest (Ruhl 1990). A method of glasshouse screening for potassium uptake was proposed by Ruhl (1989) for early assessment of rootstock for impact on fruit pH. A pilot study was conducted to determine the validity of this method. A long term field trial at the CSIRO Sunraysia, Victoria has been monitored for fruit pH at harvest. This trial consisted of 60 rootstocks grafted with Shiraz scions arranged as 5 replicated blocks. Ten of these rootstocks were included in the glasshouse assay for potassium screening. The petioles of the ungrafted glasshouse vines were harvested after six weeks treatment with hydroponic solution containing supplemental potassium. Figure 6.3.1 shows that there is also a strong correlation (adjusted R^2= 0.50) between the petiole potassium content of ungrafted vines receiving the high level of potassium (15mM) and the 8 year mean fruit pH of scions grafted to these rootstocks at the CSIRO Sunraysia field trial. A similar relationship was observed between the petioles collected from ungrafted field-grown rootstocks at anthesis and the 8 year mean fruit pH at harvest (data not shown).

The uptake of potassium by grapevines is of particular importance to the Australian wine industry due to the relatively high levels of this ion that can be found in the soil of many viticultural areas. The identification of rootstocks which have lower potassium uptake is an important screening method for breeding rootstocks directed for commercial use in Australia.

Figure 6.3.1 Correlation of ungrafted vine petiole potassium and grafted vine fruit pH. The potassium content of petioles of ungrafted vines grown in flood tanks supplemented with 15mM K^+ for six weeks was compared with scion fruit pH from a long term field trial with these rootstock grafted to Shiraz.

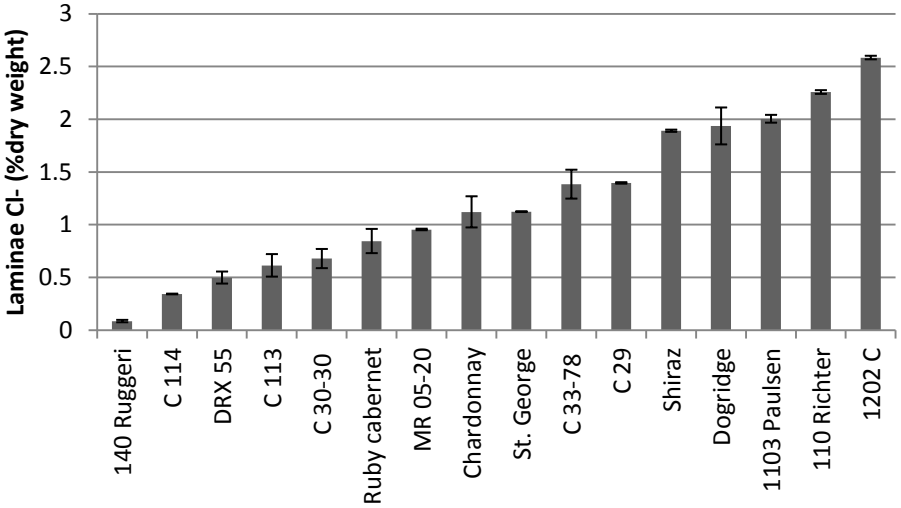


6.4 SALT EXCLUSION SCREENING

The evaluation of grapevine capacity to exclude chloride was evaluated in a pilot study conducted in the CSIRO glasshouse on the Waite Campus, South Australia. Control genotypes were included with known salt exclusion ability. The rootstock 140 Ruggeri has the greatest ability to exclude salt accumulation in the lamina (0.09% Cl^- per dry weight), while 1202 Couderc had the highest level of accumulation (2.58% Cl^- per dry weight)(Figure 6.4.1). Field grown vines with supplemental salt applications, the scions of 140 Ruggeri consistently accumulated relatively less Cl^- in the fruit and leaves compared to the scions of 1202 Couderc (Tregeagle et al. 2006). While 1103 Paulsen is considered a good Cl^- excluder in short term studies (Downton 1977, Walker et al. 2002), long term exclusion is in doubt

(Tregeagle et al. 2006). Our results of application of high salt in the medium (50mM Cl^-) are in agreement with the long term salt exclusion ability of 140 Ruggeri, 1103 Paulsen, and 1202 Couderc. Further replication of this method is required to confirm these results and the potential to use this method as a screening method in rootstock breeding.

Figure 6.4.1 Chloride exclusion of grapevines in glasshouse assay. Grapevines were grown in a flood tank for six weeks, soaked twice a day for one hour in a Hoagland solution with 50mM Cl^-. The ability to exclude chloride was assessed by collection of laminae from the the basal half of the vine.



6.5 EVALUATION OF ROOTSTOCK NURSERY

6.5.1 *Vigour*

The vigour potential of the 11 families in the rootstock nursery was examined at the conclusion of the 2008-2009 season. The family with the highest mean vigour was MC 68 (67.76g) and the lowest was MC 1 (34.12g) (Figure 6.5.1). The one way ANOVA of mean family pruning weight was found to be statistically different (P=<0.001). The pairwise multiple comparison (Tukey Test) showed a statistical difference (P<0.050) between MC 68 and MC 1 (P=0.023), as well as between MC 86 and MC 1 (P=<0.001). There was only a mild correlation (adjusted R^2=0.11) between the nursery pruning weight for the 11 family means and the ungrafted vine pruning weight from the mothervines (Figure 6.5.2) collected in the non-drought imposed year (2008). However, there was a much better relationship (adjusted R^2=0.61) between the scion pruning weight of nursery vine genotypes and the long term grafted trial at CSIRO Sunraysia site (Figure 6.5.3). There may be several factors to explain this, and as detailed in section 6.6, rootstocks may have a different rate of growth

compared to the scion. Another possible explanation is that the nursery was a replicated trial arranged in a randomised complete block manner, whereas the corresponding mothervines are single vine replicates assorted in small linear blocks according to family. Based on these results however, the vigour potential of a rootstock is believed to be better assessed in the nursery than as an ungrafted mothervine. In addition, if the pruning weight of MC 1 and MC 67 are considered outliers, then the linear regression of grafted nursery pruning weights and ungrafted mothervine pruning weights improves dramatically (adjusted R^2=0.40).

Figure 6.5.1 Mean scion vigour of rootstock families in a nursery. The mean pruning weight of scions of 11 rootstock families was assessed on 3 year old nursery vines. The differences in the mean values were found to be significantly different (P=<0.001). Bars with different letters were significantly different in a pairwise mutliple comparision (Tukey Test, P<0.050)

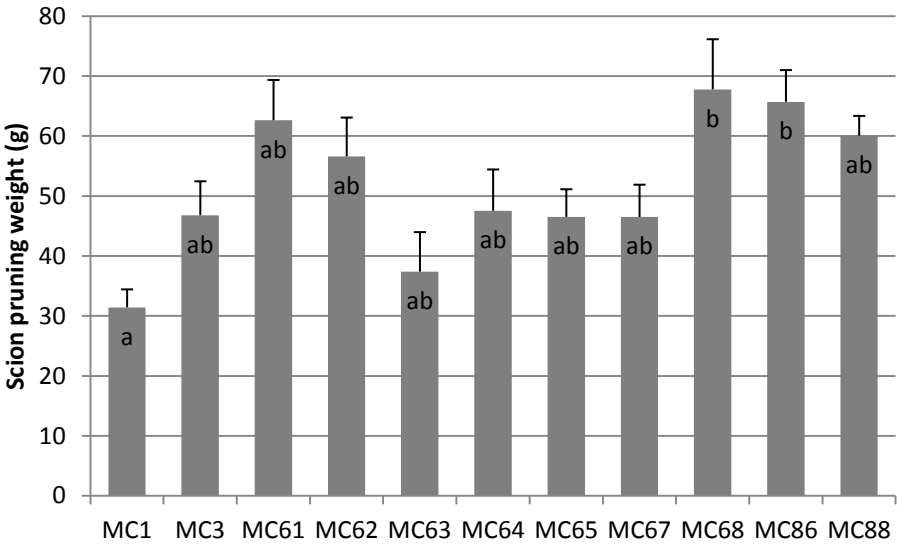


Figure 6.5.2 The correlation between pruning wood weights of ungrafted rootstocks and grafted nursery vines. The annual pruning weight of families of dormant ungrafted mothervines shows only a weak correlation with the scion pruning weight of grafted 3 year old vines in a nursery.

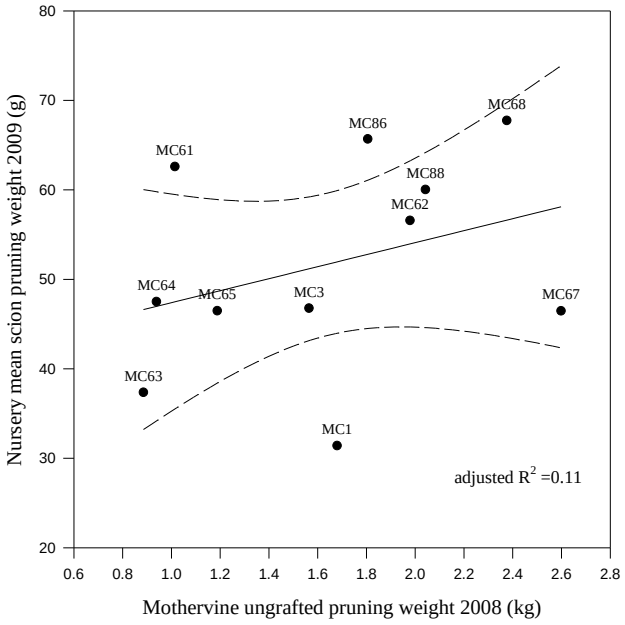
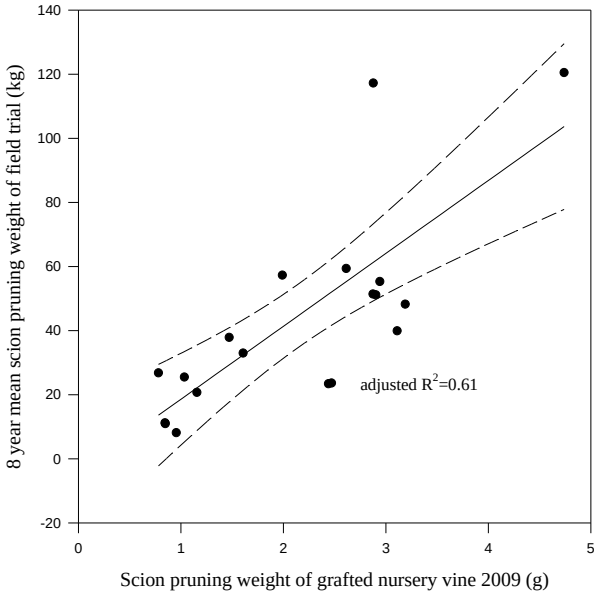


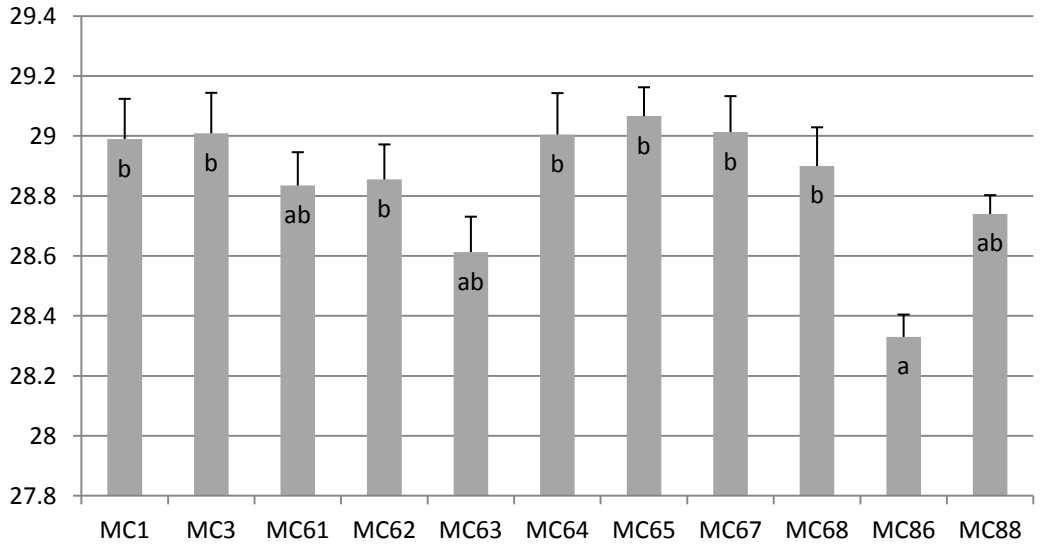
Figure 6.5.3 Correlation between pruning wood weights of grafted nursery vines and grafted field trial vines. The correlation between the scion pruing weight of 3 year old grafted nursery vines shows a strong correlation with the 8 year mean pruning weight of grafted vines in a field trial. Both the nursery and the field trial were replicated blocks grafted with Shiraz.



6.5.2 *Drought tolerance*

The transpiration efficiency of a plant is proposed to be negatively correlated with the ratio of C_{13} to C_{12} incorporated into a plant as a result of photosynthesis (Farquhar et al. 1982, Gibberd et al. 2001). Family MC 86 had the lowest mean δC value (28.33), which was significantly different (P = <0.001) from 7 other families (Figure 6.5.4). Family MC 65 has the highest mean ratio (29.07), which was only significantly different (P = <0.001) from 4 other families (Figure 6.5.5) in a pairwise comparison. It should be noted that range of δC from these vines indicate that plants were near complete hydration, and these values may not have correlation with mature vines in a field setting.

Figure 6.5.4 δC of nursery vines. The carbon isotope discrimination ratio of rootstock families of grafted nursery vines was measured from scion laminae at the conclusion of the 2006-2007 season. Vines were well watered throughout the season. . The differences in the mean values were found to be significantly different (P=<0.001). Bars with different letters were significantly different in a pairwise mutliple comparision (Fisher LSD, alpha=0.05)



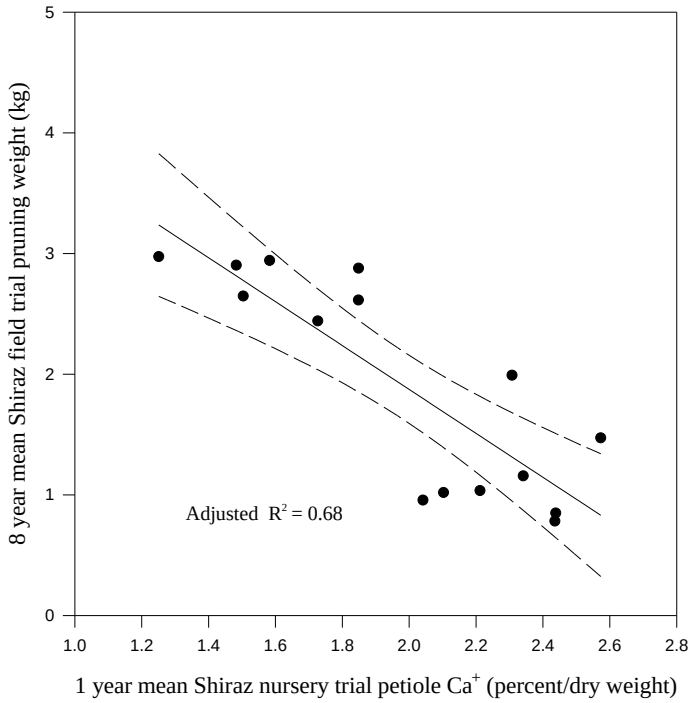
The effect of reduced irrigation on scion growth was measured in the nursery by collection of dormant cane pruning weight at the conclusion of the 2008-2009 season after the implementation of two years treatment of high irrigation on 3 blocks and low irrigation on 3 blocks. The mean soil moisture of the high irrigation treatment was 37% in the 2008-2009 season, while the blocks receiving low irrigation treatment had mean soil moisture content of 16% for the season. A comparison of mean pruning weight for all 310 genotypes between the two irrigation treatments was found to be statistically significant (P=<0.001) when analysed by Mann-Whitney Rank Sum test. The mean pruning weight of the well watered

blocks was 48.3g compared to 29.5 for the blocks with restricted irrigation. Further comparison of the individual family performance is presented in section 6.9.3.

6.5.3 *Nutrient uptake*

Mineral analysis of scion petioles was conducted for grafted nursery vines that were also present in a long term field trial in Sunraysia. As anticipated, petiole potassium concentrations at anthesis (2008-2009 season) of the nursery vines were found to be highly correlated with 8 year mean fruit pH of the mature grafted vines at Sunraysia site (adjusted R^2= 0.40). Unexpectedly, there was found to be a strong inverse relationship (adjusted R^2= 0.68) between nursery vines scion petiole calcium content at anthesis and mature vine 8 year scion pruning weight (Figure 6.5.6).

Figure 6.5.5 Correlation between petiole calcium concentration and vine vigour. The petiole calcium concentration of grafted nursery vines was assessed at anthesis from scions grafted onto rootstocks that were also present in a long term field trial. The 8 year mean scion pruning weight of the field trial vines has a strong negative correlation with the scion petiole Ca^+ concentration of grafted vines in the nursery. All rootstocks were grafted with Shiraz scions.



6.6 SCION-ROOTSTOCK COMPATIBILITY

The relationship of vigour to fruit quality for wine production has been reported previously (Clingeleffer 1996). To briefly summarize, a field trial was established in 1989 to select for rootstocks which have low transport of potassium into the scion. The trial consisted of 5 standard commercial rootstocks and 55 hybrid rootstock vines, all with Shiraz as the scion. As a result of this trial, 3 genotypes were selected for commercial release (Merbein 5489Ф, Merbein 5512Ф , and Merbein 6262Ф). The wine produced from these new rootstocks consistently has higher anthocyanin levels (absorbance, AU 520 values) and phenolic levels (absorbance, AU 280) (Figure 6.6.1). A large component of this increase in colour and phenolics appears to be related to the low to moderate vigour these vines impart to the scions, as well as the development of smaller berries and lower winter pruning weight. The scion vigour can be measured by the wood density of the trunk. Generally, more vigorous vines have a lower wood density due to the radial tracheid diameter being larger than less vigorous vines. The greater the penetrance of the rod is associated with lower density. The penetrance of the pilodyn above the graft union was found to have a strong correlation with yield, pruning weight, berry weight, berry colour (AU 520), and berry phenolic level (AU 280) (Figure 6.6.2). Interestingly, the penetrance of the trunk below the graft union had no significant relationship with vigour or fruit quality. This implies that the rootstock and scion are growing at different rates. Further investigation of how the graft union controls the exchange of water, nutrients and photosynthates needs to be explored, as this may be a component of what determines water use efficiency and fruit quality.

Figure 6.6.1 Correlation between fruit anthocyanin and vine vigour traits. The 2 year mean total anthocyanin content of Shiraz wine has a strong negative correlation with a). 6 year vine mean berry weight, b). 6 year mean yield per vine, and c). 6 year mean dormant vine cane pruning weight.

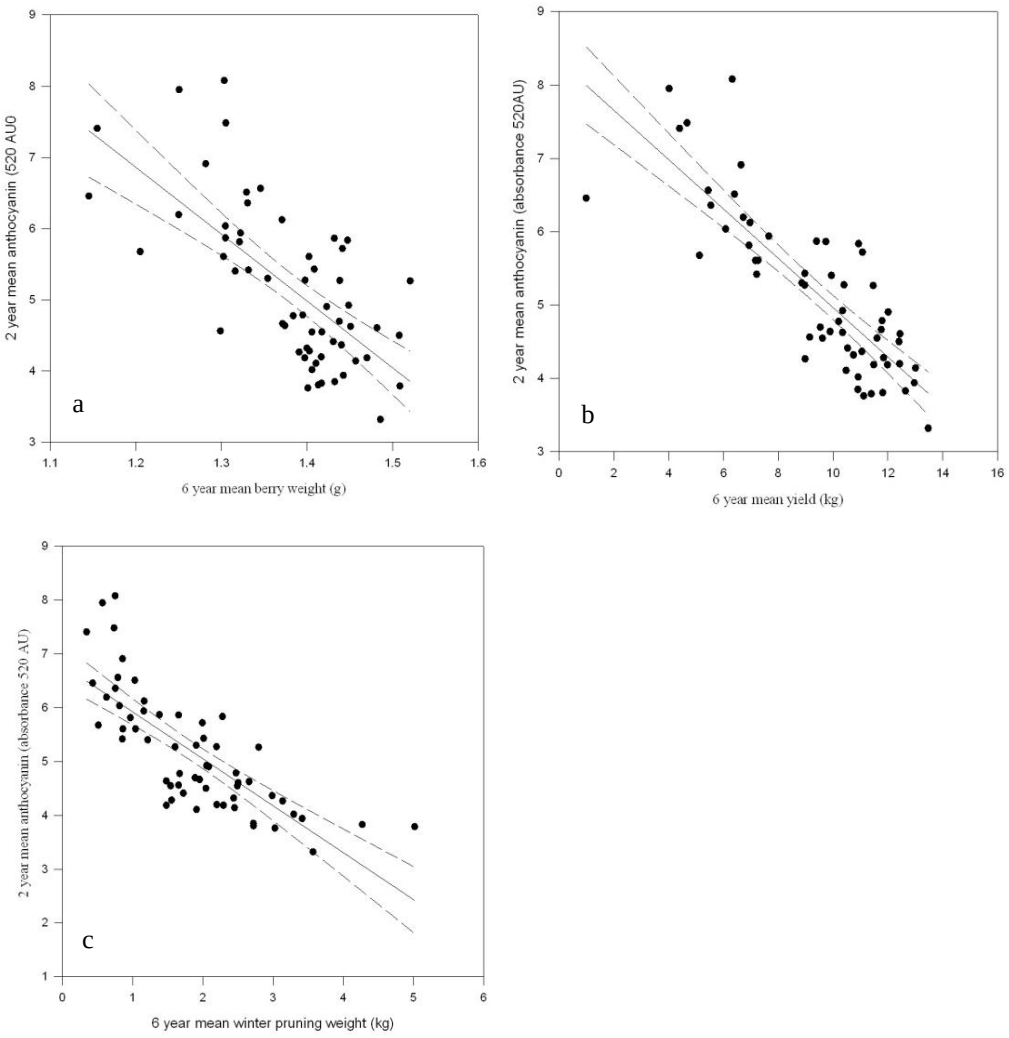
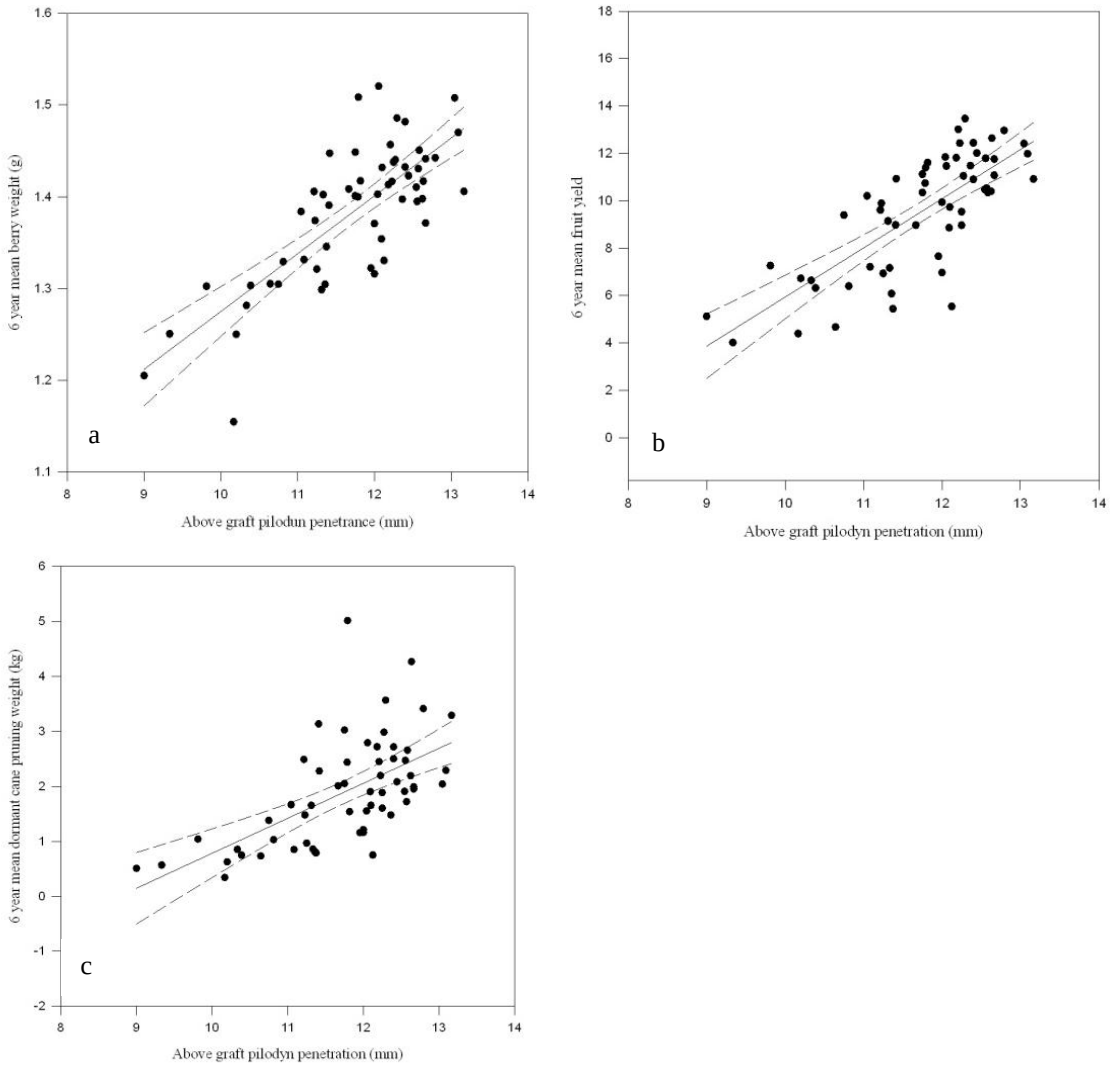


Figure 6.6.2 Correlation of scion wood density and vine vigour traits. The mean total pilodyn penetrance content of Shiraz scions has a strong correlation with a). 6 year vine mean berry weight, b). 6 year mean yield per vine, and c). 6 year mean dormant vine cane pruning weight. Pilodyn penetrance is the inverse of wood density.



6.7 CHARACTERIZATION OF ROOT ARCHITECTURE AND DROUGHT RESPONSE OF GRAPEVINE GERMPLASM

The root architecture of 14 grapevine families was scored upon removal of vines from a germplasm vineyard in Merbein, Victoria in 2011. The mean value for the root angle (on a 1-3 scale, see Table 6.7.1 for more detail) was 2.12 (SEM±0.05), with the most vertical roots in family MC 2 (1.69) and the most horizontal roots in family MC 62 (2.33). Family MC 68 had the most branching roots (2.17) and MC 67 had the least branching (2.61), with the mean

for all families 2.40 (SEM±0.03). The thickest mean root value was 2.39 (MC 67) and the thinnest mean root value was 1.79 (MC 61). The mean value for root thickness for all families was 2.06 (SEM±.05). Family 62 had the most mean number of roots per vine (52.64) and MC2 had the least mean number of roots per vine (14.08). The mean number of roots for all families was 35.65 (SEM±2.72). The grading scale for this trait was a small range as it was primarily being used to individual with extreme phenotypes such as completely plunging roots or highly branched roots. No association with an individual overall root architecture and drought response could be identified. The application of this scoring system is discussed in relation to drought response in greater detail in section 7.1.1.

Analysis of the ratio of mean root diameter of the 6 largest roots and trunk diameter for 3 families (MC 34, MC 86, and MC 88) revealed two distinct growth patterns. One group showed a ratio of 4:1 trunk diameter to mean root diameter, and the other group had 2:1 ratio (Figure 6.7.1). The group with the ratio of 4:1 trunk diameter to mean root diameter, had a mean trunk diameter of 38.79 mm (SEM=±1.14) and mean thickness of the 6 largest roots of 8.66mm (SEM=±0.22). The mean root number for this group was 39.38 (SEM=±2.16). This group had an overall root architecture that was between intermediate and horizontal, tended to have an equal distribution of final root angle with the 6 largest roots mean angle of 46.35° relative to the soil surface. The largest single root had a mean value of 24.5mm (SEM=±0.82) and a final angle of 42.5°. The mean decrease in pruning weight after 2 years of no irrigation was 47% (SEM=±5.8%). The mean pruning weight before implementation of drought was 1.73kg/vine (SEM=±0.14kg).

The group with the ratio of 2:1 trunk diameter to mean root diameter, with a mean trunk diameter of 30.76 mm (SEM=±1.05) and mean thickness of the 6 largest roots of 13.36mm (SEM=±0.44). The mean root number for this group was 30.21 (SEM=±1.81). This group had an overall root architecture that was between intermediate and horizontal, tended to have an equal distribution of final root angle with the 6 largest roots mean angle of 53.78° relative to the soil surface. The largest single root had a mean value of 21.5mm (SEM=±0.72) and a final angle of 52.4°. The mean decrease in pruning weight after 2 years of no irrigation was 57% (SEM=±7.6%). The mean pruning weight before implementation of drought was 1.39kg/vine (SEM=±0.12kg).

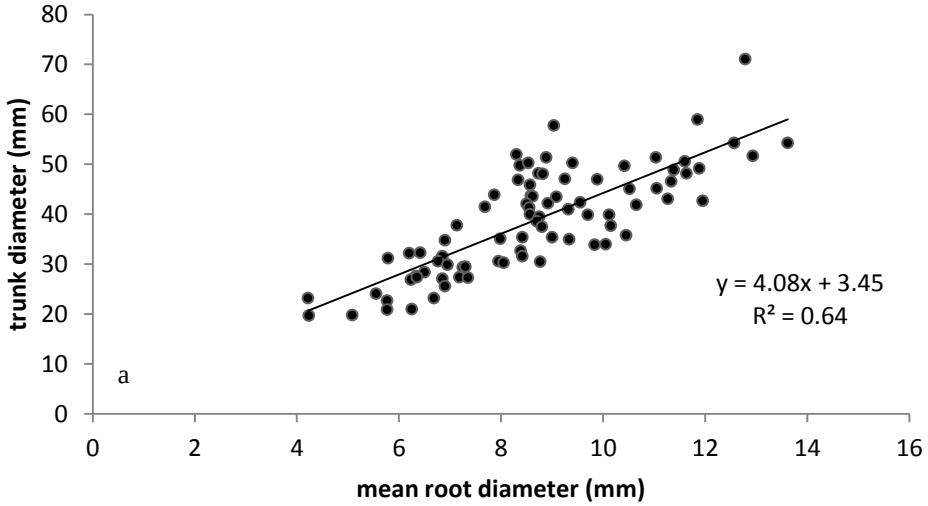
These results imply that more vigorous vines have a higher ratio of trunk to root diameter, with more roots of less girth. The 6 largest roots of the more vigorous vines have a more shallow root angle (between 0°-45° relative to the soil line) than the lower vigour vines, as well as the overall root distribution.

The range in mean root diameter was from 4.05mm (SEM=±0.47mm) to 28.82mm (SEM=±2.12mm). Family MC 34 had the lowest mean value for root diameter (9.39mm) while MC 86 and MC 88 had comparable mean root diameters of 12.42mm and 13.12mm respectively (Figure 6.7.2).

Table 6.7.1 Root characteristics of 14 grapevine families. The root traits of 14 grapevine families were examined upon removal of mature vines. The mean root angle was an estimate of final root orientation, vines with >90% of roots vertical were rated "1", vines with >90% or roots horizontal were rated "3", with all other rated "2" and considered intermediate. Mean root type was scored "1" if >90% of roots were fine and multi-branched, "3" if >90% of roots were non-branching, and "2" if roots were a mixture. The mean root thickness was rated "1" if >90% of roots were thin relative to the trunk, "3" if >90% of roots were thick relative to the trunk and "2" if roots were a mixture. Total number of roots was counted as primary roots which emerged from the root crown.

Family	Mean root angle	Mean root type	Mean root thickness	Mean number of roots
MC1	1.93	2.35	1.96	27.88
MC2	1.69	2.46	2.31	14.08
MC3	2.30	2.48	2.13	25.41
MC34	2.20	2.34	1.83	28.72
MC42	2.08	2.31	2.08	36.54
MC61	1.79	2.50	1.79	47.50
MC62	2.33	2.29	2.13	52.64
MC63	2.13	2.37	1.81	47.67
MC64	2.09	2.58	2.23	34.37
MC65	2.21	2.50	1.96	30.04
MC67	2.11	2.61	2.39	42.89
MC68	2.26	2.17	2.00	37.36
MC86	2.25	2.27	1.98	35.82
MC88	2.26	2.36	2.17	38.26

Figure 6.7.1 Correlation between trunk diameter and mean root diameter. Two distinct groups were found for the relationship between the mean root diameter of the 6 largest roots and the trunk diameter. a). Trunk diameter was 4.08 times greater than mean root diameter, b). Trunk diameter was 2.02 times greater than mean root diameter.



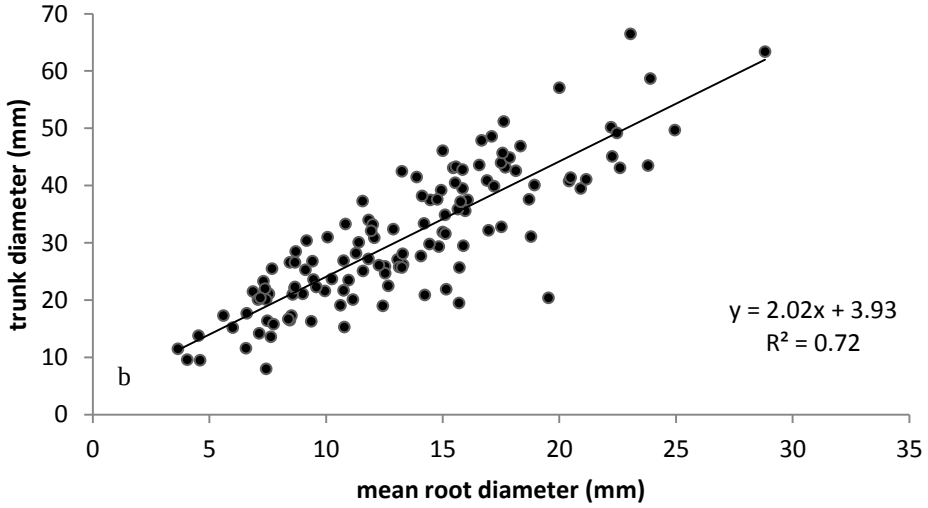
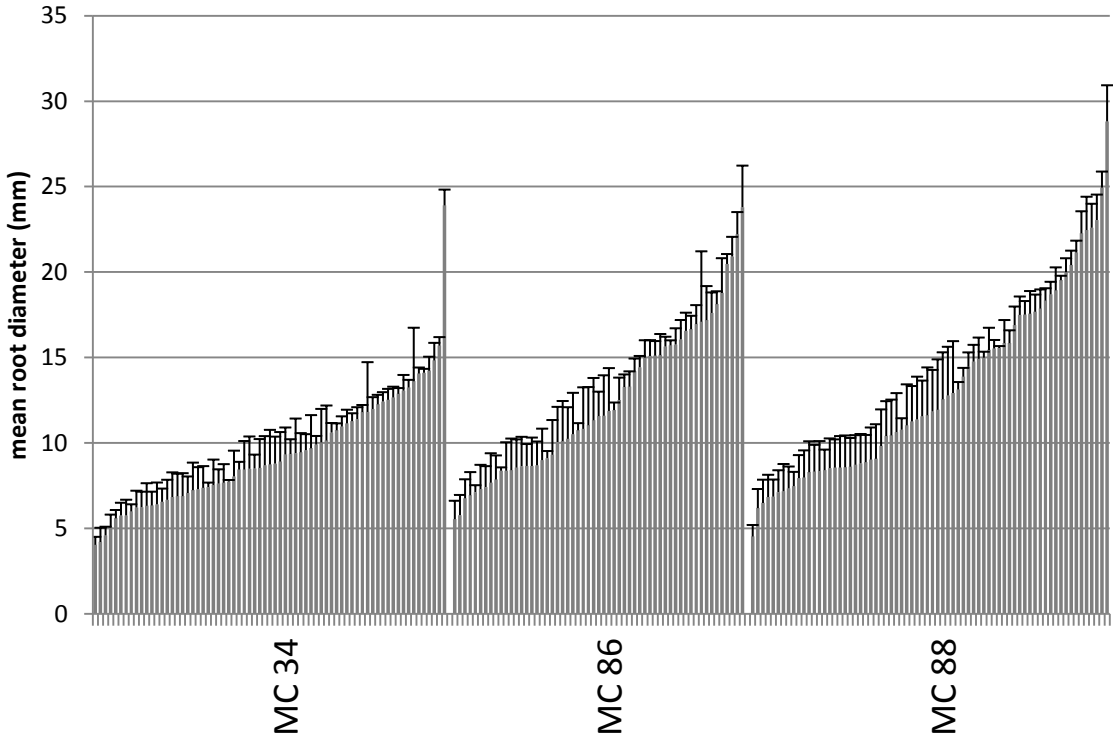


Figure 6.7.2 The mean root diameter of 3 families of grapevines. The mean root diameter of the six largest roots was measured for 3 families (MC 34, MC 86, and MC 88) upon removal of vines in 2011.



6.8 INHERITANCE OF ROOTING ABILITY

The population *V. cinerea* var. *helleri* '100' x *V. cinerea* var. *helleri* '12' is constituted from 4 unique wild accessions of *V. cinerea* var. *helleri* (Table 5.8.1). These progeny had the lowest mean rootstrike rate (17% SD±13, *n*=23). The two populations with the highest mean percentage of rooting (88% SD±13.1, *n*=35; 87% SD±16.4, *n*=15) were both derived from the seed parent Kober 5BB (*V. cinerea* var. *helleri* x *V. riparia*) with the pollen parents K 49-56 and J 17-69 respectively, which are both derived from the cross *V. xchampinii* x *V. rupestris*. The mean rootstrike rate of all the progeny populations was normally distributed (Wald statistic = 0.955, P = 0.510). Parents with the highest rate of rootstrike were *V. rupestris* St. George (100%), SO4 (95%), K 51-32 (95%), and K 41-40 (90%). The parents with the lowest root development were *V. cinerea* var. *helleri* '12' (20%), *V. cinerea* var. *helleri* '111' (45%), Merbein 6262 (50%), and *V. cinerea* '71' (61%). The highest positive effect of parents on progeny rooting in our crosses was for Schwarzmann (+6.29), and *V.*

acerifolia (+6.29). The parents with the greatest negative effect on progeny rootstrike were Ramsey (-33.0), *V. cinerea* var. *helleri* '100' (-32.59) and *V. cinerea* '71' (-28.62).

Estimates of narrow sense heritability

The heritability of rooting of dormant grapevine canes was calculated to be h^2= 0.269 with a standard error of 0.068. The estimated additive genetic variance $\hat{\sigma}_A^2 = 1.93$ on the underlying scale, with a highly significant z value of 12.72. The block variance was estimated to be $\hat{\sigma}_B^2 = 1.95$ with a non significant z value of 1.10. The Wald test for significance of fixed effects indicated that year was highly significant (*F*=211, *P*=<0.001). The ASReml-R model has taken into account that not all genotypes were propagated in all years, therefore the differences in years is not due to this factor. The estimated regression for year indicated that vines propagated in 1999 had the highest probability of rooting, with those in years 2000, 2005, and 2010 having a lower log odds of rooting of 1.27, 1.22, and 1.23 respectively. The estimated probability of a dormant cane forming roots was similarly higher in 1999 (0.82) and lower in subsequent years (2000, *P*=0.57; 2005, *P*=0.58; 2010, *P*=0.57).

All genotypes in the pedigree file were assigned an Estimated Breeding Value (EBV) which range from -3.60 (progeny of Resseguier #1 x 7651) to 3.49 (progeny of Kober 5BB x K 49-56). The EBV values were normally distributed. The parent with the lowest EBV was *V. cinerea* var. *helleri* '100' (Ressequier #2 x 7651) while Kober 5BB (*V. cinerea* var. helleri x *V. riparia*) had the highest EBV (2.86).

Estimation of pedigree and parent effect on adventitious rooting from dormant canes

The parent effects on rootstrike ranged from -73.18% (*V. cinerea* var. *helleri* '100') to 11.72% (SO4). The F test probability <0.001. The population derived from the cross of *V. cinerea* var. *helleri* '100' x *V. cinerea* var. *helleri* '12' resulted in the lowest pedigree effect (-60.17%), while the highest pedigree effect on progeny rooting (30.10%) was from the mating of Kober 5BB and K 49-56.

The control of root development of lignified grapevine cane cuttings is believed to be polygenic (El-Hamady & Olmo 1966, Smith 2010) and variable both among and between species (Mortensen 1938, Viala 1901, Williams & Antcliff 1984). The handling of material

can have a major influence on the percentage of canes which form roots (Kamiloglu 1997, Keeley 2004). While it is a widely held belief that North American species *V. cinerea* var. *helleri* and *V. cinerea* uniformly exhibit poor rootstrike, the results of our study, and others demonstrates this is a misconception. Whilst these species generally do not root well, progeny populations of randomly mated parents can produce genotypes which readily form roots. In progeny that resulted from the cross of wild accessions of *V. cinerea* var. *helleri*, Viala and Ravaz (1908) observed rootstrike between 60-85%. This value was reported by them to be as high as their accession of *V. riparia* and *V. rupestris,* which are species that are considered to root readily. The populations with the highest mean percentage of rooting were both derived from the seed parent Kober 5BB (*V. cinerea* var. *helleri* x *V. riparia*) with the pollen parents being the commercial rootstocks K 49-56 and J 17-69, which are derived from *V. xchampinii* x *V. rupestris*. Despite the presence of 25% *V. xchampinii* in their pedigree, the distribution of rooting in these populations was centred around 100% rootstrike.

The moderate heritability of the trait of adventitious rooting of dormant grape canes, combined with the strong response to selection, indicates that rapid gains can be made in few generations. Narrow sense heritability values can be viewed as an index of phenotype correlation with genotype (Visscher et al. 2008). Therefore these results indicate that reliable genetic markers can be generated for the trait of rooting. Molecular markers for rootability of grape will be an important tool for breeding new rootstocks and for development of direct producing grape cultivars.

6.9 FIELD TRIALS OF NEW ROOTSTOCK SELECTIONS

6.9.1 *New CSIRO low – medium vigour rootstocks*

Three low-medium vigour rootstocks, developed from the CSIRO grapevine rootstock breeding and evaluation program (Clingeleffer et al. 2011) have been released for larger scale industry evaluation. The purpose of wider industry evaluation was to assess their suitability in regional environments with varying abiotic stresses (i.e. temperature, water and salt) and with varying levels of soil-borne pest pressures (e.g. strains of nematodes and phylloxera). An application seeking PBR protection for the rootstocks was made in March 2005 with PBR granted in June 2009. The new rootstocks, Merbein 5489Ⓟ, Merbein 5512Ⓟ and Merbein 6262Ⓟ are described in Dry (2007).

The rootstocks were selected based on their performance in a long term trial planted in a replant situation in a nematode infested, sandy loam soil at CSIRO Sunraysia site, near Mildura, Victoria, in 1989. The trial involved 55 hybrid rootstocks and five standard rootstocks, all grafted with Shiraz. The hybrids include selections identified for their low potassium (K^+) uptake (Clingeleffer 1996). Low K^+ uptake has been a focus of Australian rootstock breeding because of its association with high pH in berries and subsequent impacts on wine quality (Ruhl 1989). A key criterion in the selection of the three rootstocks was conferred vigour assessed as pruning weight. Other criteria included berry composition, particularly K^+ concentration and pH, wine quality attributes (e.g. spectral and sensory properties) and tolerance of the soil borne pests, root-knot nematodes and phylloxera. A summary of the key performance characteristics of these rootstocks compared to 1103 Paulsen and Ramsey, measured over 3 years (2001-2003) in the previous project (CSP99/02) is included for completeness (Table 6.9.1)

Table 6.9.1 Vine vigour, must composition, yield per vine and wine characteristics of Shiraz, grafted on the three low-medium vigour rootstocks. The three new CSIRO developed low-medium vigour rootstocks (Merbein 5489Ⓟ, Merbein 5512Ⓟ and Merbein 6262Ⓟ) with Shiraz as scion, compared to Shiraz grafted on Ramsey and 1103 Paulsen, over 3 seasons (mean values, 2001-2003) at Sunraysia site, Victoria. Values with different subscripts are significantly different (P=0.05).

Shiraz/ Rootstock	Vigour	Harvest			Wine				
	Prun. Wt. (kg)	**Juice °Brix**	**Juice pH**	**Yield (kg)**	**Acid Added (g/L)**	**Wine pH**	**Colour Density (au)**	**Colour Hue**	**Total Phenolics (au)**
1103 Paulsen	4.2_c	25.6_d	4.18_d	11.8_b	4.72_d	3.57_b	6.26_{ab}	0.61_b	53.8_b
Ramsey	4.3_c	25.2_c	4.25_e	16.7_c	5.95_e	3.58_b	5.84_a	0.59_b	49.5_a
Merbein 5489	2.0_b	24.3_b	3.83_a	13.3_b	1.90_a	3.49_a	7.56_c	0.54_a	60.9_c
Merbein 5512	1.5_{ab}	24.5_b	3.91_b	8.8_a	2.72_b	3.48_a	6.55_b	0.55_a	56.8_b
Merbein 6262	1.2_a	23.7_a	3.94_b	9.9_a	2.60_{ab}	3.51_a	8.17_d	0.53_a	62.4_c

Compared to Ramsey and 1103 Paulsen, the new rootstocks grafted with Shiraz had 2-3 fold lower pruning weights, consistent with their low-medium vigour classification (Table 1). Despite having lower vigour than 1103 Paulsen, Shiraz yield on Merbein 5489Ⓟ was as good as 1103 Paulsen, whereas yields of Merbein 5512Ⓟ and Merbein 6262Ⓟ were lower by 16 % and 25%, respectively. Compared to the standard rootstocks, the new rootstocks produced lower pH in the must and enhanced wine quality attributes (i.e. brighter wines with lower

hue, higher levels of colour and phenolics), despite being harvested at slightly lower maturities. Furthermore, significantly less addition of tartaric acid was required for pH adjustment during winemaking.

Further studies in season 2007 showed that improved fruit composition and enhanced wine quality attributes could be achieved with the new rootstocks across a range of soluble solids (22-26 ^{0}Brix), indicating potential for low alcohol wine production (Clingeleffer 2008). In contrast, high levels of sugar were required to achieve good wine colour density with the standard rootstocks, particularly Ramsey. For example, in the Sunraysia trial in season 2007, a colour density of 4.4 a.u. was achieved around 22 ^{o}Brix with Merbein 5512Ⓡ and Merbein 6262Ⓡ rootstocks, compared to 25.5 ^{o}Brix with Ramsey (Table 6.9.2). Of the standard rootstocks, 140 Ruggeri produced the best results.

Table 6.9.2 Wine quality traits of vines grafted to new CSIRO rootstocks. Effect of rootstock on Shiraz wine colour density, total phenolics and sensory score (season 2007). Means have been adjusted based on the significant co-variate, total soluble solids (mean 23.6 ^{o}Brix).

Shiraz	Colour density (a.u).	Total Phenolics (a.u.)	Sensory score (max. 20)
Ramsey	0.86	25.3	11.3
1103 Paulsen	2.11	27.3	12.4
140 Ruggeri	4.23	26.7	13.8
Merbein 5489	4.76	30.6	13.6
Merbein 5512	7.24	33.4	14.5
Merbein 6262	8.42	37.5	14.2
Significance	P<0.001	P<0.01	P<0.001

6.9.2 *Tolerance of soil borne pests*

Merbein 5489Ⓡ and Merbein 5512Ⓡ were phylloxera tolerant in screening trials conducted in Rutherglen by Dr Kevin Powell, DPI Victoria, using up to three strains of phylloxera. Merbein 5489Ⓡ was tested and shown to be tolerant against the G1, G4 and G30 strains. Merbein 5512Ⓡ was tested and shown to be tolerant against the G4 and G30 strains. Merbein 6262Ⓡ showed moderate tolerance against the G4 and G30 strains, which indicates a risk of phylloxera developing on the roots, but at relatively low levels compared to *V. vinifera*.

Shiraz vines grafted to the new rootstocks and to the standards, Ramsey and 1103 Paulsen, established in a re-plant soil infested with nematodes, showed no ill effects over a period of 20 years. The ongoing existence of nematodes at this site has been confirmed in February 2011. In glasshouse screening trials, conducted prior to their release, the new rootstocks were tolerant to two strains of *M. javanica*, the most common nematode in Australia and *M. incognita*. For the new rootstocks, nematode galls and egg masses were either absent or observed at a low frequency in root systems compared to the Sultana control where they were spread throughout the root system. In a recent study conducted as part of this project, the rootstocks have also been screened in the glasshouse against a strain of *M. javanica* sourced from a vineyard where growth problems associated with high nematode numbers had been observed with vines grafted on 1103 Paulsen, a problem also reported by Walker (2009). Merbein 5489Ф and Merbein 5512Ф and the control rootstock, 1103 Paulsen, did not show tolerance to the aggressive strain of *M. javanica* whereas Merbein 6262Ф displayed a high level of tolerance.

6.9.3 *Progress with industry evaluation*

The new rootstocks, grafted with Shiraz, have been embedded in new commercial plantings by companies in a number of regions. This material was developed through rapid propagation and green grafting under glasshouse conditions by a commercial nursery, due to the limited supply of propagation material prior to and during establishment of mothervine plantings. The performance of the new rootstocks in these commercial sites has been monitored to assess their adaptability in the different regions. The first commercial planting, grafted with Shiraz and located near Griffith, New South Wales, produced a significant crop in season 2010, with two further plantings in Pomona and Padthaway bearing in 2011.

The Griffith site involves a replicated trial comparison of Merbein 5489Ф, Merbein 5512Ф and own roots. In the hot 2010 season , Merbein 5489Ф and Merbein 5512Ф had similar yields (mean of 9.0 kg/vine) compared to own-rooted vines (8.6 kg/vine) associated with more but smaller bunches and smaller berries (i.e. 1.15, 1.20, 1.26 g for Merbein 5489Ф, Merbein 5512Ф and own roots, respectively). The new rootstocks, when compared to own roots, had slightly lower levels of total soluble solids (i.e. 21.4, 20.8, 22.0 ^{o}Brix,

respectively), similar pH values (mean 4.0) and in the case of Merbein 5489Ⓡ, higher levels of titratable acidity (i.e. 5.97, 4.83, 4.94 g/L, respectively). In the cooler and wet 2011 season, samples were again collected for fruit composition, but yield data were not recorded. In 2011, a difficult season to achieve adequate levels of maturity, both Merbein 5489Ⓡ and Merbein 5512Ⓡ had higher levels of total soluble solids than own roots (20.9, 20.7 and 19.4 ^{o}Brix, respectively) with similar pH values (mean 4.0), titratable acidity levels (mean 4.7 g/L) and berry weight (mean 1.71 g). Leaf area measurements recorded at harvest indicated that Merbein 5489Ⓡ, Merbein 5512Ⓡ and own roots had similar size canopies (i.e. mean leaf area index values of 2.1). Overall, the results indicate that the field performance and juice composition of Shiraz, grafted on the new rootstocks was very similar to own rooted Shiraz.

The Pomona site involves a replicated trial comparison of Shiraz grafted on Merbein 5489Ⓡ, Merbein 5512Ⓡ and Merbein 6262Ⓡ and 140 Ruggeri. At harvest in season 2011, the new rootstocks had smaller canopies than 140 Ruggeri (i.e. leaf area index values of 2.3, 2.3, 2.1 and 3.3 for Merbein 5489Ⓡ, Merbein 5512Ⓡ, Merbein 6262Ⓡ and 140 Ruggeri, respectively). Yields of the new rootstocks in 2011 were higher than 140 Ruggeri (i.e. 17.1, 17.3, 16.6 and 14.1 kg/vine, respectively) associated with higher bunch numbers (i.e. 103, 117, 112 and 94 bunches per vine, respectively) as differences in bunch weight (mean 160 g) and berry weight (mean 1.65 g) were small. Compared to 140 Ruggeri, fruit maturity levels were slightly delayed (i.e. 20.1 ^{o}, 21.0 ^{o}, 21.2 ^{o} and 21.8^{o} Brix, respectively) with lower pH values (3.81, 3.90, 4.01 and 4.04, respectively) and similar levels of titratable acidity (mean 3.55g/L). Furthermore, the calculated yield to leaf area ratio of around 7.5 for the new rootstocks and 4.3 for 140 Ruggeri indicates that Shiraz grafted on the new rootstocks was more efficient in regard to vine growth and capacity and crop water use, determined as the crop water use index (Walker et al. 2005).

The Padthaway site involves comparisons of Shiraz grafted with Merbein 5489Ⓡ, Merbein 5512Ⓡ, Merbein 6262Ⓡ and Shiraz on Ramsey, 1103 Paulsen, 110 Richter and 140 Ruggeri. The vines were established by rapid glasshouse-based propagation, and once lignified, bare-rooted vines were heat-treated, which is a plant health requirement prior to transport from Victoria to South Australia. While establishment has been slow, sufficient reasonably mature vines of Merbein 5489Ⓡ and Merbein 5512Ⓡ were available for an initial comparative study with the other rootstocks in season 2011. Ramsey, 110 Richter and 140 Ruggeri had the highest yield (mean 8.7 kg/vine), followed by 101-14 (7.5kg/vine) and 1103 Paulsen (6.7

kg/vine) with lowest yields produced by Merbein 5489Ⓟ and Merbein 5512Ⓟ (3.2 and 5.0 kg/vine, respectively). Berry weights were highest on Ramsey (1.58 g) and smallest on 101-14 and 1103 Paulsen (1.41 and 1.46 g, respectively) with the other rootstocks, including Merbein 5489Ⓟ and Merbein 5512Ⓟ having intermediate values (1.5 g). Maturity levels were the most advanced with Merbein 5489Ⓟ (23 ^{o}Brix), followed by Merbein 5512Ⓟ (22.5) and Ramsey (22.0 ^{o}Brix) with maturation of the other rootstocks delayed (mean 21.5 ^{o}Brix). There was no rootstock effect on pH (3.55) or titratable acidity (6.0 g/L). Overall, the results are indicating site and season effects on the relative performance of the rootstocks, confirming earlier research with standard rootstocks that demonstrates significant genotype x environment interactions (S.R. Sykes et al, unpublished data). Of particular note in the Griffith trial was the observed similarity in vine performance and fruit composition between Shiraz on own roots and Shiraz on Merbein 5489Ⓟ and Merbein 5512Ⓟ. Furthermore, compared to the standard high vigour rootstocks such as Ramsey and 1103 Paulsen, the lower conferred vine vigour of the new rootstocks is leading to both production and fruit composition benefits. The data are tending to support earlier data on improved crop water use index, and although preliminary, there is evidence of improved fruit composition and wine quality attributes, including at lower alcohol levels.

Studies to assess the adaptability of the rootstocks in various locations and regions with various scion varieties and their tolerance of different pests and diseases will be ongoing as the new rootstocks become more widely distributed. Given that different levels of tolerance have been found with different nematode populations, it is likely that the new rootstocks will not be suited to all conditions, as has also been found with other rootstocks, including recent introductions from the USA (Walker & Cox 2011, Walker & Clingeleffer 2009). Given that the rootstocks grafted with Shiraz vary in vigour and yield to pruning weight ratio, different management strategies will need to be considered in order to obtain best performance in specific situations, particularly under conditions of limited water supply.

Adoption of these rootstocks may facilitate integrated approaches to vine management involving high density plantings and low-input, lighter pruning systems to improve productivity (yield /ha) and water use efficiency, enhance wine quality attributes and reduce production costs, not only in the warmer regions but in cool climates where high input, vertical shoot positioned systems are used (Clingeleffer 2010, Clingeleffer et al. 2011).

6.9.4 *Material availability*

Three nurseries (in NSW, South Australia, and Western Australia) have been licensed to propagate and distribute grafted vines of Merbein 5489Ⓟ, Merbein 5512Ⓟ and Merbein 6262Ⓟ(Figure 6.9.1). Mothervine plantings have also been established in Victoria and South Australia.

Figure 6.9.1 Mothervine planting of new CSIRO rootstocks. The three new CSIRO rootstocks have been established as large scale mothervine plantings at licenced nurseries to provide material for commercial grafting.





6.9.5 *Evaluation of next generation of rootstocks*

The next generation of rootstocks currently being evaluated for field performance has been assesed for three years at the CSIRO Sunraysia site. In 2011, all scion varieties were

evaluated for fruit quality characteristics of soluble solids (°Brix) and pH at harvest. Through a combination of selection criterea (see Table 6.2.2), only 4 rootstock genotypes were used for small scale wine production in 2011 (missing is C03, which is present in the Padthaway trial and the original Sunraysia trial planted in 1989). Due to heavy rainfall during the growing season and cooler than average temperatures, Chardonnay was harvested at a lower than average soluble solids or sugar content (6.9.2). Three of the experimental rootstocks had a final juice pH lower than the commercial rootstocks 1103 Paulsen, 140 Ruggeri, and Ramsey. Scions of the roostock C29 produced the second highest juice sugar content (18.4 °Brix) and the second lowest pH (3.49).

The yield and quality of Cabernet Sauvignon was adversely affected by the incidence of disease in the uncharacteristically wet 2010-2011 season (see section 5.7 for more detail). The °Brix were several degrees lower than would be expected for the commercial rootstocks. The juice of scions grafted to 1103 Paulsen and C113 reached the highest sugar content (21.9 °Brix), the lowest juice pH was from vines grafted to 140 Ruggeri (3.8) (Figure 6.9.3).

Shiraz scions produced juice with similar sugar content and pH as Cabernet Sauvignon. Across the seven rootstocks, the grand mean of soluble solids and pH was 21.1 °Brix and 4.0 pH for Shiraz and 21.2 °Brix and 3.9 pH for Cabernet Sauvignon. Shiraz juice from vines grafted on Ramsey had the highest mean pH 4.07 and the highest sugar content was from scions grafted to C114 (21.9 °Brix)(Figure 6.9.4).

The average berry weight in 2011 ranged from 1.18-1.33 g for Chardonnay, 0.85-1.9g for Cabernet Sauvignon, and 1.64-2.05g for Shiraz in 2011 (Figure 6.9.5). No clear trend in rootstock effect on berry size could be observed across the three scion varieties in 2011.

The mean number of Chardonnay grape bunches per vine (Figure 6.9.6) was highest on vines grated to 1103 Paulsen (183.6) and lowest for vines grafted to C29 (134.2). Cabernet Sauvignon scions ranged from 118.3 (grafted on C20 rootstock) to 144.6 (grafted on 1103 Paulsen rootstock) mean bunches per vine, with the rootstock C113 generating a comparable 144 bunches per vine. Shiraz scions grafted to 1103 Paulsen showed a sharp decline in mean vine bunch numbers (72.5) compared to the other scion varieties. The rootstock 140 Ruggeri produced the highest mean bunch number for Shiraz (115.2), with the lowest mean bunch number per vine found on scions grafted to C113 (66). The mean total yield per vine (Figure

6.9.7) followed the general trend of bunch number per vine for Chardonnay, with vines grafted to 1103 Paulsen having the highest mean yield per vine (18.18 kg) and scions grafted to C29 the lowest mean yield per vine (14.02 kg). Despite the rootstocks 1103 Paulsen and C113 stimulating a similar number of Cabernet Sauvignon clusters per vine, C113 had a higher mean yield per vine (7.34 kg) than 1103 Paulsen (5.56 kg), which was the lowest for Cabernet Sauvignon. Shiraz scions grafted to 140 Ruggeri had the highest mean yield per vine (13.3 kg), closely followed by C114 (13.2 kg/vine) and Ramsey (13.18 kg/vine), with lowest mean yield per vine produced on scions grafted to 1103 Paulsen (8.1 kg).

The three year (2009-2011) yield and fruit quality parameters were measured for Cabernet Sauvignon and Chardonnay.

The juice pH at harvest mean value for 3 years of Cabernet Sauvignon (Figure 6.9.8) was a narrow range, being highest for scions grafted to C113 (4.02) and lowest for vines with C29 rootstocks (3.92). Juice pH of Chardonnay from 2009-2011 (Figure 6.9.8) was also in a narrow range, with C114 and C20 being the lowest (3.66) and C113 the highest (3.75).

Cabernet Sauvignon grafted to C114 had the highest 3 year mean yield per vine (14.87 kg) and the scions grafted to C29 had the lowest mean yield per vine (9.85 kg) (Figure 6.9.9). 140 Ruggeri induced the highest 3 year mean fruit yield for Chardonnay (20.69 kg) while C29 was again the lowest yield rootstock at 12.11 kg/per vine (Figure 6.9.9).

The berry soluble solid level was measured at harvest for 20 rootstock hybrids and 3 commercial rootstocks with Cabernet Sauvignon and Chardonnay scions at the Sunraysia trial for 3 years (Figure 6.9.10). Cabernet Sauvignon grafted to C20 had the highest mean sugar content (23.92 °Brix) and scions grafted to C49 had the lowest (18.55 °Brix). Chardonnay had a lower mean sugar content at harvest over the three year period, with 8 rootstocks producing scion fruit between 22-23 °Brix fruit (C12, C23, C34, C42, C44, C54, C113, and C841) the two rootstocks with the lowest scion sugar content were C04 (19.55 °Brix) and C26 (19.54 °Brix).

The 3 year mean fruit quality parameters of pH and titratable acid for Cabernet Sauvignon and Chardonnay grafted 20 hybrid rootstocks under investigation and the 3 commercial rootstocks is summarized in Table 6.9.3. Cabernet Sauvignon pH ranged from 3.80 (C16) to

4.02 (C20 and C113), with a mean pH for all rootstocks of 3.92 (SEM±0.019). Titratable acidity for Cabernet Sauvignon grafted to the 23 rootstocks ranged from 4.88g/l (C29) to 6.0 g/l (C55) with a mean of 5.45g/l (SEM±0.061). The fruit pH of Chardonnay scions was generally lower than Cabernet Sauvignon, with scions grafted to C28 having the lowest 3 year mean pH (3.61) and scions grafted to C44 having the highest 3 year mean pH (3.79). The average of all fruit pH at harvest over the 3 years was 3.70 (SEM±0.01). The titratable acidity of Chardonnay fruit at harvest for the 3 years was similar to Cabernet Sauvignon across the 23 rootstocks with a grand total mean of 5.15g/l (SEM±0.14). In contrast to Cabernet Sauvignon, the fruit of Chardonnay had a larger range of titratable acidity, from 4.12g/l (C49) to 7.55g/l (C841).

The Ravaz index is the ratio of total fruit yield to total weight of canes pruned from the dormant vines. This is considered as an indicator for the balance of the vine between vegetative and reproductive growth. The Ravaz index was calculated using the 3 year mean yield (kg) and pruning weight (kg) for Cabernet Sauvignon and Chardonnay scions (Figure 6.9.11). Cabernet Sauvignon had a lower mean Ravaz index (3.37, SEM±0.44) than Chardonnay (10.22, SEM±0.83) when averaged across all the rootstocks in the trial. The rootstock C49 had the highest Ravaz index for Cabernet Sauvignon, and C112 had the lowest value for the Ravaz index (1.35). The highest Ravaz index for rootstocks grafted to Chardonnay was 18.68 (C29) and the lowest was 5.21 (C114).

Figure 6.9.2 Chardonnay fruit traits Sunraysia (2011). The °Brix and pH of juice at harvest of 4 potential new rootstocks compared to 3 commercial rootstocks (1103 Paulsen, 140 Ruggeri, and Ramsey).

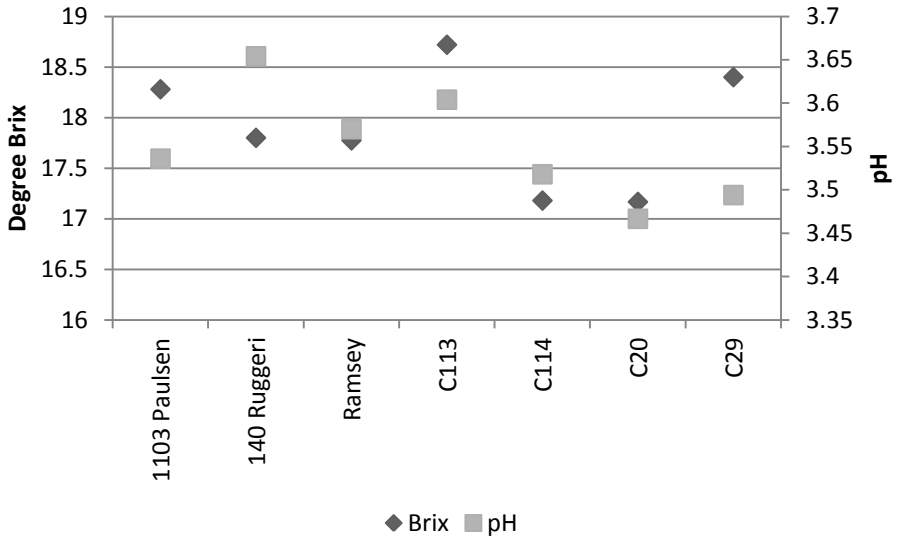


Figure 6.9.3 Cabernet Sauvignon fruit traits Sunraysia (2011). The °Brix and pH of juice at harvest of 4 potential new rootstocks compared to 3 commercial rootstocks (1103 Paulsen, 140 Ruggeri, and Ramsey).

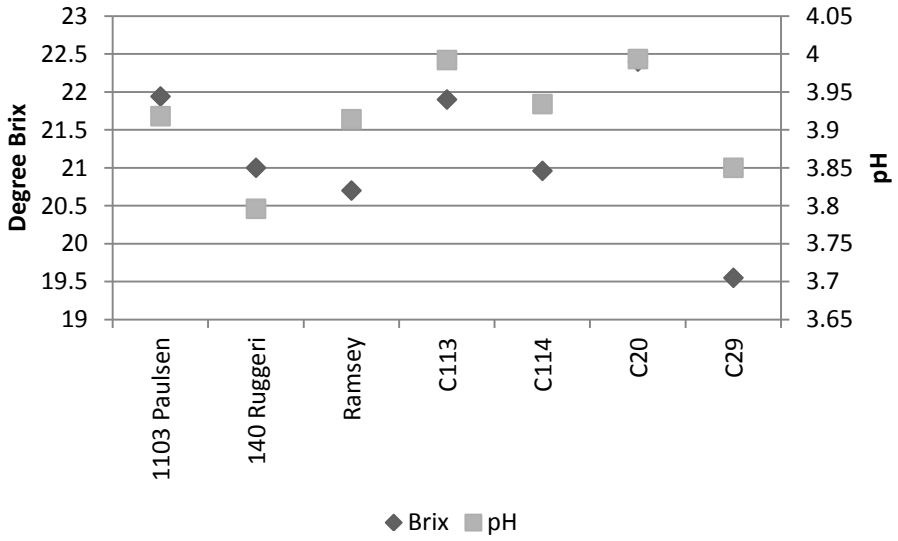


Figure 6.9.4 Shiraz fruit traits Sunraysia (2011). The °Brix and pH of juice at harvest of 4 potential new rootstocks compared to 3 commercial rootstocks (1103 Paulsen, 140 Ruggeri, and Ramsey).

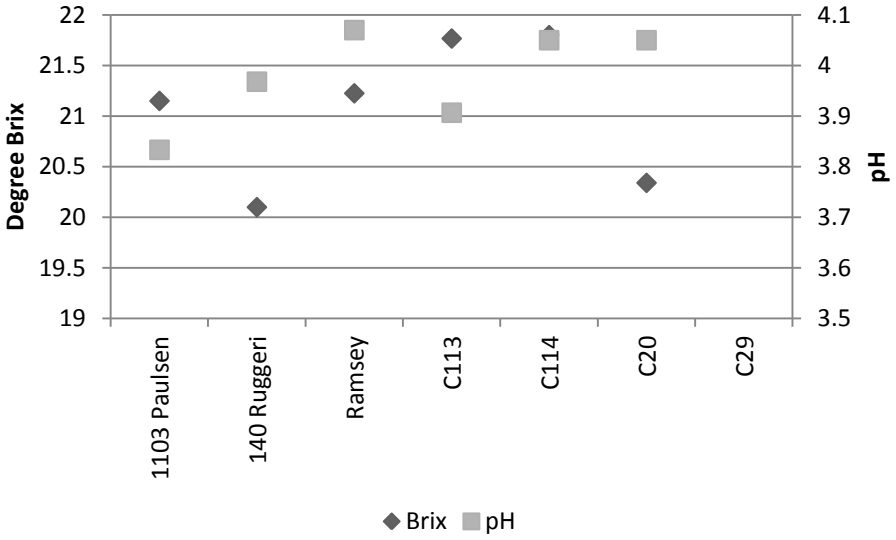


Figure 6.9.5 Average berry weight Sunraysia (2011). The average berry weight of Cabernet Sauvignon, Chardonnay, and Shiraz fruit at harvest of 4 potential new rootstocks compared to 3 commercial rootstocks (1103 Paulsen, 140 Ruggeri, and Ramsey).

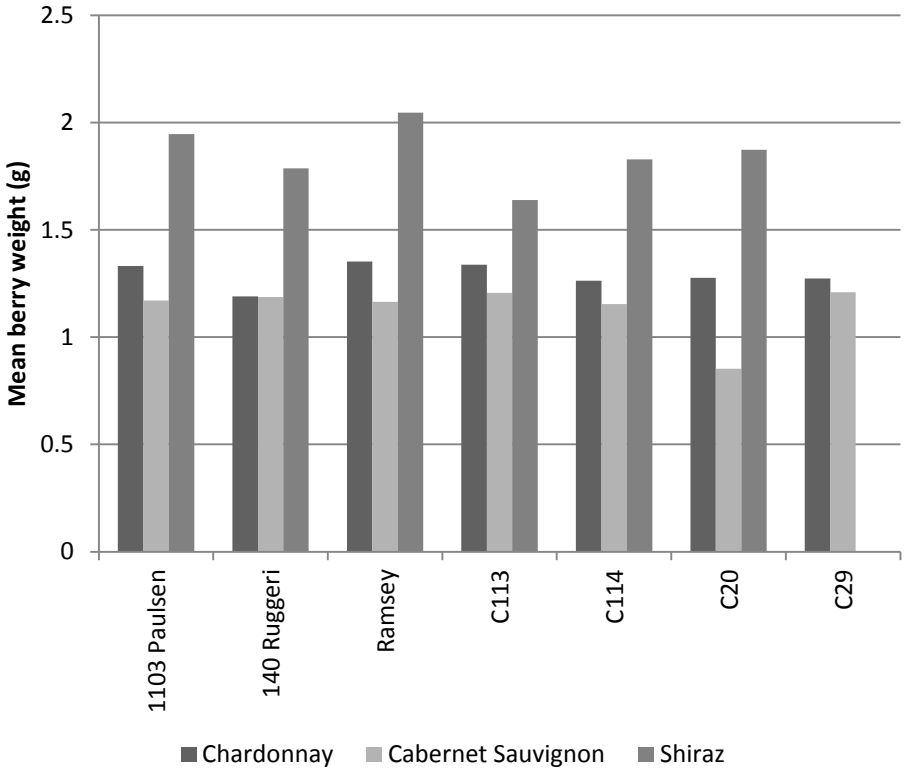


Figure 6.9.6 Average bunch number per vine Sunraysia (2011). The average number of cluster per vine of Cabernet Sauvignon, Chardonnay, and Shiraz scions at harvest of 4 potential new rootstocks compared to 3 commercial rootstocks (1103 Paulsen, 140 Ruggeri, and Ramsey).

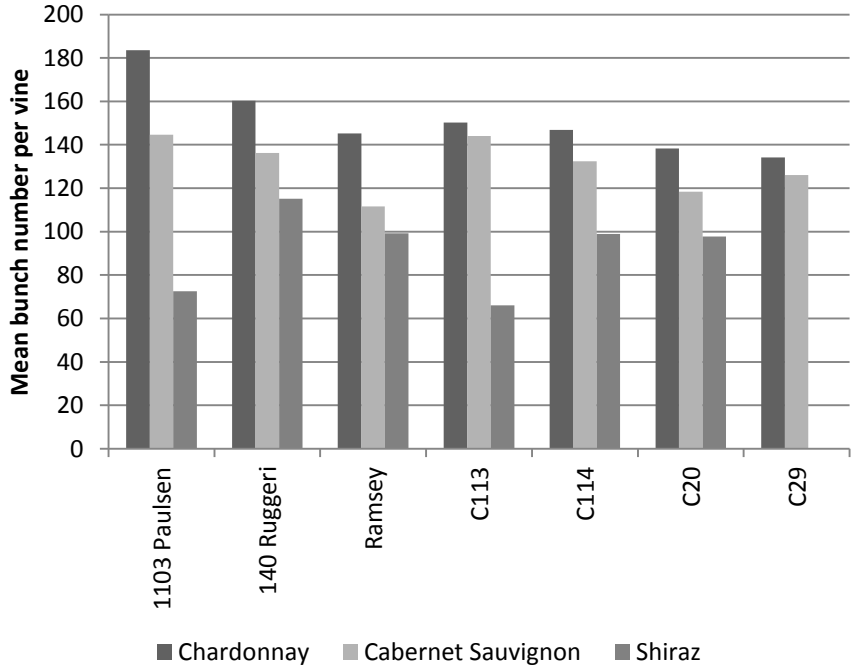


Figure 6.9.7 Average fruit yield Sunraysia (2011). The average yield per vine of Cabernet Sauvignon, Chardonnay, and Shiraz grafted to 4 potential new rootstocks compared to 3 commercial rootstocks (1103 Paulsen, 140 Ruggeri, and Ramsey).

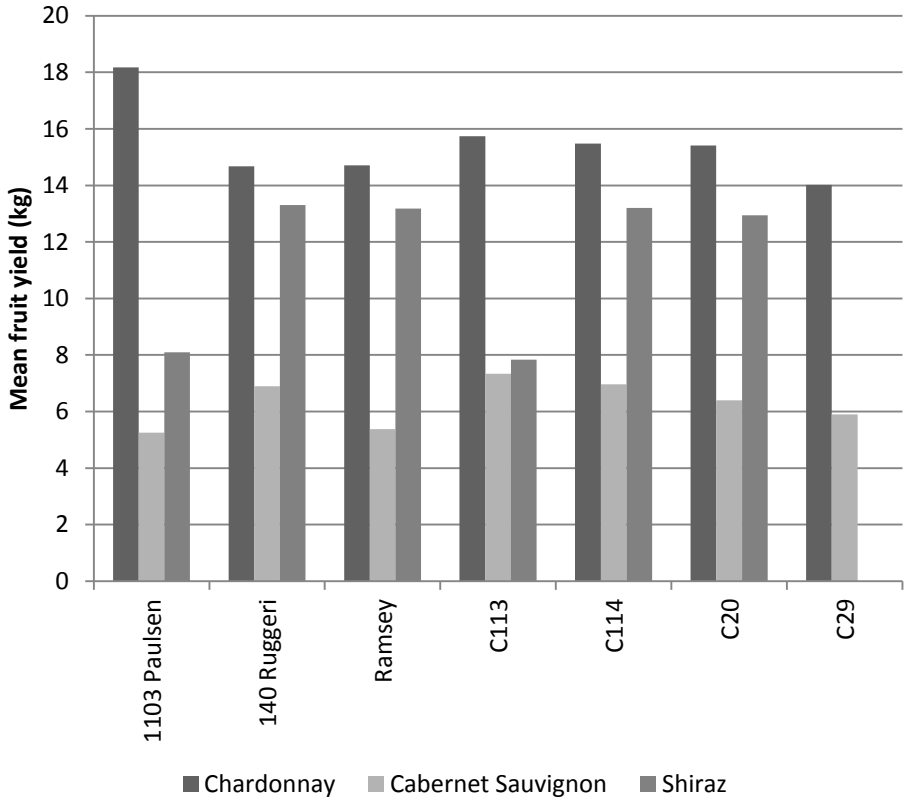


Figure 6.9.8 The 3 year mean for grape juice pH Sunraysia (2009-2011). The 3 year average berry (grape juice) pH at harvest of Cabernet Sauvignon and Chardonnay scions grafted to 4 potential new rootstocks compared to 3 commercial rootstocks (1103 Paulsen, 140 Ruggeri, and Ramsey).

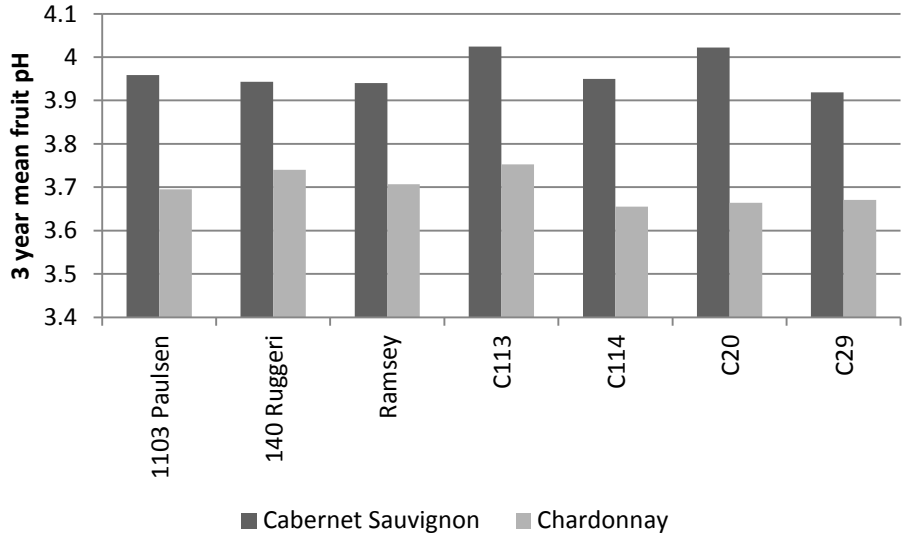


Figure 6.9.9 The 3 year mean yield per vine Sunraysia (2009-2011). The 3 year average yield per vine of Cabernet Sauvignon and Chardonnay scions grafted to 4 potential new rootstocks compared to 3 ommercial rootstocks (1103 Paulsen, 140 Ruggeri, and Ramsey).

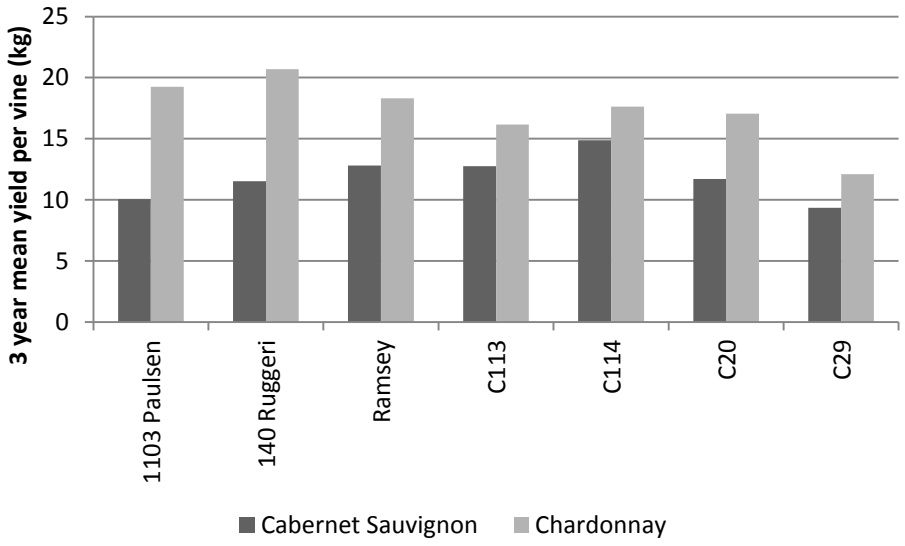


Figure 6.9.10 The 3 year mean harvest fruit soluble solids Sunraysia (2009-2011). The 3 year average berry (grape juice degrees Brix) at harvest of Cabernet Sauvignon and Chardonnay scions grafted to 20 experimental rootstocks compared to 3 commercial rootstocks (1103 Paulsen, 140 Ruggeri, and Ramsey).

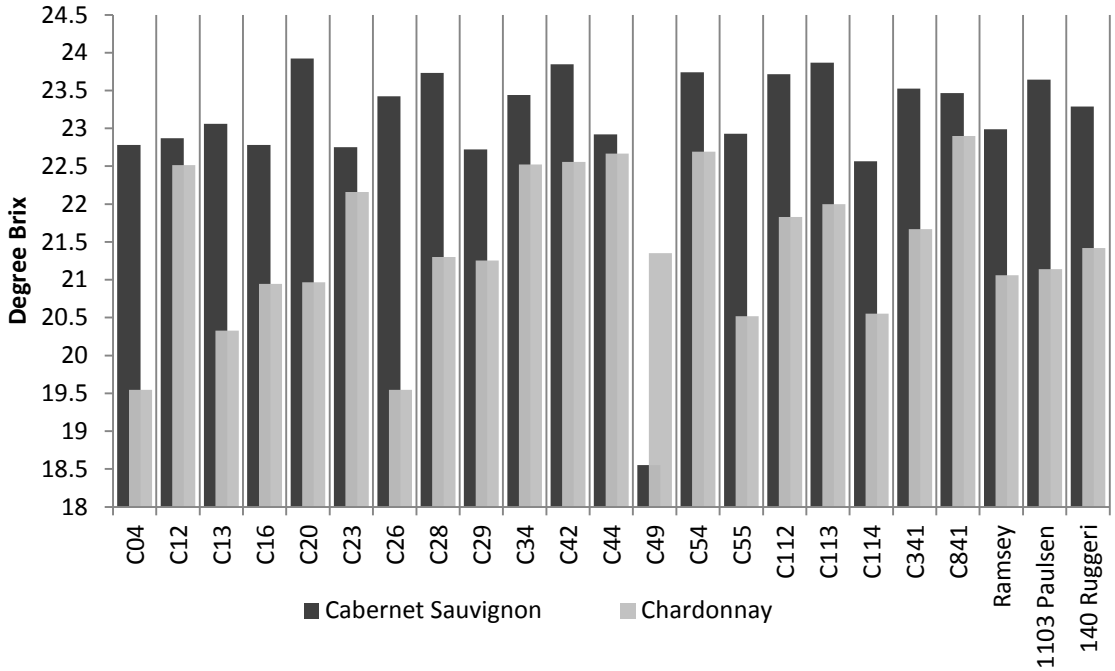
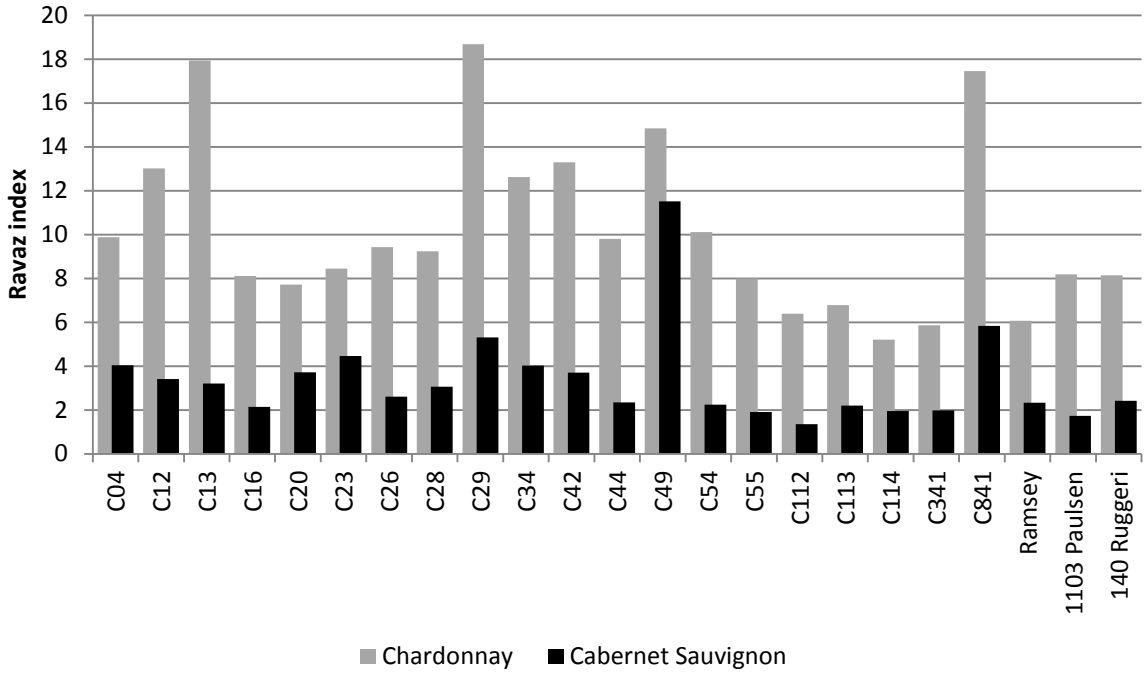


Table 6.9.3 The 3 year mean fruit pH and titratable acidity Sunraysia (2009-2011). The 3 year mean pH and titratable acidity of fruit (grape juice) of Cabernet Sauvignon and Chardonnay scions grafted to 20 experimental rootstocks compared to 3 commecial rootstocks (1103 Paulsen, 140 Ruggeri, and Ramsey).

	Chardonnay 3 year mean		**Cabernet Sauvignon 3 year mean**	
Rootstock	**pH**	**Titratable acid (g/l)**	**pH**	**Titratable acid (g/l)**
C04	3.64	5.09	3.84	5.45
C12	3.68	4.68	3.87	5.50
C13	3.70	4.75	3.92	5.29
C16	3.66	5.24	3.80	5.80
C20	3.66	5.53	4.02	5.35
C23	3.68	5.13	3.88	5.15
C26	3.65	6.10	4.00	5.28
C28	3.61	5.14	3.87	5.52
C29	3.67	4.45	3.92	4.88
C34	3.73	5.04	3.87	5.46
C42	3.79	4.98	3.93	5.69
C44	3.79	4.32	3.94	5.33
C49	3.72	4.12	3.59	4.98
C54	3.73	4.91	3.94	5.56
C55	3.64	5.86	3.94	6.00
C112	3.76	4.93	3.97	5.81
C113	3.75	4.97	4.02	5.28
C114	3.66	5.16	3.95	5.85
C341	3.74	5.16	3.96	5.34
C841	3.67	7.55	3.97	4.95
Ramsey	3.71	4.87	3.94	5.76
1103 Paulsen	3.70	5.36	3.96	5.55
140 Ruggeri	3.74	5.18	3.94	5.52

Figure 6.9.11 The 3 year mean Ravaz index Sunraysia (2009-2011). The 3 year average ratio of total fruit yield to dormant cane pruning weight of Cabernet Sauvignon and Chardonnay scions grafted to 20 experimental rootstocks compared to 3 commercial rootstocks (1103 Paulsen, 140 Ruggeri, and Ramsey).



6.9.6 *Selection of potential new rootstocks from CSIRO breeding program*

Sunraysia trial and Padthaway trials

The field trials established at Sunraysia and Padthaway have not yet come into bearing, and therefore have not been evaluated for fruit quality traits. As a result of screening methods, the vigour potential, potassium uptake, root-knot nematode resistance, and rootstrike have been assessed for these vines. Field performance of this trial will provide an indication of the efficacy of screening methods.

Nematode resistance

The parents used to generate the 12 families have been screened in the glasshouse for resistance to the McLaren Vale strain of *M. javanica*. Genotypes selected for field trials have been screened for root-knot nematode (RKN) resistance.

Table 6.9.4 *Meloidogyne javanica* root-knot nematode resistance of rootstock families. The resistance (R) and susceptibility (S) of parents of the 310 rootstock genotypes examined in the nursery at Merbein, Victoria. Based on other selection criteria, 123 of the progeny have also been scored.

Family	Seed Parent	Pollen Parent	R:S
MC1	R	S	-
MC2	R	S	-
MC3	R	T	-
MC61	R	S	1:1
MC62	R	R	-
MC63	R	S	7:1
MC64	R	S	-
MC65	R	T	1:1
MC67	R	R	3:1
MC68	R	S	1:1
MC86	R	R	47:5
MC88	R	R	26:27

Phylloxera resistance

The phylloxera resistance of the seed parent *V. cinerea* '71' was examined in the detached-root bioassay. Neither the G1 nor G4 strain of phylloxera developed to the adult stage on the roots of *V. cinerea* '71', indicating a high level of tolerance. *V. cinerea* '71' is the seed parent in 9 of the 12 families, and the parent of 268 of the 310 experimental rootstocks. The pattern of segregation of this trait is unknown, however researchers have identified genetic markers derived from *V. cinerea* that segregates with this trait (Zhang et al. 2009). In their test population with *V. vinifera*, phylloxera resistance behaves as a single dominant gene. Research is ongoing to determine the efficacy of this molecular marker in our populations.

Salt exclusion

The ability of ungrafted vines to exclude chloride in a glasshouse assay has been examined on two members of the MC 86 family. These two vines showed distinct differences in salt toxicity symptoms after 6 weeks of irrigation with a 50mM Cl^- solution. Examination of leaf Cl^- levels revealed that the vine with no salt burn symptoms had a content of 0.68% Cl^-/dry leaf weight, whilst the vine displaying salt toxicity symptoms at the leaf margins contained 1.38% Cl^-/dry leaf weight. Gong et al. (2011) determined that chloride exclusion is a

continuous trait in a population constituted from parents with high or low salt exclusion capacity. Further examination of the segregation of this trait is required to determine if molecular markers can be identified that are associated with salt exclusion.

Vigour potential

The vigour potential the 310 experimental genotypes was examined as both ungrafted mature vines and replicated grafted vines in the nursery. The rootstock family MC 67 had the highest mean pruning weight of ungrafted vines in 2008 (2.60 kg), however in the 3 subsequent years, family MC 62 had the highest mean pruning weights of ungrafted vines (2.56 kg, 1.67 kg, and 4.18 kg)(Table 6.9.5 and Figure 6.9.12). Similarly, family MC 42 had the lowest mean pruning weight of ungrafted vines in 2008 (0.70 kg), and MC 34 had the lowest mean pruning weight in subsequent years (0.72 kg, 0.54 kg, 1.04 kg). Families MC 62 and MC 67 have two highest 3 year mean pruning weight (2.60 kg and 2.24 kg respectively. These two families are half sibling groups, with both sharing the seed parent *V. cinerea* '71'. Additionally both groups pollen parents are ½ *V. xchampinii* , which is may be contributing factor to the high vigour, however, family 61 shares ¾ of its pedigree with these two groups and is one of the lowest vigour families in all years.

The scion pruning weight of the 310 genotypes in the nursery was weighed at the conclusion of the 2008-2009 season for all 6 blocks. Three of the blocks had vines which received sufficient irrigation (37% mean soil moisture)(Table 6.9.6). Family MC 68 had the highest mean scion pruning weight (67.76 g), closely followed by family MC 86 (65.19 g). The families which induced the lowest vigour in scions were MC 1 (31.42 g mean pruning weight) and MC 63 (37.37 g mean pruning weight).

Drought tolerance

The effect of drought conditions was measured on ungrafted vines in the field and grafted vines in the nursery. Ungrafted field vines were well established mature vines, the drought conditions imposed were more severe, as no supplemental irrigation was applied for three seasons. Nursery vines were in their third year of growth when 3 of the 6 blocks received a reduction in the irrigation applied (see section 5.5.2).

The pruning weights of ungrafted mature vines were collected in 2009, 2010, and 2011 at the conclusion of seasons that received no supplemental irrigation, the annual rainfall in those years was 229mm, 289 mm and 924 mm respectively. As there was a record high level of annual rainfall in the 2010-2011 season, the effect of drought were considered as the reduction in vigour from 2008 to 2010 (Table 6.9.5). While family MC 62 had the higest mean pruning weights in 3 of the 4 seasons, family MC 64 had the lowest reduction in pruning weights from 2008 to 2010 (-13.15%) and MC 62 had the second lowest reduction in pruning weights (-15.68%). Family MC 2 had the largest reduction in ungrafted vine pruning weights (-82.82%) in the period, dropping from 1.24 kg to 0.21kg. In the nursery, family MC 62 had the lowest drop in vigour from the reduced irrigation treatment (-13.57%). Scions on this family had the highest mean pruning weight of all families (39.61 g) in the blocks with the reduced irrigation. However, the ungrafted vines did not respond the same as grafted vines to lower irrigation in all cases (Figure 6.9.13). This data requires careful interpretation, as the treatments were not identical and the ungrafted vines were not replicated. One possible explanation may be the different strategies grape species may have evolved to cope with water stress, namely drought tolerance (e.g. better water used efficiency or finer roots for greater surface area to uptake water) or drought avoidance (deeper penetrating roots to explore for water). For example, family MC 62 had high vigour as both grafted and ungrafted vines with a low reduction in vigour under reduced irrigation, the overall root structure of ungrafted mature vines was rated 2.33 on a scale of 1-3, (with 1 being >90% of roots were vertical, 2 was a continuum between horizontal and vertical roots, and 3 being >90% of roots horizontal), this was the highest value of all 14 families. Additionally, MC 62 had the highest mean number of roots emerging from the root crown (52.36). One possible interpretation is that the drought adaptation of this family is to have more roots for greater total root surface area, with roots found closer to the soil surface. In contrast, family MC 2 had the most vertical overall mean root architecture (1.69), but also the lowest mean number of roots (14.08). This family had an 82.82% largest reduction in ungrafted vine pruning weight (it should be noted it was not present in the grafted vine nursery). It may be possible that while deep rooting may be a mechanism of drought avoidance, this strategy may not always be effective due to impenetrable soil layers, or perhaps requires a minimum root threshold of root proliferation. Family MC 64 had the lowest reduction in ungrafted vine mean pruning weight from 2008 to 2010 (-13.15),but had a much larger relative decline as grafted vines in the nursery under the reduced irrigation treatment (-26.4%). The decline in the nursery is comparable to MC 63 (-29.68%), both families had similar values for root

angle (2.09 and 2.12 for MC 64 and MC 63 respectively), the major difference being in total mean root number of 34.37 for MC 64 and 47.67 for MC 63. Yet as ungrafted vines, MC 63 had a 23.44% decline in pruning weight from 2008 to 2010. In this case, the greater root number of MC 63 did not result in greater drought tolerance as ungrafted vines, despite similar root architecture and performance in the nursery. It is clear that drought tolerance is a complex trait that will require greater investigation to isolate and identify in grapevine rootstocks.

Table 6.9.5 The response of ungrafted rootstocks families to drought. The mean family pruning weight with full irrigation in 2008 and again in 2010, after 2 years of non-irrigation of ungrafted mothervines in Merbein, Victoria.

Family	2008 mean pruning weight (kg)	2010 mean pruning weight (kg)	2008 - 2010 percent change	2011 mean pruning weight (kg)
MC1	1.68	0.74	-55.73	2.72
MC2	1.24	0.21	-82.82	1.31
MC3	1.56	0.74	-52.67	2.95
MC34	0.83	0.54	-34.32	1.04
MC61	1.01	0.26	-74.01	1.48
MC62	1.98	1.67	-15.68	4.18
MC63	0.89	0.68	-23.44	1.44
MC64	0.94	0.82	-13.15	2.13
MC65	1.19	0.53	-55.73	1.82
MC67	2.60	1.26	-51.48	3.73
MC68	2.38	1.01	-57.35	3.65
MC86	1.81	0.59	-67.15	2.31
MC88	2.04	1.04	-49.31	2.81

Figure 6.9.12 The yearly dormant vine pruning weight for rootstock families. The annual growth of ungrafted mothervines for rootstock families was measured in a non-irrigated mothervine block at Merbein, Victoria to asses vine response to drought like conditions

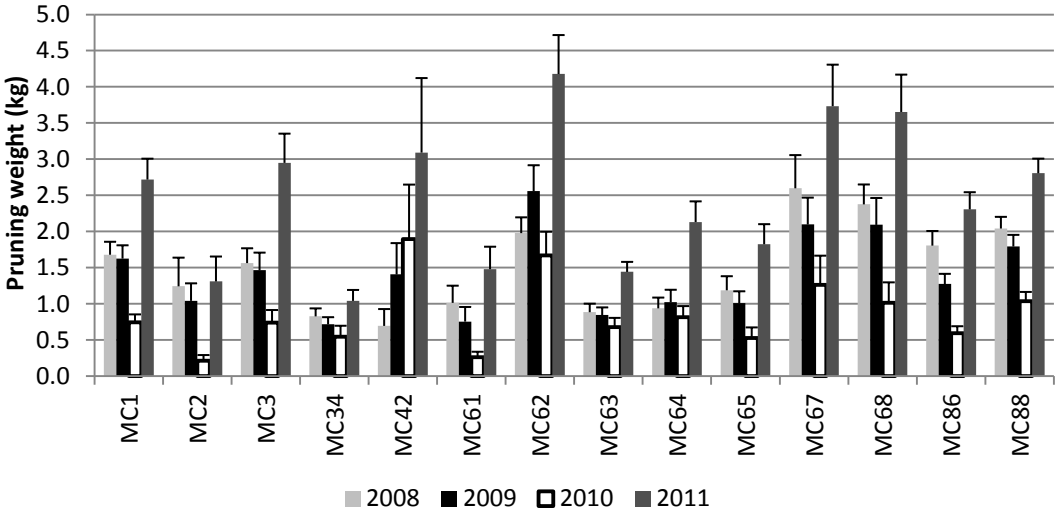
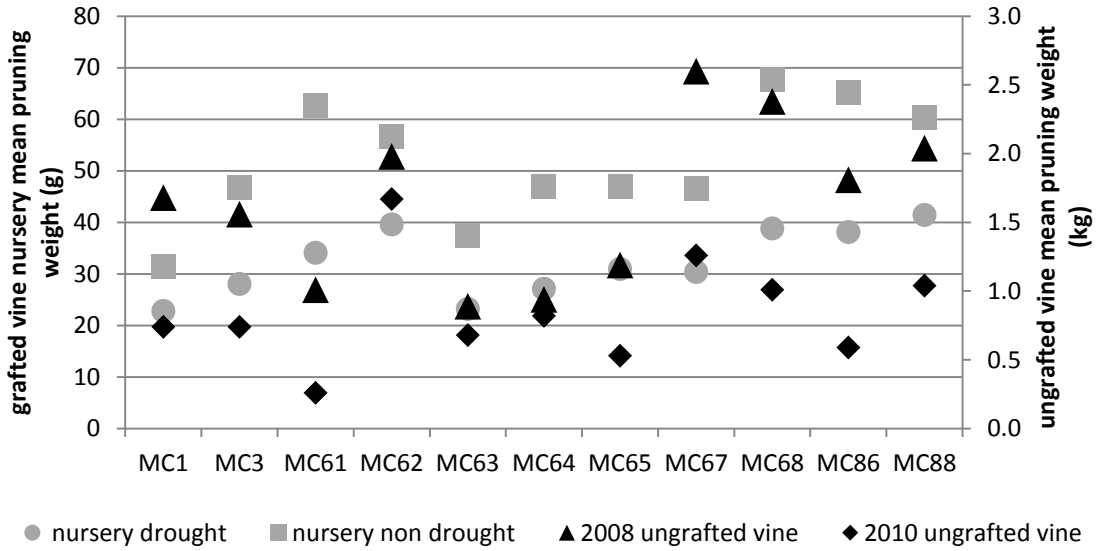


Table 6.9.6 Nursery trial scion vigour. The scion pruning weight (g) of 11 rootstock families was measured in 2009 for nursery vines receiving sufficient irrigation treatment.

Family	2008 nursery scion pruning weight (g)
MC1	31.41
MC3	46.77
MC61	62.60
MC62	56.58
MC63	37.37
MC64	47.00
MC65	46.94
MC67	46.48
MC68	67.75
MC86	65.19
MC88	60.28

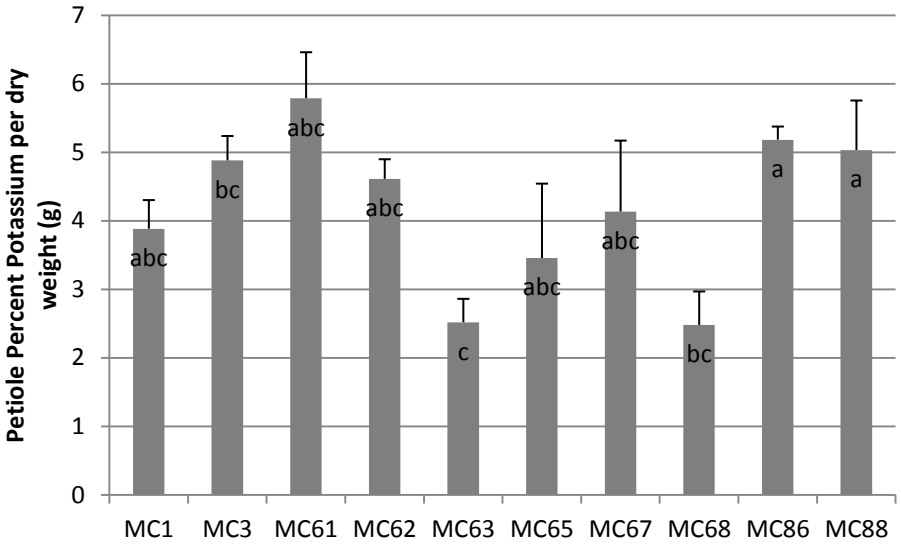
Figure 6.9.13 The effect of limited irrigation on ungrafted and grafted grapevine rootstock families. The mean pruning weight of ungrafted mothervines in 2008 (final year of irrigation) and in 2010 (after two years of witholding irrigation) compared with three-year-old grafted vine pruning weight in a nursery where 3 blocks received sufficient irrigation (37% mean soil moisture) and where 3 blocks received low irrigation (16% mean soil moisture in the 2008-2009 season.



Potassium uptake

The potassium content of petioles of 145 experimental vines in the rootstock nursery were examined on the basis of these genotypes displaying high rootstrike and having a minimal impact on scion vigour reduction under low irrigation. The family with the lowest mean potassium accumulation (2.48% dry weight) of ungrafted vines was MC68, while the highest petiole potassium concentration was 5.79% for family MC61.

Figure 6.9.14 Potassium uptake of rootstock families. The petiole potassium concentration (%K^+/dry weight) for 145 grafted (10 families) vines in a rootstock nursery. The differences in the mean values were found to be significantly different (P=<0.001). Bars with different letters were significantly different in a pairwise mutliple comparision (Holm-Sidak, P<0.05)



Propagation

The rootstrike of the vines was measured and Estimated Breeding Values (EBV) were calculated for all genotypes. Propagation results were used to help in selection of experimental rootstock for the field trial established at CSIRO Sunraysia site, in 2010. Vines with a high EBV were retained for the germplasm breeding program, as it is predicted these genotypes have a higher probability of producing progeny with a high rate of rootstrike.

7.Outcome/Conclusion

The project has involved the assessment of rootstock genotypes at various stages of development, ranging from newly propagated seedlings derived from controlled crosses; ungrafted hybrid populations established in the field in previous projects; replicated trials with advanced selections identified in the previous project and grafted with major scion varieties; regional trial sites established with the low-medium vigour CSIRO rootstocks with commercially available rootstocks for comparison. The outcomes of these studies have significant ramifications for industry and in particular for the development of future breeding strategies based on knowledge of the inheritance of key traits, and for application of rapid screening to minimise the number of selections established in field trials. The implementation of marker assisted selection, e.g. for nematode and phylloxera resistance would greatly facilitate this process.

From an industry perspective, the studies have shown that 30 of the 69 rootstocks varieties in Australia, including the widely planted 1103 Paulsen rootstock, allow reproduction of an aggressive strain of *M. javanica* in glasshouse assays. In regard to bio-security, it will be important to establish how widespread the aggressive strain of *M. javanica* is in soils in the main viticultural regions to both minimise further spread and to provide growers with information in regard to rootstock choice. While DNA typing techniques have been developed to identify the major nematode species found in Australian soils in this study, soil testing will require use of glasshouse screening procedures to segregate virulent and non-virulent strains of *M. javanica*. The 5 new rootstocks identified for further development based on their field performance and wine quality attributes when grafted with Chardonnay, Cabernet Sauvignon and Shiraz offer significant potential. The inability of a specific strain of root-knot nematode to reproduce on these 5 rootstocks, as well as relatively high tolerance to phylloxera in a glasshouse bioassays, may offer growers with alternative rootstocks to address this significant problem. Their performance of the 5 rootstocks in plantings at Padthaway is yet to be determined.

Ongoing studies with the low-medium vigour CSIRO rootstocks, Merbein 5489Φ, Merbein 5512Φ and Merbein 6262Φ have shown that these rootstocks are a potentially powerful tool to manipulate berry composition (i.e. break the nexus between ‘sugar ripeness’ and ‘flavour

ripeness') with capacity to produce acceptable and preferred red wines at lower levels of alcohol than can be achieved with standard rootstocks such as Ramsey and 1103 Paulsen. Because of the importance of the issue of wine alcohol content to the Australian industry, further study into the impact of rootstock on berry maturation and the development of anthocyanin, tannin and flavour compounds and wine body is required. Initial studies with the three rootstocks prior to their release had shown that they had good root-knot nematode tolerance (Dry 2007). When tested against the aggressive strain of *M. javanica* isolated from McLaren Vale, Merbein 5512Ⓟ and Merbein 6262Ⓟ were shown to be tolerant wheras as Merbein 5489Ⓟ was sensitive, further highlighting the issue of variable performance relating to nematode type and strain and the implications of this for rootstock choice. The project has also facilitated industry evaluation of the three CSIRO rootstocks which were granted Plant Breeders Rights in 2009, initially through the use of a nursery with rapid propagation and green grafting facilities to overcome the issue of limited material. The subsequent licensing of three nurseries, one in Sunraysia, one in South Australia and one in Western Australia, and establishment of mother vine plantings in Victoria and South Australia have provided access options to growers in all regions. The advent of the worst drought in living memory with its severe impact in south eastern Australia, coupled with industry fruit over-supply issues and subdued demand and pricing for fruit, had a severe negative impact on new plantings, with many established vine nurseries struggling for survival as demand for planting material severely decreased. Accordingly, demand for rootstocks was much reduced during this time. However, with mature mothervine plantings now available and evidence of increasing interest by growers in new plantings, there is greater optimism that more growers will be interested to evaluate the new rootstocks.

In the current study, more than 50 genotypes have been selected with good propagation characteristics, appropriate vigour and drought tolerance. Plants of these genotypes, grafted with Shiraz, have been included in a replicated trial to assess performance under well-watered and deficit irrigation treatments which will produce the first crop in 2013. A further subset of these genotypes grafted with Shiraz has also been planted at Padthaway to assess performance in a cooler region than where they were developed and selected (Sunraysia). It is likely that a number of drought tolerant rootstocks will be identified in these trials for further development once the trials are completed and phylloxera and nematode tolerance assessed.

A further 160 genotypes have been retained for future breeding and evaluation purposes from ungrafted hybrid populations planted at Merbein. The parentage of this material covers a diverse range of *Vitis* species. These genotypes have been retained because they were the best performers in the various screening studies involving selection for drought tolerance, low potassium uptake, salt exclusion and propagation characteristics including genotypes with the highest breeding values for rootability.

The development of efficient, high throughput, rapid screening techniques to improve rootstock breeding efficiencies, and to minimise the number of selections established in field trials, has been an important component of this project. Multi-trait glasshouse screening techniques using the same plants have been developed and applied for root-knot nematode tolerance, potassium uptake and salt exclusion. The appropriateness of the potassium uptake screen has been verified using well established field trials with grafted plants. Furthermore, the evaluation of a large number of genotypes in a nursery situation has provided a very useful approach to assess vine vigour, plant mineral status and drought tolerance without the establishment of long term field trials.

In addition to the development of rapid screening techniques, the project has also generated significant new knowledge to underpin future strategies for efficient rootstock breeding through targeted parental selection based on the known inheritance of key traits which may also be augmented by the use of use molecular markers to rapidly assist selection at the very early seedling stage. In this regard, the studies have shown that the breeding parent *Vitis cinerea* is an excellent source of nematode resistance and is potentially also a source of phylloxera resistance. The results indicate potential to develop genetic markers for some key traits such as phylloxera, nematode resistance and rootability. The use of marker assisted selection at the seedling stage would complement the glasshouse based rapid screening techniques developed in this project and reduce numbers for screening as grafted plants in the nursery (i.e. for vigour and drought tolerance) and ultimately for planting in field trials.

In conclusion, the project has continued the development of new rootstocks for the Australian winegrape industry that provide protection against the Australian bio-types of soil borne pests

(i.e. nematodes and phylloxera); are suited to the Australian environment, particularly soils which are high in potassium; address water related issues of water use efficiency, drought and salinity and which provide enhanced wine quality attributes. The project has provided new rootstock genotypes for further development and assessment and demonstrated that the low-moderate vigour CSIRO rootstocks have significant potential to facilitate production of wines with lower alcohol contents. As part of the project, rapid multi-trait glasshouse and nursery based screening techniques have been developed and implemented to significantly improve the efficiency of new rootstock selection. Furthermore, the project has provided underpinning new knowledge on the inheritance of key traits that can be used in the future breeding strategies for the selection of parents with high breeding values and to refine the development of molecular markers to facilitate marker assisted selection to further improve breeding efficiency and minimise the number of rootstock genotypes selected for field evaluation as grafted plants.

8.Recommendations

Germplasm maintenance.

GWRDC and CSIRO, through co-investment in this and previous projects, have committed to delivering improved rootstocks to secure the development of an economically sustainable, resilient Australian wine grape industry. To underpin future rootstock research and development of new rootstock genotypes it is recommended that key rootstock germplasm and established trial sites developed as part of this project be maintained as part of the current project, 'Rootstocks to meet future challenges of the Australian wine industry' CSP11/01. This material includes:

- ***Rootstock seed sources*** from targeted breeding which will be maintained under refrigeration at the CSIRO Waite campus.
- ***The rootstock variety and species collection*** at the CSIRO site and some material already established at SARDI for 'ready access'.
- ***Drought tolerant genotypes*** (i.e. approx. 300) requiring establishment and maintenance at CSIRO.
- ***Key rootstock populations*** from targeted breeding for establishment and maintenance at CSIRO. These include those generated for nematode and phylloxera resistance using *V.cinerea* and segregation populations for nematode, phylloxera and salt tolerance.
- ***Promising rootstock genotypes*** used in grafted vine trials, with confirmed nematode and phylloxera tolerance, and other selected genotypes from multiple screening studies, for establishment and maintenance at key sites.
- ***Planted rootstock trials***. These include replicated rootstock trials planted at the CSIRO site and at Padthaway with promising genotypes, including drought tolerant genotypes and new rootstock genotypes which have been shown to have good nematode and phylloxera resistance, good production and wine quality characteristics with potential for wider scale industry evaluation.

Further research

The project has identified a number of research opportunities which should be pursued to (1) complete the evaluation of generated material to identify new rootstocks for use by industry;

(2) to develop and utilise new and improved techniques to rapidly screen breeding lines and increase breeding efficiencies; and, (3) provide new scientific knowledge on key rootstock traits which may be incorporated in future breeding. These include:

- Complete the evaluation of rootstock trials which have been established in the current project (CSP 05/03) at the CSIRO site and at Padthaway to identify new rootstocks for commercial release and wider scale industry evaluation. This will also require conduct of DUS comparator trials for PBR purposes.
    1. The Sunraysia trial with Shiraz rootstock grafted on more than 50 genotypes which were selected for good propagation characteristics and drought tolerance in a nursery situation. This trial, which will include well watered and deficit irrigations treatments, will produce the first crop in 2013.
    2. The Sunraysia trial with 26 rootstock genotypes grafted with Chardonnay, Cabernet Sauvignon and Shiraz from which 5 selections have been identified for further development that have good nematode and phylloxera resistance and good production and wine quality characteristics when managed with limited water supply.
    3. Padthaway trial with the same rootstock genotypes as in 2 above and the three CSIRO low medium vigour rootstocks. This trial will produce some crop in 2012. The data will augment ongoing information collected at the CSIRO site on the 5 most promising selections identified for further development prior to their release.
    4. Padthaway trial established with a subset of those in 1 above to evaluate vine performance and drought tolerance in a different climate with supplementary irrigation.
- In collaboration with commercial nurseries, conduct propagation/grafting trials with the 5 new promising selections to provide information on these characteristics prior to their release and establish mothervine plantings to facilitate rapid adoption if released to industry.
- Establish new rootstocks trials with the 5 promising selections identified in the current project in commercial vineyards covering a range of environments to assess Genotype x Environment interactions and broad scale performance.

- Screen drought tolerant genotypes (approx. 300) identified under non-irrigated conditions at Merbein for root-knot nematode tolerance, potassium uptake discrimination, and salt exclusion capacity to reduce numbers for further study. Confirm drought tolerance of remaining genotypes in a nursery situation as grafted plants and select the most promising drought tolerant types for further development and inclusion in larger scale evaluation trials.
- Develop and apply genetic markers for rapid screening of breeding lines for nematode resistance based on current studies conducted in this project which show that resistance, at least in *Vitis cinerea*, segregates as a single dominant gene. There is potential to develop international collaboration in this area. CSIRO is currently developing collaborative research in this area with Peter Cousins, USDA, Geneva, New York.
- Develop genetic markers for rapid screening of rootstock breeding lines for phylloxera resistance and confirm results using conventional screening techniques conducted by DPI, Victoria, Rutherglen.
- Confirm that measurement of calcium uptake is a useful screening/classification tool for the assessment of vigour of rootstock genotypes at the early stages in the breeding pipeline, i.e. as small seedlings.
- Implement a targeted, rapid rootstock breeding and selection program based on enhanced knowledge of the inheritance of key characteristics and application of rapid screening techniques developed in the current project for root-knot nematode, potassium uptake, salt exclusion, vigour, and potential use of markers for phylloxera (see above).
- Continue to monitor the performance of the 3 CSIRO low-medium vigour rootstocks, Merbein 5489Ⓟ, Merbein 5512Ⓟ and Merbein 6262Ⓟ, established in commercial vineyards. Consideration should be given to optimising their performance and production efficiency through changes to irrigation strategies and use in high density plantings (i.e. closer row x vine spacing) as identified by Clingeleffer et al. (2011)
- The studies with the 3 low-medium vigour rootstocks have demonstrated the potential of specific rootstocks as a possible tool to break the nexus between sugar ripeness and colour and flavour development, leading to higher colour and potential flavour at lower total soluble solids than standard rootstocks. Because of the requirement to

produce wines with lower alcohol in Australia it is recommended that more study be undertaken to assess the effects of rootstocks on maturation and the development of anthocyanin, tannin and flavour compounds and wine body.

- The studies to assess the nematode resistance of potential rootstocks available in Australia have shown that 43% are not completely resistant to an aggressive strain of *M. javanica*. Potentially this is a serious issue in regard to biosecurity, particularly as many of the own rooted plantings on sandy soils are redeveloped in the future. It is recommended that a major study to investigate how widespread the aggressive nematode strain of *M. javanica* is in Australian vineyards. This will require soil sampling, extraction of nematodes and screening under glasshouse conditions using 1103 Paulsen. In the longer term, it is recommended that molecular techniques be developed to distinguish the aggressive nematode strain from less virulent *M. javanica.*

Appendix 1: Communication

Project results and updates have been presented at various research and industry forums. These included:-

- The Australian Rootstock Research Forum, Irymple, May 2008.
- The 14th Australian Wine Industry Technical Conference Rootstock Workshop (July 2010) with presentations by Peter Clingeleffer (Adapting to changing environments through the use of rootstocks) and Brady Smith (Rootstock breeding and development for Australian conditions).
- Poster presentation to the 14th Australian Wine Industry Technical Conference Smith et al. 2010. Development of grapevine rootstocks for Australian conditions (poster and paper included in proceedings). Adelaide, July 3-8, 2010
- The ASVO/ Phylloxera Board of SA, Below Ground Seminar (July 2011)
- The 17th GiESCO Symposium, Asti , Italy, Aug 29th -Sept. 4th 2011(Clingeleffer et. al 2011)
- Presentations to wine companies, Australian Vintage (2007), Orlando (2011) and Accolade wines (2011)
- The Riverland Vine Improvement Group, August 2011
- CSIRO Plant Industry Waite Campus Seminar, July 2011

In addition, the rootstock studies have also been presented as part of addresses on grapevine management in national and international forums. These include:-

- The ASVO, 'Viticultural practices to moderate wine alcohol content', seminar October 2007 (Clingeleffer 2008).
- The 16th International Symposium, GiESCO 2009, July 12-15th University of California, Davis, USA. pp. 259-264. (Clingeleffer 2009)
- The Eighth International Symposium on Grapevine Physiology and Biotechnology. Adelaide (Clingeleffer 2010)

Tastings of small scale-wines from rootstock trials were presented to McGuigan-Simeon in 2007 (2006 wines), the Australian Rootstock Research Forum in May 2008 and the ASVO/ Phylloxera Board of SA, Below Ground Seminar (July 2011).

Papers have also been published in scientific journals, conference proceedings and industry journals. They are listed below.

Published papers

Clingeleffer, P.R. (2008) Viticultural practices to moderate wine alcohol content. In: Allen, M., Cameron, W., Johnstone, R.S. and Pattison P., eds. Towards best practice through innovation in winery processing: Brenton Langbein Theatre, Tanunda, South Australia, Wednesday, 17 October 2007. Adelaide: Australian Society of Viticulture and Oenology, 37-39.

Clingeleffer PR (2010) Plant management research-status, what it can offer to address the challenges and limitations. In: Special edition of the Eighth International Symposium on Grapevine Physiology and Biotechnology. Australian Journal of Grape and Wine Research. 16 (S1) 25-32.

Clingeleffer PR (2009) Integrated vine management to minimise the impacts of seasonal variability on yield, fruit composition and wine quality. In: *Proceedings 16th International Symposium, GiESCO 2009*, July 12-15th University of California, Davis, USA. pp. 259-264.

Clingeleffer, P.R., Smith, B.P., Edwards, E.J., Collins, M.J., Morales, N.B. and Walker, R.R. (2011) Rootstocks, a tool to manipulate vine growth characteristics, fruit composition and wine quality attributes, water use efficiency and drought tolerance. In: Novello, V., Bovio, M. and Cavalletto, S., eds. Proceedings 17th GiESCO, Asti-Alba(CN), Italy, August 29th - September 2nd 2011, pp 451- 454.

Clingeleffer, P.R., Smith, B.P., Edwards, E., Collins, M., Morales, N.B., Davis, H., Sykes, S., and Walker, R.R. (2011b) Industry puts low-medium vigour rootstocks to the test. Wine and Viticulture Journal 3, 72-76. (Appendix 6)

Clingeleffer, P.R., Smith, B.P., Davis, H.P., Edwards, E.J., Collins, M.J., Morales, N.B., Boettcher, A., Singh, D.P. and Walker, R.R.(2011) Rootstock breeding and development for Australian conditions: Update on current work. In. Proceedings ASVO Below ground Seminar, Mildura,July 28-29th 2011 (In press).

Dry, N. (2007) Grapevine rootstocks: selection and management for South Australian vineyards. (Lythrum Press in association with Phylloxera and Grape Industry Board of South Australia: Adelaide).

Jones, T.H., Cullis, B.R., Clingeleffer, P.R. and Ruhl, E.H (2009) Effects of novel hybrid and traditional rootstocks on vigour and yield components of Shiraz grapes. Australian Journal of Grape and Wine Research 15, 284-292.

Korosi, G.A., Powell, K.S., Clingeleffer, P.R., Smith, B., Walker, R.R. and Wood, J. (2011). New hybrid rootstock resistance screening for Phylloxera under laboratory conditions. Acta Horticulturae. 904:53-58

Smith, B.P., Clingeleffer, P.R., Morales, N. B, Thomas, M.R and Walker R.R. (2010) Development of Australian rootstocks with nematode root-knot resistance and low potassium transport. In: Proc. 10th International Conference on Grapevine Breeding and Genetics, Geneva, New York (in press).

Smith, B.P., Clingeleffer, P.R., Morales, N. B, Thomas, M.R and Walker R.R. (2010) Development of rootstocks for Australian conditions. In: Proceedings 14th Australian Wine Industry Technical Conference. Adelaide July 3-8 2010.

Smith, B.P. (2011) History of Grapevine Propagation and Grafting in Australia (2011) In: Proceedings of the 21st International Geisenheim Conference on Grapevine propagation and Grafting (in press).

Smith, B.P., Wheal, M.S., Jones, T.H. Morales, N. B and Clingeleffer, P.R. (2011) Heritability of adventitious rooting of grapevine dormant canes. Australian Journal of Grape and Wine Research (submitted).

Walker, R.R. and Clingeleffer, P.R. (2009) Rootstock attributes and selection for Australian conditions. Rootstock Symposium, American Society of Enology and Viticulture, Napa Valley, June 2009, Australian Viticulture13(4), 70-76.

Appendix 2: Intellectual property

The key IP issue relates to the development of new rootstocks for adoption by industry with PBR protection. An application seeking PBR protection for three low-medium vigour rootstocks was made in March 2005 with PBR granted in June 2009. The new rootstocks are Merbein 5489Ф, Merbein 5512Ф and Merbein 6262Ф. As part of this project they were grafted with Shiraz and established across a range of environments. This process was facilitated through licensing arrangements with commercial nurseries (see below). The stakeholders of this material are CSIRO, GWRDC and HAL Ltd.

Five new rootstocks with potential for release have been identified in this project based on their field performance under limited water supply, nematode tolerance and phylloxera tolerance and wine attributes. These selections have been provided to two of CSIRO's licensed nurseries under testing agreement to assess l propagation characteristics. .

Furthermore, 50 new rootstock selections with good rooting and grafting, appropriate vigour and drought tolerance have been identified. They have been grafted with Shiraz and established in a replicated field trial for assessment under well-watered and deficit irrigation regimes. In the longer term, a number of these selections may also be identified for commercial release through an established pathway.

Three nurseries (New South Wales, South Australia, and Western Australia)have been licensed to propagate and distribute grafted vines of Merbein 5489Ф, Merbein 5512Ф and Merbein 6262Ф. Mothervine plantings have been established in Victoria and South Australia.

Appendix 3: References

Aballay, E., P. Persson, and A. Martensson (2009) Plant-parasitic nematodes in Chilean vineyards. Nematropica 39, 85-97.

Anwar, S.A. and M.V. McKenry (2002) Penetration and development of *Meloidogyne arenaria* on two new grape rootstocks. J. Nem. 34, 143-145.

Bloodworth, P.J., W.B. Nesbitt, and K.R. Barker Resistance to root-knot nematodes in *Euvitis* x *Muscadinia* hybrids. Proceedings of the Third International Symposium of Grape Breeding, Davis, CA Dept. Vitic. and Enol., University of California Davis. 275-292.

Boubals, D. (1992) Au sujet de la rεsistance a l'anguillule de la vigne (*Meloidogynae* sp.) a travers le monde. Le Progres Agricole et Viticole Annee 109, 118.

Cain, D.W., M.V. McKenry, and R.E. Tarailo (1984) A new pathotype of root-knot nematode on grape rootstocks. J. Nem. 16, 207-208.

Chitambar, J.J. and D.J. Raski (1984) Reaction of grape rootstocks to *Pratylenchus vulnus* and *Meloidogyne* spp. J. Nem. 16, 166-170.

Cirami, R.M., M.G. McCarthy, and T. Glenn (1984) Comparison of the effect of rootstock on crop, juice and wine composition in a replanted nematode-infested Barossa Valley vineyard. Aust. J. Exp. Agric. Anim. Husb. 24, 283-289.

Clingeleffer, P. (1996) Rootstocks : a means to control scion pH, ion composition and spectral parameters and meet juice and wine quality specifications. In: Quality Management in Viticulture., Eds. R. Hamiliton and P.F. Hayes ASVO, Adelaide 22-25.

Clingeleffer, P. (2010) Plant management research-status, what it can offer to address the challenges and limitations. Aus. J. Grape and Wine Res. 16 (S1), 25-32.

Clingeleffer, P.R. (1996) Rootstocks: A means to control scion pH, ion composition and spectral parameters and meet juice and wine quality specifications. Proceedings of the Quality Management in Viticulture: ASVO Viticulture Seminar, Mildura Arts Centre, August 2 1996 ASVO.

Clingeleffer, P.R. (2007) Viticultural practices to moderate wine alcohol content. Proceedings of the Towards best practice through innovation in winery processing, Tanunda, South Australia ASVO, 37-39.

Clingeleffer, P.R., B.P. Smith, E.J. Edwards, M.J. Collins, N.B. Morales, H. Davis, S. Sykes, and R.R. Walker (2011) Industry puts low-medium vigour rootstocks to the test. Wine and Vit. J. 3, 72-76.

Cousins, P., D. Johnston, and S. Switras-Meyer USDA ARS research in grape rootstock breeding and genetics. Proceedings of the International Grapevine Phylloxera Symposium, Fremantle, Australia ISHS 51-58.

Cousins, P. and M. Lauver, Response to root-knot nematodes in a *Vitis monticola* hybrid population, in *XXVI International Horticultural Congress*, edited by P.L. Forsline, C. Fideghelli, H. Knuepffer, A. Meerow, J. Nienhus, K. Richards, A. Stoner, E. Thörn, A.F.C. Tombolato, and D. Williams. ACTA Horticultrae, Toronto, Canada, 2003, Vol. 623, 319-324.

Cousins, P., M. Lauver, and L. Boyden (2003) Genetic analysis of root-knot nematode resistance derived from *Vitis mustangensis*. Proceedings of the 8th International Conference on Grape Genetics and Breeding, Vols 1 and 2, 149-155.

Cousins, P. and M.A. Walker (2001) A technique for screening grape germplasm for resistance to *Meloidogyne incognita*. Plant Dis. 85, 1052-1054.

Cousins, P. and M.A. Walker (2002) Genetics of resistance to *Meloidogyne incognita* in crosses of grape rootstocks. Theor. and Appl. Gen. 105, 802-807.

Downton, W.J.S. (1977) Chloride accumulation in different species of grapevine. Scientia Horticulturae 7, 249-253.

Dry, N. (2007) Grapevine rootstocks: selection and management for South Australian vineyards. Lythrum Press in association with Phylloxera and Grape Industry Board of South Australia: Adelaide, South Australia.

El-Hamady, M.M. and H.P. Olmo (1966) Inherent growth regulation in rooting of Vitis. Unpublished manuscript. Department of Viticulture and Enology, University of California, Davis.

Farquhar, G.D., M.H. O'Leary, and J.A. Berry (1982) On the Relationship Between Carbon Isotope Discrimination and the Intercellular Carbon Dioxide Concentration in Leaves. Aust. J. Plant. Path. 9, 121-137.

Ferris, H. and M.V. McKenry (1974) Seasonal fluctuation in the spatial distribution of nematode poplulations in a California vineyard. J. Nem. 6, 203-210.

Ferris, H., S.M. Schneider, and M.C. Stuth (1982) Probability of penetration and infection by root-knot nematode, *Meloidogyne arenaria,* in grape cultivars. Am. J. Enol. Vitic. 33, 31-35.

Firoozabady, E. and H.P. Olmo (1982) The heritability of resistance to root-knot nematode (*Meloidogyne incognita acrita* Chit) in *Vitis vinifera* x *V. rotundifolia* hybrid derivatives. Vitis 21, 136-144.

Galet, P. (1998) Grape Varieties and Rootstock Varieties. Oenoplurimedia: Chaintre, France.

Gibberd, M.R., R.R. Walker, D.H. Blackmore, and A.G. Condon (2001) Transpiration efficiency and carbon isotope discrimination of grapevines grown under well-watered conditions in either glasshouse of vineyard. Aust. J. Grape and Wine Res. 3, 110-117.

Gilmour, A.R., B.J. Gogel, B.R. Cullis, and R. Thompson, (2009) ASReml User Guide Release 3.0. V.I. Ltd Hempstead, UK, 2009.

Gong, H., Blackmore, D., Clingeleffer, P., Sykes, S., Jha, D., Tester, M., and Walker, R. (2011) Contrast in chloride exclusion between two grapevine genotypes and its variation in their hybrid progeny. J. Exp. Bot. 62, 989-999.

Granett, J., A.C. Gohenn, L.A. Linder, and J.J. White (1987) Evaluation of grape rootstocks for resistance to type A and B grape phylloxera. Amer. J. Enol. Vit. 38, 298-300.

Granett, J., A. Walker, J. de Benedictis, G. Fong, H. Lin, and E. Weber (1996) California grape phylloxera more variable than expected. California Agric. 50, 9-13.

Hale, C.R. and C.J. Brien (1978) Influence of Salt Creek rootstocks on composition and quality of Shiraz grapes and wine. Vitis 17, 139-146.

Harris, A.R. (1984) Distribution of plant parasitic nematodes in horticultural crops in the Gol Gol, Mildura, Nangiloc, Robinvale and Swan Hill districts. Aus. Plant Path. 13, 52-55.

Hugall, A., C. Mortiz, J. Stanton, and D.R. Wolstenholme (1994) Low, but strongly structured mitochondrial DNA diverstiy in root knot nematodes (*Meloidogyne*). Genetics 136, 903-912.

Johnston, B., T. Healy, J. I'ons, and M. McGregor (1992) Rural research - The pay-off. Occasional paper no. 7.

Kamiloglu, Ö.a.T., S. (1997) A comparison of three methods producing grafted vines. Acta Horticulturae 441, 395-398.

Keeley, K., Preece, J.E., Taylor, B.H., and Dami, I.E. (2004) Effects of high auxin concentrations, cold storage, and cane position on improved rooting of *Vitis aestivalis* (Michx.) 'Norton' cuttings. American Journal of Enology and Viticulture 55, 265-268.

Kingston, K.B., K.S. Powell, and P.D. Cooper (2007) Grape phylloxera external morphology observations under scanning electron microscopy. ACTA Hort. 733, 107-114.

Korosi, G.A., Powell, K.S., Clingeleffer, P.R., Smith, B., Walker, R.R. and Wood, J. (2011). New hybrid rootstock resistance screening for Phylloxera under laboratory conditions. Acta Horticulturae. 904:53-58

Lider, L.A. (1954) Inheritance of resistance to a root-knot nematode (*Meloidogyne incognita* var. *acrita* Chitwood) in *Vitis* spp. Proc. Helminthol Soc. Wash. 21, 53-60.

Loubser, J. (1988) Occurrence and pathogenicity of root-knot nematodes (*Meloidogyne* species) in South African vineyards. S. African J. of Enol. Vitic. 9, 21-27.

Lowe, K.M. and M.A. Walker (2006) Genetic linkage map of the interspecific grape rootstock cross Ramsey (*Vitis champinii*) x Riparia Gloire (*Vitis riparia*). Theoretical and Applied Genetics 112, 1582-1592.

McKenry, M.V. and J.O. Kretsch, It is a long road from the finding of a new rootstock to the replacement of a soil fumigant, in Intl. Research Conference on Methyl Bromide Alternatives and Emissions Reductions San Diego, CA, 1995. 33, 1-3.

McLeod, R.W. and M.Y. Gendy (1996) Seasonal behaviour of *Meloidogyne javanica* and host race identity of rootknot nematodes and citrus nematode, T*ylenchulus semipenetrans*, associated with grapevines. Aust. Grapegrower & Winemaker, Adelaide.

McLeod, R.W. and C.C. Steel (1999) Rootknot nematodes from vineyards and comparisons between crop species as hosts for *Meloidogyne* spp. Aus. J. Grape and Wine Res. 5, 104-108.

Mortensen, E. (1938) Nursery tests with grape rootstocks. Proc. Am. Soc. Hort. Sci. 36, 153-157.

Nicol, J.M., G.R. Stirling, B.J. Rose, P. May, and R. Van Heeswijck (1999) Impact of nematodes of grapevine growth and productivity: current knowledge and future directions, with special reference to Australian viticulture. Aus. J. Grape and Wine Res. 5, 109-127.

Quader, M., I.T. Riley, and G.E. Walker (2001) Distribution pattern of root-knot nematodes (*Meloidogyne* spp.) in South Australian vineyards. Australas. Plant Pathol. 30, 357-360.

Ruhl, E.H. (1989) Uptake and distribution of potassium by grapevine rootstocks and its implication for grape juice pH of scion varieties. Aus. J. Exp. Ag. 29, 707-712.

Ruhl, E.H. (1989) Uptake and distribution of potassium by grapevine rootstocks and its implication for grape juice pH of scion varieties. Aus. J. Exp. Ag. Ani. Hus. 29, 707-712.

Ruhl, E.H. (1990) Better rootstocks for wine grape production. The Aust. Grapegrower and Winemaker 316, 113-115.

Ruhl, E.H., P.R. Clingeleffer, P.R. Nicholas, R.M. Cirami, M.G. McCarthy, and J.R. Whiting (1988) Effect of rootstocks on berry weight and ph, mineral-content and organic-acid concentration of grape juice of some wine varieties. Australian Journal of Experimental Agriculture 28, 119-125.

Sauer, M.R. (1962) Distribution of plant parasitic nematodes in irrigated vineyards at Merbein and Robinvale. Aus. J. Exp. Ag. Ani. Hus. 2, 8-11.

Sauer, M.R. (1972) Rootstock trials for sultana grapes on light textured soils. Aus. J. Exp. Ag. Ani. Hus. 12, 107-111.

Smith, B.P. (2010) Genetic and molecular mapping studies on a population derived from *Vitis vinifera* x *Muscadinia rotundifolia* and genetic diversity of wild *Muscadinia rotundifolia* Ph.D. Genetics, Department of Viticulture and Enology, University of California Davis, Davis.

Snyder, E. (1939) Susceptibility of grape rootstocks to root knot nematode. USDA Circular 405.

Stirling, G.R. (1976) Distribution of plant parasitic nematodes in South Australian vineyards. Aust. J. Exp. Agric. Anim. Husb. 16, 588-591.

Stirling, G.R. and R.M. Cirami (1984) Resistance and tolerance of grape rootstocks to South Australian populations of root-knot nematode. Aust. J. Expr. Agric. Anim. Husb. 24, 277-282.

Terlidou, M.C. (1974) Effect of root-knot nematode *Meloidogyne javanica* (Treub) Chitwood in vine nurseries (m. franz. Zus.). Vitis 12, 316-319.

Tregeagle, J.M., J.M. Tisdall, D.H. Blackmore, and R.R. Walker (2006) A diminished capacity for chloride exclusion by grapevine rootstocks following long-term saline irrigation in an inland versus a coastal region of Australia. Aust. J. of Grape and Wine Res. 12, 178-191.

Umina, P.A., A.M. Corrie, K.S. Herbert, V.L. White, K.S. Powell, and A.A. Hoffmann (2007) The use of DNA markers for pest management – clonal lineages and population biology of grape phylloxera. ACTA Hort. 733, 183-195.

Viala, P.a.R., L. (1901) American Vines: Their Adaptation, Culture, Grafting, and Propagation. Robert S Brain: Melbourne, Australia.

Visscher, P.M., N.R. Wray, and W.G. Hill (2008) Heritability in the genomics era - Concepts and misconceptions. Nat. Rev. Genet. 9, 255-266.

Walker, G.E. (1997) Ramsey is not resistant to all local populations of root knot nematode. Aust. Grapegrower and Winemaker. 402A, 113-115.

Walker, G.E. (2009) Resistance of some commonly used rootstocks to root-knot nematode: The problem of rootstock selection. Aust. & N.Z. Grapegrower and Winemaker. 547, 16-23.

Walker, G.E. and C. Cox (2011) Resistance of RS-3 and RS-9 rootstocks to local nematode populations. Aus. and N.Z. Grapegrower and Winemaker 567, 35-37.

Walker, G.E. and G.R. Stirling (2008) Plant-parasitic nematodes in Australian viticulture: Key pests, current management practices and opportunities for future improvements. Australas. Plant Pathol. 37, 268-278.

Walker, M.A., P.R. Clingeleffer, G.H. Kerridge, E.H. Ruhl, P.R. Nicholas, and D.H. Blackmore (1998) Effects of the rootstock Ramsey (*Vitis champini*) on ion and organic acid composition of grapes and wine, and on wine spectral characteristics. Australian Journal of Grape and Wine Research 4, 100-110.

Walker, M.A., H. Ferris, and M. Eyre (1994) Resistance in *Vitis* and *Muscadinia* species to *Meloidogyne incognita*. Plant Dis. 78, 1055-1058.

Walker, R. and P. Clingeleffer (2009) Rootstock attributes and selection for Australian conditions. Am. J. Enol. Vit. 13, 70-76.

Walker, R.R., D.H. Blackmore, P.R. Clingeleffer, and R.L. Correll (2002) Rootstock effects on salt tolerance of irrigated field-grown grapevines (Vitis vinifera L. cv. Sultana). 1. Yield and vigour inter-relationships. Australian Journal of Grape and Wine Research 8, 3-14.

Walker, R.R., M.R. Gibbered, and R.M. Stevens Improving vineyard water use efficiency. . Proceedings of the 12th Australian Wine Industry Technical Conference, Melbourne, Australia ASVO. 152-158.

Williams, P.L. and A.J. Antcliff (1984) Successful propagation of *Vitis Berlandieri* and *Vitis cinerea* from hardwood cuttings. Am. J. Enol. Vitic. 35, 75-76.

Zhang, J., L. Hausmann, R. Eibach, L. Welter, R. Töpfer, and E. Zyprian (2009) A framework map from grapevine V3125 (Vitis vinifera ‘Schiava grossa’×‘Riesling’) × rootstock cultivar ‘Börner’ (Vitis riparia×Vitis cinerea) to localize genetic determinants of phylloxera root resistance. Theo. and Appl.Gen. 119, 1039-1051.

Zijlstra, C., D.T.H.M. Donkers-Venne, and M. Fargette (2000) Identification of *Meloidogyne incognita, M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays. Nematology 2, 847-853.

Appendix 4: Staff

Mr. Peter Clingeleffer	Project supervisor
Dr. Tim Jones	Chief Investigator (Postdoctoral fellow March, 2006 - January 2008)
Ms. Hilary Davis	Research officer (February 2008 –November 2008)
Dr. Brady Smith	Chief Investigator (November 2008- June 2011)
Ms. Norma Morales	Technical support
Dr. Steve Sykes	Plant breeder and PBR officer
Dr. Rob Walker	Manager

Appendix 5: Budget reconciliation

Sent separately to GWRDC

Appendix 6 Performance of the CSIRO low-medium vigour rootstocks

Clingeleffer, P.R., Smith, B.P., Edwards, E., Collins, M., Morales, N.B., Davis, H., Sykes, S., and Walker, R.R. (2011b) Industry puts low-medium vigour rootstocks to the test. Wine and Viticulture Journal 3, 72-76.

Performance of the CSIRO low-medium vigour rootstocks (Merbein 5489Φ, Merbein 5512Φ and Merbein 6262Φ)

Peter Clingeleffer[1], Brady Smith[1], Everard Edwards[1], Marissa Collins[1], Norma Morales[1], Hilary Davis[2], Steve Sykes[2] and Rob Walker[1]

[1] CSIRO Plant Industry, Waite Campus, Hartley Grove, Urrbrae, South Australia 5064 and [2] CSIRO Plant Industry, Merbein, Victoria 3505.

Background.

Three low-medium vigour rootstocks, developed from the CSIRO grapevine rootstock breeding and evaluation program (Clingeleffer 2008, 2010), were released for larger scale industry evaluation in the mid 2000s. The purpose of wider industry evaluation was to assess their suitability in regional environments with varying abiotic stresses (i.e. temperature, water and salt) and with varying levels of soil-borne pest pressures (e.g. strains of nematodes and phylloxera). An application seeking PBR protection for the rootstocks was made in March 2005 with PBR granted in June 2009. The new rootstocks, Merbein 5489Φ, Merbein 5512Φ and Merbein 6262Φ are described in Dry (2007).

The rootstocks were selected based on their performance in a long term trial planted in a replant situation in a nematode infested, sandy loam soil at Koorlong, near Mildura, Victoria, in 1989. The trial involved 55 hybrid rootstocks and five standard rootstocks, all grafted with Shiraz. The hybrids include selections identified for their low potassium (K) uptake (Clingeleffer 1996). Low K uptake has been a focus of Australian rootstock breeding because of its association with high pH in berries and subsequent impacts on wine quality (Ruhl 1989). A key criterion in the selection of the three rootstocks was conferred vigour assessed as pruning weight. Other criteria included berry composition, particularly K concentration and pH, wine quality attributes (e.g. spectral and sensory properties) and tolerance of the soil borne pests, root-knot nematodes and phylloxera.

Key performance characteristics of the new low-medium vigour rootstocks

Key performance characteristics of the three rootstocks, measured over 3 seasons (2001-2003), are presented in Table 1. Compared to Ramsey and 1103 Paulsen, the new rootstocks grafted with Shiraz had 2-3 fold lower pruning weights, consistent with their low-medium vigour classification. Despite having lower vigour than 1103 Paulsen, Shiraz yield on Merbein 5489Φ was as good as 1103 Paulsen, whereas yields of Merbein 5512Φ and Merbein 6262Φ were lower by 16 % and 25%, respectively. Compared to the standard rootstocks, the new rootstocks produced lower pH in the must and enhanced wine quality attributes (i.e. brighter wines with lower hue, higher levels of colour and phenolics), despite being harvested at slightly lower maturities. Furthermore, significantly less addition of tartaric acid was required for pH adjustment during winemaking (Table 1). Further studies in season 2007 showed that improved fruit composition and enhanced wine quality attributes could be achieved with the new rootstocks across a range of soluble solids (22-26 ^{0}Brix), indicating potential for low alcohol wine production (Clingeleffer 2008). In contrast, high levels of sugar were required to achieve good wine colour density with the standard rootstocks, particularly Ramsey. For example, in the Koorlong trial in season 2007, a colour density of 4.4 a.u. was achieved around 22 ^{o}Brix with Merbein 5512Φ and Merbein 6262Φ rootstocks, compared to 25.5 ^{o}Brix with Ramsey.

Table 1. Vine vigour, must composition, yield per vine and wine characteristics of Shiraz, grafted on the three low-medium vigour rootstocks (Merbein 5489Ф, Merbein 5512Ф and Merbein 6262Ф) compared to grafting on the standard rootstocks, Ramsey and 1103 Paulsen, over 3 seasons (mean values, 2001-2003) at Koorlong. Values with different subscripts are significantly different (p=0.05).

Shiraz/ Rootstock	Vigour	Harvest			Wine				
	Prun. Wt. (kg)	Juice °Brix	Juice pH	Yield (kg)	Acid Added (g/L)	Wine pH	Colour Density (au)	Colour Hue	Total Phenolics (au)
1103 Paulsen	4.2_{c}	25.6_{d}	4.18_{d}	11.8_{b}	4.72_{d}	3.57_{b}	6.26_{ab}	0.61_{b}	53.8_{b}
Ramsey	4.3_{c}	25.2_{c}	4.25_{e}	16.7_{c}	5.95_{e}	3.58_{b}	5.84_{a}	0.59_{b}	49.5_{a}
Merbein 5489	2.0_{b}	24.3_{b}	3.83_{a}	13.3_{b}	1.90_{a}	3.49_{a}	7.56_{c}	0.54_{a}	60.9_{c}
Merbein 5512	1.5_{ab}	24.5_{b}	3.91_{b}	8.8_{a}	2.72_{b}	3.48_{a}	6.55_{b}	0.55_{a}	56.8_{b}
Merbein 6262	1.2_{a}	23.7_{a}	3.94_{b}	9.9_{a}	2.60_{ab}	3.51_{a}	8.17_{d}	0.53_{a}	62.4_{c}

The capacity of the rootstocks to exclude chloride and sodium is yet to be fully tested in regions with higher water and soil salinity levels. However, at the relatively low salinity Koorlong site in season 2010, Shiraz grafted on Merbein 5489Ф accumulated concentrations of chloride in laminae at harvest equivalent to Ramsey and 1103 Paulsen (mean of 0.18 % dry weight), whereas Merbein 5512Ф and Merbein 6262Ф had higher concentrations (0.33±0.13 and 0.42±0.06 % dry weight, respectively).

Tolerance of soil borne pests

Merbein 5489Ф and Merbein 5512Ф were phylloxera tolerant in screening trials conducted in Rutherglen by Dr Kevin Powell, DPI Victoria, using up to three strains of phylloxera. Merbein 5489Ф was tested and shown to be tolerant against the G1, G4 and G30 strains. Merbein 5512Ф was tested and shown to be tolerant against the G4 and G30 strains. Merbein 6262Ф showed moderate tolerance against the G4 and G30 strains, which indicates a risk of phylloxera developing on the roots, but at relatively low levels compared to *Vitis vinifera*.

Shiraz vines grafted to the new rootstocks and to the standards, Ramsey and 1103 Paulsen, established in a re-plant soil infested with nematodes, showed no ill effects over a period of 20 years. The ongoing existence of nematodes at this site has been confirmed in February 2011. In glasshouse screening trials, conducted prior to their release, the new rootstocks were tolerant to two strains of *Meloidogyne javanica*, the most common nematode in Australia and *Meloidogyne incognita*. For the new rootstocks, nematode galls and egg masses were either absent or observed at a low frequency in root systems compared to the Sultana control where they were spread throughout the root system. In a recent study, the rootstocks have also been screened in the glasshouse against a strain of *Meloidogyne javanica* sourced from a vineyard where growth problems associated with high nematode numbers had been observed with vines grafted on 1103 Paulsen, a problem also reported by Walker (2009). Merbein 5489Ф and Merbein 5512Ф and the control rootstock, 1103 Paulsen, did not show tolerance to the aggressive strain of *Meloidogyne javanica* whereas Merbein 6262Ф displayed a high level of tolerance.

Performance in seasons 2010 and 2011, i.e. under deficit and well watered conditions

Further studies have been undertaken with the aim to further investigate water use efficiency and drought tolerance of Merbein 5489Ф, Merbein 5512Ф and Merbein 6262Ф compared to standard rootstocks. Data has been collected from the Koorlong site over two very contrasting seasons, 2010 and 2011. In season 2010, which was hot and dry, the vines were converted to drip irrigation with a significant deficit applied over the season (i.e. total irrigation of 3.6 ML/ha). Prior to 2010, the vines had been irrigated with overhead sprinkler irrigation. The 2011 season was cool with a number of significant, record breaking rain events which reduced the ability to control soil moisture levels and produced significant levels of disease, including both downy and powdery mildew.

Under deficit conditions in season 2010 there was a significant reduction in vine growth (Figure 1) with much lower pruning weights across all rootstocks compared to the earlier data presented in Table 1. Ramsey had a 45% reduction in pruning weight (2.4 kg/vine) compared to a reduction of 70 % with 1103 Paulsen (1.3 kg/vine), 73 % with Merbein 5512Φ (0.4 kg/vine), 86% with Merbein 5489Φ (0.3 kg/vine) and 82% with Merbein 6262Φ (0.2 kg/vine). There was a 5-fold difference in peak leaf area index (LAI), ranging from 2.5 for Ramsey, 1.5 for 1103 Paulsen, 1.9 for Merbein 5512Φ, 1.6 for Merbein 5489Φ to 0.5 for Merbein 6262Φ. Similarly there was a 3.6 fold difference in yield, ranging from 20.7 kg/vine with Ramsey, 11.9 kg/vine for 1103 Paulsen, 6.4 kg/vine for Merbein 5512Φ, 6.3 kg/vine for Merbein 5489Φ to 3.3 kg/vine for Merbein 6262Φ. Compared to the data in Table 1, yields of Ramsey and 1103 Paulsen were maintained under the deficit treatment whereas they were reduced by 53%, 27% and 66% for Merbein 5489Φ, Merbein 5512Φ and Merbein 6262Φ, respectively.

Compared to season 2010, there were significant changes in vine performance under the well watered and cooler conditions in 2011. LAI values were higher for Ramsey (3.9), 1103 Paulsen (3.2) and Merbein 6262Φ (1.8) but similar to the previous season for Merbein 5489Φ (2.0) and Merbein 5512Φ (1.9). Yields (kg/vine) were highest for 1103 Paulsen (16.2), followed by Merbein 5512Φ (15.3), Merbein 5489Φ (11.9), Ramsey (11.2) and Merbein 6262Φ(9.1). These responses were associated with differences in bunch number per vine and bunch weight. Bunch numbers were highest for 1103 Paulsen (164) followed by Merbein 5512Φ (156), Ramsey (148), Merbein 6262Φ (117) and Merbein 5489Φ (115). Merbein 5489Φ had the largest bunches (107 g) followed by 1103 Paulsen (100g), Merbein 5512Φ (97 g), Merbein 6262Φ (81 g) and Ramsey (76 g). It was noted that the high vigour Ramsey vines had significant losses due to downy mildew infection of bunches compared to the other rootstocks. Furthermore, measurements of photosynthesis indicate a loss of leaf function of Shiraz grafted on Ramsey at veraison, which may also be attributed to the effects of disease.

Overall the yield and growth characteristics indicate that the new low-moderate vigour rootstocks were less robust under deficit irrigation treatments following conversion to drip irrigation. These responses are most likely associated with development of smaller and shallower root systems than the standard rootstocks (Clingeleffer and Wheal, unpublished data). However, under well watered, cooler conditions, vine growth and yield of the new rootstocks was restored to expected levels.

Progress with industry adoption

Three nurseries, K.C. Vines and Rootstocks in Sunraysia, Glenavon Nursery in South Australia and ARM Nursery in Western Australia have been licensed to propagate and distribute grafted vines of Merbein 5489Φ, Merbein 5512Φ and Merbein 6262Φ. Mother vine plantings of the new rootstocks have been established in Victoria and South Australia.

The new rootstocks, grafted with Shiraz have been embedded in new commercial plantings by companies in a number of regions. This material was developed through rapid propagation and green grafting under glasshouse conditions by a commercial nursery, due to the limited supply of propagation material prior to establishment of mother-vine plantings. The performance of the new rootstocks in these commercial sites has been monitored to assess their adaptability in the different regions. The first commercial planting of the new rootstocks, grafted with Shiraz and located near Griffith, New South Wales, produced a significant crop in season 2010 with two further plantings in Sunraysia and Padthaway bearing in 2011.

The Griffith site involves a replicated trial comparison of Merbein 5489Φ, Merbein 5512Φ and own roots. In the hot 2010 season , Merbein 5489Φ and Merbein 5512Φ had similar yields (mean of 9.0 kg/vine) compared to own-rooted vines (8.6 kg/vine) associated with more but smaller bunches and smaller berries (i.e. 1.15, 1.20, 1.26 g for Merbein 5489Φ, Merbein 5512Φ and own roots, respectively). The new rootstocks, when compared to own roots, had slightly lower levels of total soluble solids (i.e. 21.4, 20.8, 22.0 ^{o}Brix, respectively), similar pH values (mean 4.0) and in the case of Merbein 5489Φ, higher levels of titratable acidity (i.e. 5.97, 4.83, 4.94 g/L, respectively). In the cooler and wet 2011 season, samples were again collected for fruit composition but yield data were not recorded. In 2011, a difficult season to achieve adequate levels of maturity, both Merbein 5489Φ and Merbein 5512Φ had higher levels of total soluble solids than own roots (20.9, 20.7 and 19.4 ^{o}Brix, respectively) with similar pH values (mean 4.0), titratable acidity levels (mean 4.7 g/L) and berry weight (mean 1.71 g). Leaf area measurements recorded at harvest indicated that Merbein 5489Φ, Merbein 5512Φ and own roots had similar size canopies (i.e. mean leaf area index values of 2.1).

The Sunraysia site involves a replicated trial comparison of Shiraz grafted on Merbein 5489Ф, Merbein 5512Ф and Merbein 6262Ф and 140 Ruggeri. At harvest in season 2011, the new rootstocks had smaller canopies than 140 Ruggeri (i.e. leaf area index values of 2.3, 2.3, 2.1 and 3.3 for Merbein 5489Ф, Merbein 5512Ф, Merbein 6262Ф and 140 Ruggeri, respectively). Yields of the new rootstocks were higher than 140 Ruggeri (i.e. 17.1, 17.3, 16.6 and 14.1 kg/vine, respectively) associated with higher bunch numbers (i.e. 103, 117, 112 and 94 bunches per vine, respectively) as differences in bunch weight (mean 160 g) and berry weight (mean 1.65 g) were small. Compared to 140 Ruggeri, fruit maturity levels were slightly delayed (i.e. 20.1, 21.0, 21.2 and 21.8 ^{o}Brix, respectively) with lower pH values (3.81, 3.90, 4.01 and 4.04, respectively) and similar levels of titratable acidity (mean 3.55g/L). Furthermore, the calculated yield to leaf area ratio of around 7.5 for the new rootstocks and 4.3 for 140 Ruggeri indicates that Shiraz grafted on the new rootstocks was more efficient in regard to vine growth and capacity and crop water use, determined as the crop water use index (Walker et al. 2005)

The Padthaway site involves comparisons of Shiraz grafted with the 3 new rootstocks Merbein 5489Ф, Merbein 5512Ф, Merbein 6262Ф with a number of standard rootstocks (i.e. Ramsey, 1103 Paulsen, 110 Richter and 140 Ruggeri). The vines were established by rapid glasshouse-based propagation, and once lignified, bare-rooted vines were heat-treated, which is a plant health requirement prior to transport from Victoria to South Australia. While establishment has been slow, sufficient reasonably mature vines of Merbein 5489Ф and Merbein 5512Ф were available for an initial comparative study with the other rootstocks in season 2011. Ramsey, 110 Richter and 140 Ruggeri had the highest yield (mean 8.7 kg/vine), followed by 101-14 (7.5kg/vine) and 1103 Paulsen (6.7 kg/vine) with lowest yields produced by Merbein 5489Ф and Merbein 5512Ф (3.2 and 5.0 kg/vine, respectively). Berry weights were highest on Ramsey (1.58 g) and smallest on 101-14 and 1103 Paulsen (1.41 and 1.46 g, respectively) with the other rootstocks, including Merbein 5489Ф and Merbein 5512Ф having intermediate values (1.5 g). Maturity levels were the most advanced with Merbein 5489Ф (23 ^{o}Brix), followed by Merbein 5512Ф (22.5) and Ramsey (22.0 ^{o}Brix) with maturation of the other rootstocks delayed (mean 21.5 ^{o}Brix). There was no rootstock effect on pH (3.55) or titratable acidity (6.0 g/L).

Conclusions

Overall, the results are indicating site and season effects on the relative performance of the rootstocks, confirming earlier research with standard rootstocks that demonstrates significant genotype x environment interactions (S.R. Sykes et al, unpublished data). Of particular note in the Griffith trial was the observed similarity in vine performance and fruit composition between Shiraz on own roots and Shiraz on Merbein 5489Ф and Merbein 5512Ф. Furthermore, compared to the standard high vigour rootstocks such as Ramsey and 1103 Paulsen, the lower conferred vine vigour of the new rootstocks is leading to both production and fruit composition benefits. The data are tending to support earlier data on improved crop water use index, and although preliminary, there is evidence of improved fruit composition and wine quality attributes, including at lower alcohol levels.

Studies to assess the adaptability of the rootstocks in various locations and regions with various scion varieties and their tolerance of different pests and diseases will be ongoing as the new rootstocks become more widely distributed. Given that different levels of tolerance have been found with different nematode populations, it is likely that the new rootstocks will not be suited to all conditions, as has also been found with other rootstocks, including recent introductions from the USA (Walker 2009, Walker and Cox 2011). Given that the rootstocks grafted with Shiraz vary in vigour and yield to pruning weight ratio, different management strategies will need to be considered in order to obtain best performance in specific situations, particularly under conditions of limited water supply.

Adoption of these rootstocks may facilitate integrated approaches to vine management involving high density plantings and low-input, lighter pruning systems to improve productivity (yield /ha) and water use efficiency, enhance wine quality attributes and reduce production costs, not only in the warmer regions but in cool climates where high input, vertical shoot positioned systems are used (Clingeleffer 2010).

Acknowledgements

The Grape and Wine Research and Development Corporation is acknowledged for its support of this research. We also acknowledge the support of the various wine companies for access to plantings to obtain the comparative data reported here.

References

Clingeleffer (1996) Rootstocks: a means to control pH, ion composition and spectral parameters and meet juice and wine quality specifications. In: Hamilton, R., Hayes, PF. Editors. Quality management in the viticulture. Mildura, Vic. Australia, S.Aust.:Australian Society of Viticulture and Oenology, 1996, pp. 22-25.

Clingeleffer, P.R. (2008) Viticultural practices to moderate wine alcohol content. In: Allen, M., Cameron, W., Johnstone, R.S. and Pattison P., editors. Towards best practice through innovation in winery processing : Brenton Langbein Theatre, Tanunda, South Australia, Wednesday, 17 October 2007. Adelaide: Australian Society of Viticulture and Oenology, pp. 37-39.

Clingeleffer, P.R. (2010) Plant management research-status, what it can offer to address the challenges and limitations. In: Proceedings Eighth International Symposium on Grapevine Physiology and Biotechnology. Adelaide, South Australia: Australian Society of Viticulture and Oenology. Australian Journal of Grape and Wine Research, **16**(S1), 25-32.

Dry, N. (2007) Grapevine rootstocks: selection and management for South Australian vineyards. (Lythrum Press in association with Phylloxera and Grape Industry Board of South Australia: Adelaide).

Ruhl, E.H. (1989) Uptake and distribution of potassium by grapevine rootstocks and its implication for grape juice pH of scion varieties. Australian Journal of Experimental Agriculture **29**, 707-712.

Walker, G.E. (2009) Resistance of some commonly used rootstocks to root-knot nematode: the problem of rootstock selection. Australian and New Zealand Grapegrower and Winemaker, **547**, 16-22.

Walker, G.E. and Cox, C. (2011) Resistance of RS-3 and RS-9 rootstock s to local nematode populations. Australian and New Zealand Grapegrower and Winemaker, **567**, 35-36.

Walker, R.R., Gibberd, M.R. and Stevens, R.M. (2005). Improving vineyard water use efficiency. In: Blair, R.J., Williams, P.J., Pretorius, I.S., editors. Proceedings of the 12th Australian Wine Industry Technical Conference, Melbourne, Vic.: Australian Wine Industry Technical Conference Inc., Urrbrae, S. Australia, pp.152-158.



Figure 1. Canopy growth of Ramsey (left), Merbein 5512 (middle) and Merbein 6262 (right) managed with a deficit irrigation, season 2010.