Grape Juice Composition and Wine Quality from Salt Excluding Rootstocks and Characterisation of the Chloride Exclusion Mechanism



FINAL REPORT to
GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **CSP 06/05**

Principal Investigator: **Dr Rob Walker**

Research Organisation: **CSIRO Plant Industry**

Date: **30 September 2010**

Title: Grape Juice Composition and Wine Quality from Salt Excluding Rootstocks and Characterisation of the Chloride Exclusion Mechanism

Authors: [1]Rob Walker, [1]Haijun Gong, [1]Peter Clingeleffer, [1]Deidre Blackmore, [2]Mark Tester and [2]Deepa Jha

[1]CSRO Plant Industry, Waite Campus, Adelaide
[2]Australian Centre for Plant Functional Genomics, Waite Campus Adelaide

Publisher: CSIRO Plant Industry

Copyright and Disclaimer:
© 2008 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.

Important Disclaimer:
CSIRO 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) exclude 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.

Acknowledgements: We thank the following people for important contributions to this project: Peter Rogers (Provisor/CSIRO) for winemaking, Briony Liebich (Provisor/SARDI) for wine sensory evaluation, Dr Mandy Walker for guidance with work to investigate presence of candidate Cl^- transporter genes in grapevines and studies on expression levels in rootstock genotypes differing in salt exclusion capacity, staff of two cooperating Australian wine companies for assistance with field assessments, and David Emanuelli for occasional assistance with collection of field data. We acknowledge also the assistance of Tapas Biswas and Tim Pitt for contribution and installation of Solusamplers in the Chardonnay study (site 2), and to Dr Ray Correll for assistance with analyzing these data.

Table of Contents:

1. Abstract: 1

2. Executive summary: 1

3. Background: 4

4. Project Aims and Performance Targets: 6

4.1 Projects Aims: 6
4.2 Outputs and performance targets: 6

5. Methods: 9

5.1. Yield, laminae, juice and wine ion concentrations, composition and sensory qualities of wine of Chardonnay and Shiraz on own roots and a range of rootstocks in a saline environment: 9
5.2. Effects of salinity on inorganic/organic anion inter-relationships in grape berries: 12
5.3. Variation for Cl^- and Na^+ exclusion in a family from a cross between grapevine rootstocks K 51-40 and 140 Ruggeri: 14
5.4. Characterisation of the Cl^- exclusion mechanism: 15
5.5. Assessment of candidate Cl^- transporter genes (collaboration with Australian Centre for Plant Functional Genomics, ACPFG): 17

6. Results: 18

6.1 Yield, laminae, juice and wine ion concentrations, composition and sensory qualities of wine of Chardonnay and Shiraz on own roots and on a range of rootstocks in a saline environment: 18
6.2 Effects of salinity on inorganic/organic anion inter-relationships in grape berries: 39
6.3 Variation for Cl^- and Na^+ exclusion in a family from a cross between grapevine rootstocks K 51-40 and 140 Ruggeri: 42
6.4 Characterisation of the Cl^- exclusion mechanism: 46
6.5 Assessment of candidate Cl^- transporter genes (collaboration with Australian Centre for Plant Functional Genomics, ACPFG): 49

7. Discussion: 51

7.1 Yield, laminae, juice and wine ion concentrations, composition and sensory qualities of wine of Chardonnay and Shiraz on own roots and on a range of rootstocks in a saline environment: 51
7.2 Effects of salinity on inorganic/organic anion inter-relationships in grape berries: 53
7.3 Variation for Cl^- and Na^+ exclusion in a family from a cross between grapevine rootstocks K 51-40 and 140 Ruggeri: 54
7.4 Characterisation of the Cl^- exclusion mechanism: 55
7.5 Candidate Cl^- transporter genes involved in Cl^- exclusion: 56
7.6 General: 57

8. Outcomes/Conclusions: 58

9. Recommendations: 59

10. Appendix 1: Communication: 60

10.1 Presentations 60
10.2 Conference Proceedings 60
10.3 Conference Posters 61
10.4 Industry Journal Articles 61
10.5 Peer Reviewed Journal Papers 61
10.6 General Communications 61

11. Appendix 2: Intellectual Property and/or valuable information arising from the research: 62

12. **Appendix 3: References cited in the report** **62**

13. **Appendix 4: Staff (A list of all staff that have been engaged on the Project).** **65**

14. **Appendix 5: Budget reconciliation** **66**

1. Abstract:

Yield, juice and wine composition and wine sensory qualities of Chardonnay and Shiraz on own roots and a range of rootstocks in a saline environment at Padthaway, South Australia, were assessed, and the mechanism of chloride (Cl^-) exclusion in grapevines was investigated.

Rootstocks provided few benefits relative to own roots for Chardonnay, with similar yields, or lower yield in the case of 101-14, and wines that were assessed to have sensory profiles generally similar to those from own roots. Grape juice and wine Cl^- concentrations from grafted vines were lower than for own-rooted vines. Rootstocks 140 Ruggeri and 1103 Paulsen yielded better than own-rooted Shiraz, but of the two, only 140 Ruggeri significantly reduced wine Cl^- and sodium (Na^+) concentrations. With Shiraz, rootstocks Ramsey, 1103 Paulsen and 140 Ruggeri resulted in lower wine colour density, total and ionized anthocyanins than own-rooted vines and vines grafted with Schwarzmann and 101-14 rootstocks. Rootstocks also changed the wine sensory profile relative to own-rooted vines. Salty taste in Shiraz wines was not consistently detected for wines having Cl^- in the range 265 to 854 mg/L.

Roots of good and poor Cl^- excluders were shown to have similar rates of Cl^- uptake and to transport Cl^- primarily in the root symplast. Good Cl^- excluders were shown to restrict entry of Cl^- to xylem, leading in some cases to build-up of Cl^- in roots. Cl^- exclusion in a 140 Ruggeri x K 51-40 hybrid family is controlled by more than one gene. Candidate Cl^- transporter genes were assessed, but to date, none have been strongly linked with Cl^- exclusion.

2. Executive summary:

Some Australian viticultural regions experience problems associated with high salinity in irrigation water, which in turn can lead to high soil salinities and consequent impacts on vine performance and wine quality. The Australian Food Standards Code currently imposes a limit of 607 mg/L of Cl^- in wine. Whilst there is no current limit for Na^+ in Australian wine, the maximum permissible levels in wine vary widely among countries, ranging from 60 mg/kg (Switzerland) to 500 mg/L (Canada). Rootstocks with capacity for salt exclusion and for quality wine production in a saline environment present a significant opportunity to mitigate the negative impacts of salinity in irrigation water and soil and to reduce Na^+ and Cl^- content of wines.

Previous studies on rootstock performance in areas affected by salinity have concentrated mainly on scion yield, basic grape and wine composition, pH , titratable acidity, concentrations of ions such as Cl^-, Na^+ and potassium (K^+), and for red grape varieties, wine spectral properties such as colour density and hue, and total and ionized anthocyanins. Very few studies, to our knowledge, and none that we are aware of in Australia, have focused not only on these parameters, but also on the sensory properties of wines from the grapevines. This broader range of information will assist grape growers in regions affected by salinity to make informed decisions on rootstock choice.

For Chardonnay, we used two sites to investigate rootstock performance. Rootstocks were Ramsey, 1103 Paulsen, 140 Ruggeri, Schwarzmann and 101-14. One was a site established in 1992 and one was a more recent site established in 2003. At the site with older vines, there was no significant difference between yields of grapevines on own

roots (12.1 kg/vine) and on Ramsey and 1103 Paulsen rootstocks (11.7 to 13.2 kg/vine). Yield of Chardonnay on 140 Ruggeri rootstock (10.5 kg/vine) was lower than that of Ramsey (13.2 kg/vine), but not significantly different from own roots and 1103 Paulsen (11.7 to 12.1 kg/vine). Mean Chardonnay grape juice Cl^- concentrations were less than 100 mg/L, but mean grape juice Na^+ concentrations reached 100-135 mg/L for Ramsey, 140 Ruggeri and Schwarzmann. Difference testing of Chardonnay wines conducted in two of the three seasons revealed no significant differences between wines from grafted and own-rooted vines.

The second site with younger Chardonnay vines contained the same rootstocks as at the older site, but did not contain an own-rooted control. At this site, yields were not significantly different between the rootstocks (range 8.7 to 9.7 kg/vine). Grape juice Cl^- and Na^+ concentrations were all much lower (less than 25 mg/L for Cl^- and less than 22 mg/L for Na^+) than those recorded for Chardonnay vines at the longer established site.

Shiraz on 140 Ruggeri and 1103 Paulsen produced best yields (6.5 to 7.2 kg/vine). Grape juice Cl^- concentrations were highest in vines grafted to 1103 Paulsen (176.4 mg/L), followed by vines on own roots (136.1 mg/L) and Ramsey (132.1 mg/L), with 140 Ruggeri the lowest (52.8 mg/L). Grape juice Na^+ concentrations were also highest in 1103 Paulsen (112.8 mg/L) and lowest in 140 Ruggeri (52.8 mg/L). Across the three seasons, colour density and total and ionized anthocyanins were similar for wines made from vines on own roots, Schwarzmann and 101-14 rootstocks, which were all significantly higher than wines made from vines on Ramsey, 1103 Paulsen and 140 Ruggeri rootstocks. Wines made from vines on 1103 Paulsen and 140 Ruggeri had the lowest total phenolics and ionized anthocyanins. Difference testing revealed differences between wine made from vines on own roots and from all grafted vines. In 2009, the wines from own roots, 101-14 and Schwarzmann rootstocks were all perceived with the highest dark berry aroma and higher stewed/dried fruit flavours. The own roots and 101-14 rootstock treatments also showed more persistent fruit flavours.

The fermentation process affected Cl^- concentrations more than Na^+ concentrations for both Chardonnay and Shiraz. For example, with Chardonnay, Cl^- and Na^+ concentrations increased 1.4-fold and 1.1-fold respectively, whereas with Shiraz, the respective increases were 2.2-fold and 1.25-fold respectively. These increases occurred mainly during the first few days of fermentation. Highest Chardonnay wine Cl^- and Na^+ concentrations were 127.5 mg/L (own roots) 101.6 mg/L (Ramsey), respectively. Highest Shiraz wine Cl^- and Na^+ concentrations were 383.0 mg/L (own roots) and 111.9 mg/L (1103 Paulsen), respectively. Free Na^+ concentrations (the excess of Na^+ over Cl^-) were in all cases less than 60 mg/L. Salty taste was not consistently detected in wine profiling studies. Moreover, in a trial conducted with Shiraz wines spiked with salt to give wine Cl^- concentrations in the range 265 to 854 mg/L, the trained panel could not consistently detect salty taste across the range of salt-spiked wines.

Partitioning studies of inorganic ions and organic acids between skin and pulp of mature grape berries from Chardonnay and Shiraz vines on own roots and grafted to Ramsey, 1103 Paulsen and 140 Ruggeri rootstocks showed that grape berry skin was a significant repository of ions. Concentrations of K^+, Cl^- and Na^+ in skin were on average 4.4, 10.8 and 2.0 times the concentrations in pulp. These differences implicated Cl^- in particular, but possibly also malate and tartrate as counter ions balancing the higher Na^+ and K^+ concentrations in skin cells. There was also a significant negative linear regression between malic acid and Cl^- concentration in the skin.

The higher malic acid and lower Cl^- concentarations in skin of most grafted Chardonnay and Shiraz vines may indicate competition for similar transporter proteins involved in loading into skins. Alternatively, higher salt concentrations in skins may be associated with accelerated malic acid catabolism.

Rootstock 140 Ruggeri was one of the best excluders of Cl^- in each of the rootstock trials. Flux studies, involving rooted leaves as a model plant system and using $^{36}Cl^-$ to compare 140 Ruggeri with the poor Cl^- excluder K 51-40, have shown similar uptake rates of Cl^- into roots of the two genotypes, but significantly lower root to shoot transport of $^{36}Cl^-$ in 140 Ruggeri. 140 Ruggeri also accumulated higher concentrations of Cl^- in roots than did K 51-40. Differences in Cl^- uptake between the genotypes could not be accounted for by apoplastic transport, indicating Cl^- uptake by grapevine roots primarily follows the symplastic pathway. This implicates Cl^- transporter proteins, most likely in the stele, in the Cl^- exclusion mechanism, but studies on a range of candidate Cl^- transporter genes have yet to identify a transporter whose expression levels correlate with the difference in Cl^- exclusion between 140 Ruggeri and K 51-40.

Our work investigating laminae and wood Cl^- and Na^+ concentrations in Shiraz vines pre- and post vine dormancy revealed that laminae Cl^- and Na^+ concentrations were generally of the order 10-fold higher than wood concentrations (when expressed on a dry weight basis). Furthermore, we found no evidence for a significant reduction of wood Cl^- or Na^+ concentrations in the period pre- to post dormancy.

The project has provided information that will be of assistance to grape growers who may be considering adopting rootstocks in regions affected by salinity, and in particular provides information on the performance of rootstocks from both a yield and wine quality perspective.

We acknowledge the in-kind contributions of the Australian wine companies that made available trial sites for the conduct of the research. In particular, the collection on a regular basis of data from 30 Solusamplers was a major undertaking by one of the companies. This work has demonstrated positive correlations between laminae Cl^- and Na^+ concentrations and Solusampler EC readings. Also of interest in that work, was the demonstration of so far unexplained reductions in Cl^- and Na^+ concentrations in Chardonnay laminae (expressed on a dry weight basis) between flowering and veraison, before increasing again in laminae after veraison.

We also acknowledge the contribution of the Australian Centre for Plant Functional Genomics (ACPFG) for assistance with $^{36}Cl^-$ flux studies and work to identify and test a range a candidate Cl^- transporters that potentially have a role in the Cl^- exclusion process.

3. Background:

Water is a precious resource which is in demand for agricultural production and for the sustainable health of river systems and riverine environments. Increasing demands for water in combination with periods of drought have resulted in water restrictions in some regions in some years. All water resources available for grape production in Australia contain some level of dissolved salts. For example, approximate electrical conductivities (dS/m) of irrigation water in some of the major viticultural regions are as follows: Griffith-Leeton (0.2-0.3 dS/m), Cowra (0.3-0.7 dS/m), Sunraysia (0.2-0.4 dS/m), Riverland (0.4-0.5 dS/m), Coonawarra (1.0-1.5 dS/m), McLaren Vale (0.3-3.0), Barossa Valley (0.5-3.0), Padthaway (1.5 -2.5 dS/m) and south west WA (0.5-0.7 dS/m).

In a region requiring an irrigation application of 6 ML/ha during the course of a season with water having a salinity of 0.3 dS/m (192 mg/L), some 1.15 tonnes of salt is added per hectare during the season. Build-up of salts in the soil across seasons is generally prevented by a combination of leaching rainfalls in winter (although it is recognized that these can be unpredictable), use of appropriate drainage e.g. tile drains, and by careful irrigation management to ensure adequate leaching fractions are applied. Regular monitoring of soil water content and soil salinity are essential. In periods of water restrictions however, while less water and less total salt is added, less water is available for leaching and salts will build-up in the rootzone.

In a major survey of Cl^- and Na^+ concentrations in over 1200 Australian grape juices across 17 viticultural regions, Leske et al. (1997) found a mean concentration of Na^+ across all samples of 55 mg/L and a mean concentration of Cl^- (expressed as soluble Cl^-) of 141 mg/L. In a similar survey of Australian wines, Downton (1977) found mean Cl^- concentrations (expressed as soluble Cl^-) of 242 and 74 mg/L in Australian red and white wines respectively. These values (for Cl^-) compared with 49 mg/L for French red wines, 37 mg/L for red wines from Austria and Italy and 21 mg/L for white wines from Germany, France and Portugal. The mean Cl^- and Na^+ concentrations in Australian grape juice as reported by Leske et al. (1977) demonstrated that over 87% of the juices tested had a Na^+ concentration of less than 100 mg/L (expressed as soluble Na^+) and over 83% had a Cl^- concentration of less than 241 mg/L (expressed as soluble Cl^-). The results take no account of variety and rootstock differences. Certain rootstocks, for example, Ramsey, 1103 Paulsen and 140 Ruggeri limit the accumulation of Cl^-, whereas K 51-40 results in greater Cl^- accumulation in Shiraz or Chardonnay grape juice than in Shiraz or Chardonnay on own roots. Some scions accumulate more Cl^- than others, irrespective of rootstock e.g. Shiraz accumulates more Cl^- than Chardonnay (Walker et al. 1998).

For red varieties such as Shiraz, juice Cl^- concentrations of 240-250 mg/L can result in wines potentially exceeding the 607 mg/L upper limit for Cl^- (Leske et al. 1997). This is due to extraction of additional Cl^- from skins during winemaking resulting in wine Cl^- concentrations which can be 2-3 times higher than juice Cl^- concentrations. Thus, while rootstocks such as Ramsey, 1103 Paulsen and 140 Ruggeri might safeguard against this effect due to their superior ability for Cl^- exclusion and their ability to sustain this superior ability over the longer term at some sites such as Padthaway (Tregeagle et al. 2006), much less is known about the quality of wines obtained from such rootstocks. Bravdo et al. (2000) reported that wine from Cabernet Sauvignon vines on rootstock 140 Ruggeri had enhanced aroma and wine quality when compared with wine from Cabernet Sauvignon vines on Ramsey rootstock grown under similar saline conditions. In that

study, three levels of saline irrigation water (1.2, 2.7 and 4.2 dS/m) were applied to Cabernet Sauvignon on Ramsey and 140 Ruggeri rootstocks in the Ramat Negev area of Israel. Both laminae and petioles of the vines on 140 Ruggeri rootstock contained less Cl^- than vines on Ramsey at all three salinity levels. Wines of Cabernet Sauvignon on rootstock 140 Ruggeri scored significantly higher in sensory evaluation than those from Cabernet Sauvignon on Ramsey.

We are not aware of any other studies focusing on the quality of wines from salt excluding rootstocks under saline field conditions. Accordingly, the study will focus on selected wine varieties grafted on Cl^- excluding rootstocks under saline conditions and will compare the various rootstock effects on grape juice composition and wine quality, including a detailed analysis of skin and pulp $Cl^-/Na^+/K^+$/tartrate/malate inter-relationships.

With regard to the mechanism of Cl^- exclusion in grapevines, our understanding of the process has been advanced through CRC for Viticulture project 2.3.5 (CRV 02/04). The research demonstrated that Cl^- excluding genotypes such as 140 Ruggeri have reduced Cl^- uptake into excised roots and rooted leaves. Reasons for the reduced uptake are unclear, but may be due to higher rates of efflux from roots of 140 Ruggeri relative to the Cl^- accumulating genotype K 51-40. Alternatively, loading of Cl^- into xylem of 140 Ruggeri roots may be less, which in turn could have a feedback effect to reduce the uptake of Cl^-.

We have also found that root morphology impacts on the chloride exclusion ability of 140 Ruggeri. The development of fewer, longer, less-branched roots in hydroponics is associated with a loss or reduced capacity for Cl^- exclusion from shoot tissue.

In previous research, hybrids between *V. berlandieri* and *V. vinifera* had low petiole Cl^- concentrations and discontinuous distributions within backcrosses to *V. vinifera*, suggesting a single dominant gene for Cl^- exclusion in *V. berlandieri* (Newman and Antcliff 1984). In other work involving cultivars Ramsey, Sultana and Ramsey x Sultana hybrids there was continuous variation in Cl^- accumulation between hybrids, suggesting control of Cl^- exclusion is a transmissible character displaying a quantitative inheritance (Sykes, 1985).

We have crossed 140 Ruggeri (a strong Cl^- excluder) with K 51-40 (a poor Cl^- excluder) to develop a family of 89 hybrids. This will provide a resource to further investigate the genetics of Cl^- exclusion in grapevines.

Thus this study will, in addition, further investigate the physiological basis and genetics of Cl^- exclusion in grapevines, including comparative rates of uptake of Cl^- into roots of the two genotypes and comparative rates of root to shoot transport between the genotypes. It will include a comparison of Cl^- exclusion between plants grown in potting mix and solution culture. Provided significant differences remain between the genotypes in solution culture, $^{36}Cl^-$ flux studies will then be undertaken using rooted leaves with roots in saline solution containing $^{36}Cl^-$. The inheritance of Cl^- exclusion will also be assessed in the hybrid family from the cross between 140 Ruggeri and K 51-40.

In particular, the hybrid family will be used to look for (a) a major gene effect, i.e. a clear 1:1 segregation, providing the Cl^- excluder is heterozygous, and (b) variation between

individuals within the family by using replicate vines. Concurrent with this work will be a collaboration with the Australian Centre for Plant Functional Genomics to investigate candidate Cl^- transporter genes that may be involved in the Cl^- exclusion process.

We also plan to investigate changes in soil salinity throughout the season using devices enabling continuous monitoring of soil salinity, and to determine whether the vine is able to redistribute accumulated salt between different vine tissues, in particular following periods of significant rainfall, when salinity levels may be reduced.

Information gained from the project will enable more informed choices to be made with respect to which of the salt excluding rootstocks are best with regard to yield and wine quality when grown under saline conditions, and with the improved knowledge of Cl^- exclusion gained from the research, a basis will be provided for optimum rootstock management and for the eventual identification of a gene or genes linked to Cl^- exclusion, with consequent benefits for rootstock improvement.

4. Project Aims and Performance Targets:

These are the planned outputs and performance targets as they appeared in the original application, or as otherwise approved.

4.1 Projects Aims:

* Evaluate selected salt excluding rootstocks for vine performance and in particular, grape juice composition and wine compositional and sensory qualities

* Assess chloride/tartrate inter-relationships in berry skins and their effects on tartrate/malate ratios and wine sensory scores

* Further characterize the Cl^- exclusion mechanism in rootstocks such as 140 Ruggeri using glasshouse and laboratory based studies

4.2 Outputs and performance targets:

2006-2007:

Output: Project established
Performance target: Contract signed: postdoctoral fellow appointed and commenced (November 2006)

Output: Site soil and water characterization
Performance target: Soil pH, ECe, SAR and ion data obtained (May 2007)

Output: harvest completed and samples processed
Performance target: 07 yields measured, small scale winemaking completed, juice samples analysed (June 2007)

Output: Rooted leaf and hybrid family experiments
Performance target: Cuttings taken for hybrid family experiment (June 2007)

Output: Communication to stakeholders
Performance target: Annual progress report (February 2007)

2007-2008:

Output; Compositional analysis 07 wines and berries
Performance target: Acids, spectral and other quality measures completed (December 2007)

Output: Sensory analysis 07 wines
Performance target: Analysis completed by Provisor (December 2007)

Output: Harvest completed and samples processed
Performance target: 08 yields measured, small scale wines completed, juice and skin samples analysed (June 2008).

Output: Hybrid family experiment
Performance target: Hybrid family experiment established (October 2007) and samples processed (June 08).

Output: Communication to stakeholders
Performance target: Annual progress report (February 2008); Industry journal article (June 08)

2008-2009:

Output: Compositional analysis of 08 wines
Performance target: Acids, spectral and other quality measures completed (December 2008)

Output: Sensory analysis of 08 wines
Performance target: Analysis completed by Provisor (December 2008)

Output: Harvest completed and samples processed
Performance target; 09 yields measured, small scale winemaking completed, juice and skin samples analysed (June 2009)

Output: Rooted leaf experiment
Performance target: Hybrid family lines rooted leaf experiment established; Cl^- fluxes measured and samples processed (June 2009)

Output: Communication to stakeholders
Performance target: Annual progress report (February 2009); Industry journal article (June 2009)

2009-2010:

Output: Compositional analysis of 09 wines.
Performance target: Acids, spectral and other quality measures completed (October 2009)

Output: Sensory analysis of 09 wines
Performance target: Analysis completed by Provisor (October 09)

Output: Hybrid lines rooted leaf experiment
Performance target: Experiment established (December 2009 and heterologous characterization continued (May 2010)

Output: Communication to stakeholders
Performance target: Annual progress report (February 2010)

2010-2011:

Output: Final Report
Performance target: Final report completed and submitted (July 2010) (changed to September 2010 with approval of GWRDC).

5. Methods:

Methodology will be described for each component part as follows:

5.1. Yield, laminae, juice and wine ion concentrations, composition and sensory qualities of wine of Chardonnay and Shiraz on own roots and a range of rootstocks in a saline environment:

5.1.1 Site 1: The trial site was situated at Padthaway, South Australia, in a commercial vineyard. Two rootstock trials were situated within the same vineyard. One was a rootstock trial with Shiraz as scion while the other was a rootstock trial with Chardonnay as scion. Each was embedded within large blocks, respectively, of Chardonnay and Shiraz vines. The trials were planted in 1992 as complete randomized blocks with one year old vines. As some vines failed to establish, replants were made in 1993 to ensure the trials were complete and balanced. There were ten blocks in each of the Chardonnay and Shiraz sites. Main treatments were scion varieties, Chardonnay and Shiraz (*Vitis vinifera*) on their own roots or grafted to rootstocks Ramsey (*V. champinii*), 1103 Paulsen (*V. berlandieri x V. rupestris*), 140 Ruggeri (*V. berlandieri x V. rupestris*), K 51-40 (*V. champinii x V. riparia* 'Gloire'), Schwarzmann (*V. riparia x V. rupestris*), 101-14 Millardet et de Grasset, referred to here as 101-14 (*V. riparia x V. rupestris*), Rupestris St George (*V. rupestris*) and 1202 Couderc (*V. vinifera x V. rupestris*). Because of limitations to the number of wines that could be made, only vines on own roots and grafted to Ramsey, 1103 Paulsen, 140 Ruggeri, Schwarzmann and 101-14 were used in the trial.

Soil type, trellis type and spacings between vines and rows were as described by Walker et al. (2010). Vines were machine hedged.

Soil samples were taken post harvest in 2007, 2008, 2009 and 2010 in order to determine electrical conductivity (EC) and sodium adsorption ratio (SAR) of 1:5 (w/v) soil:water extracts. Soil sampling methodology and methods for determination of EC, Cl^- and cation concentrations were as described by Walker et al. (2010).

Rainfall between September and May in each of the 2006-2007, 2007-2008, 2008-2009 and 2009-2010 seasons was respectively 251.7, 249.6, 238.8 and 340.4 mm.

Irrigation was applied by dripper. For the Chardonnay site, total amounts delivered were 3.62, 3.60, 2.61 and 3.26 ML/ha in seasons 2006-2007, 2007-2008, 2008-2009, and 2009-2010, respectively. For Shiraz, total amounts delivered per season were 1.89, 1.60, 1.46 and 1.71 ML/ha, respectively.

Mean electrical conductivity of the irrigation water in each of 2006-2007, 2007-2008, 2008-2009 and 2009-2010 was respectively 1.82, 1.88,1.86 and 1.80 dS/m. Sodium adsorption ratio of the irrigation water was approximately 3.6.

At Padthaway, soil water monitoring to assist irrigation scheduling for Chardonnay and Shiraz was carried out using a capacitance probe (EnviroScan RT-5, Sentek Sensor Technologies, Stepney, South Australia) with the lowest sensor at 90 cm depth (Chardonnay) and 100 cm depth (Shiraz). Neutron moisture probes (ICT International,

Armidale, NSW, Australia) were also used, with access tubes to 100 cm depth. For Chardonnay vines, irrigation was not normally applied until approximately the middle of November, and thereafter once the RAW in the soil profile had been used (Tregeagle et al. 2006). For Shiraz vines, irrigation was not normally applied until the middle of December, and with the exception of a period of regulated deficit irrigation between fruit set and veraison, irrigations were applied thereafter once RAW had been used. Winter rainfall was relied on at Padthaway to provide adequate leaching of salts from the soil profile.

Berry and laminae samples were analysed for Cl^- and cations as described by Walker et al. (2010).

The small scale winemaking procedure was described by Gong et al. (2010a)

Wine sensory analyses were conducted by Provisor Pty Ltd on wines made in seasons in 2007, 2008 and 2009. The structure of sensory analyses varied between seasons.

In 2007, wine aroma and flavour profiling was conducted to quantify the key sensory properties of each wine treatment in the Chardonnay and Shiraz rootstock trials. This method was used as the volume of wine was insufficient to perform descriptive analysis due to frost damage during the 2007 growing season. Provisor's trained panel (9-10 assessors) used a standard list of Chardonnay and Shiraz wine attributes that were developed for similar wine studies. The additional flavour attribute 'savoury/salty' was added to the red wine attribute list as soil salinity was of interest in this trial. Informal assessment of Chardonnay wines showed no obvious salty taste so this attribute was not rated. In two formal rating sessions, the white and red trial wines were evaluated independently in booths. Samples were presented in triplicate in random order in 3-digit coded ISO standard tasting glasses (30 ml) and assessed at room temperature under white fluorescent lighting. The panelists rated each of the attributes on a 15 cm line scale, with anchors of 'weak' and 'intense' placed at 1 cm and 14 cm, respectively.

In 2008, both difference tests and wine flavour and aroma profiling were conducted. The triangle difference test method was used, where three coded samples were presented simultaneously, two of which were identical. In each case, rootstock grafted vines were compared against vines on own roots as standard. Difference tests were performed with 15-20 assessors. Each assessor was asked to select the odd sample in each set, and also to comment on the reasons for their choice. Bottles from the two fermentation replicates from the vineyard treatments were combined and blended in a large glass bottle to make a representative sample of each wine treatment for sensory evaluation. The samples were presented as described for 2007 wines. Wine aroma and flavour profiling was conducted by Provisor's trained panel. The wines were evaluated in a series of four sessions. The first three sessions were conducted to familiarize panelists with the samples and to determine the suitability of the attribute lists used in the previous vintage. The fourth session was a formal rating in which wine samples were evaluated independently in booths as in 2007.

In 2009, difference tests on Chardonnay wines were performed by 16-18 assessors using the triangle test method as described above. Wine aroma and flavour profiling was conducted for Shiraz wines by a trained panel of 12 assessors. Prior to profiling, five sessions were conducted to familiarize panelists with the samples and to determine the

suitability of the attribute list used in the previous vintage. A randomized complete block design was assumed for analyses so that the variability between panelists could be accounted for. Final rating sessions were held over two successive days and evaluation for aroma and by mouth attributes took place in the same sitting in independent booths.

In 2009, NaCl was added to a 'base' Shiraz wine to create a range in wine Cl^- concentrations, from 265 mg/l to 854 mg/L. A trained panel of 10 assessors who regularly perform wine descriptive analysis was convened for this study. Several training sessions were conducted to determine the panel's sensitivity to water and wine samples with salt added, but no training was performed with the trial samples as volumes were limited. In one formal rating session, the wines were evaluated independently in booths. Samples were presented in triplicate in random order and assessed at room temperature as described above for difference testing.

5.1.2 Site 2: The trial site was also situated at Padthaway, South Australia, in a commercial vineyard. This was a rootstock trial with Chardonnay as scion. It was planted in 2005. The rootstocks were Ramsey, 1103 Paulsen, 140 Ruggeri, Schwarzmann and 101-14. There were 65 rows, comprising 13 main blocks of 5 rows each, with each block of 5 rows comprising entire rows of each of the 5 rootstocks. Rootstocks were randomly assigned to rows within a block. Each row consisted of 200 vines. Each main block was divided into 13 sub-blocks, and one experimental sub-block was chosen at random within each main block from the 13 sub-blocks. This resulted in 13 experimental sub-blocks, making up the complete random block design across the trial site.

Rows were 2.7 m apart and vines were 1.8 m apart. Drippers (1.3 L/h) were situated every 50 cm along the row. Irrigation amounts applied were 2.89 ML/ha in 2007-08, 2.56 ML/ha in 2008-09 and 2.46 ML/ha in 2009-10. Electrical conductivity of the irrigation water was 1.61 dS/m in 2007-08, 1.62 dS/m in 2008-09 and 1.66 dS/m in 2009-10.

Solusamplers were installed at 25, 50 and 75 cm depths at each of 10 locations, with each location being within an experimental sub-block. Of the 10 locations, two were situated in the vicinity of each of the five rootstocks, effectively giving two Solusampler replicates for each rootstock, randomly assigned throughout the trial site. Solusampler measurements were made at regular intervals throughout the year. Rainfall was also recorded.

Laminae were sampled from adjacent to basal inflorescences or clusters on four occasions in each of three seasons, 2007-2008, 2008-2009 and 2009-2010. Juice samples were obtained in 2008-2009 and 2009-2010. Yields were also measured in 2008-2009 and 2009-2010. Pruning wood weights were measured in winter of both seasons.

Chloride and cations were measured in laminae and juice samples as described by Walker et al. (2010).

A further block of Shiraz vines at this site, regularly demonstrating symptoms of leaf burn throughout the course of a season, were used to investigate Cl^- and Na^+ concentrations in laminae and wood, using two sampling times pre-leaf fall and three sampling times post-budburst, to investigate whether there was any movement of salt from wood during the dormancy period.

Wood samples for analysis were cut into small pieces and ground using a Bench Top Ring Mill (Rocklabs) to a fine powder (particle size of 5 microns).

Chloride in wood samples was measured as described above for other tissues. Cations were measured by ICP analysis. Samples were prepared for ICP analysis by weighing approximately 100 mg powdered samples into a glass test tube that had been calibrated to a 20 mL volume and weight recorded for each sample. Extractions were performed in a fume hood by addition of 2 ml of AR grade 69.9% nitric acid to each tube, mixing, then standing overnight for partial digestion. Tubes were then placed in a heating block to slowly reach 125^{o}C over a 1 h period. The temperature of the heating block was held at 125^{o}C for 4 h, then cooled for 1 h. Deionised water was then added to bring each tube to 20 mL, mixed and allowed to stand overnight prior to ICP analysis.

5.2. Effects of salinity on inorganic/organic anion inter-relationships in grape berries:

5.2.1 Plant material and experimental design:

There were two sites used in the study. One was an experimental vineyard at Merbein, Victoria, and the other at a commercial vineyard in Padthaway, South Australia. The experiments were established as complete randomized block designs in both sites. The vines were planted in 1992 with 1-year-old grapevines of Chardonnay and Shiraz on their own roots and on a range of rootstocks. Some were replanted in 1993 to ensure the trials were complete and balanced. Only own-rooted and grapevines (Chardonnay and Shiraz) grafted on Ramsey, 1103 Paulsen and 140 Ruggeri rootstocks were used in the study. Soil type, row spacing, vine spacing, trellis design, pruning method and irrigation set-up were described by Gong et al. (2010a).

5.2.2 Irrigation water:

At Merbein, the desired salinity level in the irrigation water was achieved by application of a brine solution (mainly NaCl, containing small amounts of $CaCl_2$ and $MgCl_2$) to the main irrigation pipe containing Murray River water (Tregeagle et al. 2006). The final salinity of the irrigation water was 2.1 dS/m with a sodium adsorption ratio (SAR) of 10-12. At Padthaway, the Chardonnay and Shiraz vines were irrigated with bore water with an EC of approximately 1.6-1.8 dS/m and with a SAR of 3.6 (Tregeagle et al. 2006).

5.2.3 Sample collection and preparation:

At Padthaway, mature berries were sampled before harvest with berry maturity ranging from an average of 12.2-12.8 Baume for Chardonnay to 13.8-14.4 Baume for Shiraz. The samples were collected on 16 February 2007. At Merbein, berries were sampled with berry maturity ranging from an average of 12.8-13.8 Baume for Chardonnay to 15.0-17.7 Baume for Shiraz. Chardonnay samples were collected on 31 January 2007. Shiraz samples were collected on 8 February 2007.

Two replicates of 15 berries per vine (a total of 30 berries from each vine) were taken at Padthaway and Merbein. Each replicate was a sub-sample selected at random from a larger 110-berry sample per vine obtained by sampling 11 bunches at random on either side of the vine, with two berries sampled from the shoulder of each bunch, two from the middle and one from the base. Each berry was peeled carefully, and skin and pulp (seeds removed) were separated. The bulked skins were quickly washed with distilled water to remove any adhering pulp tissue and gently blotted. Bulked skin and pulp samples were frozen and stored at -20^{o}C until analysis.

5.2.4 Sample extraction:

Pulp samples were crushed with a mortar and pestle and the juice was collected and its volume was recorded. The juice samples were used for analyses and calculation of inorganic ions and organic acids/anions in the pulp. Ions in skin were extracted in deionised water (g skin:mL water = 1:3) in an autoclave at 15 psi for 15 min. After cooling at room temperature, the extracts were mixed thoroughly and centrifuged at approximately 3200 x g for 15 min (Eppendorf Centrifuge 5810 R, Eppendorf, Hamburg, Germany), after which the supernatants were collected. The pellets were re-extracted in 5 mL of deionised water. Supernatants from these two procedures were combined and made to 25 mL with deionised water (solution 1). In order to extract inorganic ions as much as possible, the remaining pellets were re-extracted in 3.5% (v/v) HNO_3 twice (5 mL and 3 mL in turn) in a boiling water bath for 30 min, after which the extracts were centrifuged at about 3200 x g for 15 min. The supernatants were collected and made to 10 mL (solution 2). Organic acid anions were extracted by de-ionised water and were examined in solution 1, while inorganic ions (Cl^-, K^+ and Na^+) were measured and concentrations calculated from solutions 1 and 2.

5.2.5 Analyses:

Inorganic and organic acids analyses were as described in Gong et al. (2010a).

5.2.6 Winemaking:

Winemaking procedure for Shiraz and Chardonnay was as described in Gong et al. (2010a).

5.2.7 Statistical analysis:

General analysis of variance (ANOVA) was conducted using Genstat Release 11.1. The block structure in Tables 8, 9, 10 and 11 was block/vine/replicate. Regression analyses were conducted using Sigma plot 10.0 for Windows. Regressions between malic acid and Cl^-, between malic acid and Na^+, or between Cl^- and Na^+ were done using data from individual vines (averaged from two replicated samples).

5.3. Variation for Cl^- and Na^+ exclusion in a family from a cross between grapevine rootstocks K 51-40 and 140 Ruggeri:

5.3.1 Vine material:

Controlled crosses were made between K 51-40 and 140 Ruggeri in 1985 and 2005 from which 80 hybrid progeny were obtained. The 'MI' hybrids were obtained in 1985, while the 'HB' hybrids were obtained in 2005. Following seed germination, the hybrids were planted and grown to mature vines in CSIRO's experimental vineyard at Merbein, Victoria. Soil characteristics, vineyard management and irrigation practices were the same for the 'MI' and 'HB' vines. Irrigation water was taken from the River Murray and was generally of low salinity (under 0.35 dS/m).

5.3.2 Vine propagation:

Cuttings of each hybrid, their parents and two standard rootstocks, namely Ramsey (*V. champinii*) and 1103 Paulsen (*V. berlandieri x V. rupestris*), were propagated in potting mix held in plastic boxes (30 x 37 cm) placed on heat beds. The heat beds were heated to approximately 25ºC to encourage callus formation and root growth, in a mist house held at or below 25ºC by evaporative cooling during August 2007 (pot trial) and September 2008 (solution culture trial). Only 60 hybrids (11 'MI' and 49 'HB' were used because the vigour of the remaining vines was weak such that cuttings, if available, were thin and did not strike. Six uniform grapevines (about 20-30 cm in height) of each hybrid, their parents and the two standard rootstocks were selected for each trial.

5.3.3 Pot trial:

Grapevines of each hybrid, their parents and two standard rootstocks were planted in a mixture of sand and red loam, 40:60 v/v, respectively, held in 4.5 L pots under glasshouse conditions in late October 2007. Vines were laid out on the bench as randomized blocks with each genotype replicated once per block. The vines were watered daily with excess nutrient solution to ensure adequate leaching via an automated drip-irrigation system. The main solution contained the following elements (mM): Ca^{2+}, 1.2; K^+, 1.7; Mg^{2+}, 0.4; NH_4^+, 0.5; NO_3^- , 4.2; SO_4^{2-}, 0.4; $H_2PO_4^-$, 0.3; H_3BO_3, 8.3 x 10^{-4}; Zn, 1.0 x 10^{-3}; Cu, 1.4 x 10^{-3}; Mn, 4.4 x 10^{-3}; Mo, 3.2 x 10^{-5}; EDTA-Fe, 3.0 x 10^{-2}. The vines were maintained as single shoots by removing lateral shoots and after 28 days salt was added to the nutrient solution at a rate of 10 mM Cl^- (with cations as Na^+:Ca^{2+}:Mg^{2+} in the ratio 6:1:1) per day until 50 mM was reached. The vines were maintained under these conditions for a further 27 days when they were destructively harvested with all laminae and petioles in the middle 60% of each shoot retained for analysis. This was achieved by discarding 20% of the shoot proximal to the apex and 20% distal to the root system. A large composite root sample (>25% of total root mass) was also retained from each vine in 4 of the blocks.

5.3.4 Solution culture trial:

Uniform grapevines were transferred to aerated solution cultures held in 450 L fibreglass tanks (surface area 1m^{-2}) under glasshouse conditions in mid-November 2008. There was one replicate vine per genotype randomly positioned in each of 6 tanks. The nutrient

solution had a similar composition as that used in the pot trial except that NH_4^+ was absent and total NO_3^- was 3.8 mM. Three weeks after vine transfer to solution culture, the concentration of EDTA-Fe was increased from 30 µM to 71 µM, and photoperiod was extended 2 hours by supplementary lighting. One week later, Cl^- was added to the culture at a rate of 12.5 mM per day (with cations Na^+:Ca^{2+}: Mg^{2+} in the ratio 6:1:1) to give a concentration of 25 mM. The youngest leaf on each vine was labeled with a loosely tied cotton thread and shoot length was measured when the Cl^- treatment started. The vines were maintained as single shoots by removing lateral shoots under these conditions for 27 days, after which total and shoot lengths between the labeled leaf and the shoot apex were recorded and they were destructively harvested. As in the pot experiment, all laminae and petioles in the middle 60% of each vine were retained for analysis. A sample, as close as possible to the complete root system of each vine, was also retained.

5.3.5 Ion analyses:

Laminae, petiole and root samples were dried at 60^{o}C for at least 72 h and finely powdered in a hammer mill to pass through a 0.5 mm mesh. Chloride concentration was measured by silver ion titration with a Buchler Cotlove Chloridometer (Nuclear Chicago, USA) from extracts prepared by digesting 20-100 mg dry samples in 4 mL of an acid solution containing 10% (v/v) CH_3COOH and 0.1 M HNO_3 overnight before analysis. Sodium was analysed by inductively coupled plasma (Spectroflame ICP, Spectro Analytical Instruments, Kleve, Germany) from extracts of 100-300 mg powdered samples digested in 2 mL of concentrated HNO_3 on a heating block and diluted to 20 mL with deionised water.

5.3.6 Statistical analysis:

Data were subjected to analysis of variance using Genstat Release 11/1 software. Where F-tests were significant (P<0.05), means were separated by least significant differences (Snedecor and Cochran 1967). Significant differences between data for ‘HB’ and ‘MI’ hybrids were explored and where none were demonstrated, the data were combined.

5.4. Characterisation of the Cl^- exclusion mechanism:

5.4.1 Preparation of rooted leaves of K 51-40 and 140 Ruggeri:

Grapevines of K 51-40 and 140 Ruggeri were established from cuttings, potted in a glasshouse and watered daily with non-saline nutrient solution (see Soil Trial section). Mature leaves were excised from the grapevines at the petiole base. The petioles were then dipped in a hormone gel containing 1.5 g L^{-1} indole-3-butryic acid (Clonex, Growth Technology, Taunton, Somerset, UK) and planted in propagating medium containing 50% vermiculite and 50% perlite (Australian Vermiculite and Perlite Pty Ltd, Campbellfield, Victoria, Australia). The propagation boxes were transferred to heating beds (30 ºC) in a mini-shadehouse within the glasshouse. The mini-shadehouse was covered with 90% shadecloth. The rooted leaves were misted with fine sprays for 1 minute, 13 times during the day between 7 am to 7 pm. About 3 weeks later, the rooted leaves were transferred to modified Hoagland solution containing the following nutrients

(mM): KNO_3, 1.0; $Ca(NO_3)_2 \cdot 4H_2O$, 1.0; $MgSO_4 \cdot 7H_2O$, 0.4; KH_2PO_4, 0.2; H_3BO_3, 4.6×10^{-2}; $MnCl_2 \cdot 4H_2O$, 9.1×10^{-3}; $ZnSO_4 \cdot 7H_2O$, 7.6×10^{-4}; $CuSO_4 \cdot 5H_2O$, 3.2×10^{-4}; $Na_2MoO_4 \cdot 2H_2O$, 2.4×10^{-4}; EDTA-Fe-Na, 7.1×10^{-2}.

5.4.2 Cl^- accumulation in rooted leaves of K 51-40 and 140 Ruggeri:

Two weeks after being transferred to modified Hoagland culture solution, the rooted leaves were subjected to 25 mM Cl^- (Na^+: Ca^{2+}: Mg^{2+} = 6:1:1) in addition to nutrient solution for up to 14 days. At each harvest, the plant was detached into lamina, petiole and roots, fresh weights determined, and the samples then dried in an oven for Cl^- analysis.

5.4.3 8-*hydroxy-1,3,6-pyrenetrisulphonic acid (PTS) uptake:*

Two weeks after being transferred to modified Hoagland nutrient solution, the plants were subjected to 25 mM Cl^- (Na^+: Ca^{2+}: Mg^{2+} = 6:1:1) without or with 100 mg/L of PTS for 24 hours, after which the plants were harvested for Cl^- and fluorescence analysis. The leaves and roots were extracted in 40 mL deionised water at 90ºC in a water bath for 2 hours. PTS fluorescence was determined at $\lambda_{excitation}$ of 400 nm and $\lambda_{excitation}$ of 520 nm with a FLUOstar Omega microplate reader (BMG LABTECH GmbH, Hanns-Martin-Schleyer-Str. 10, 77656 Offenburg, Germany).

5.4.4 *Chloride uptake studies:*

$^{36}Cl^-$ flux experiments were conducted at 25 °C under a fluorescent light bank at Australian Centre for Plant Functional Genomics, Adelaide, Australia. Two experiments were conducted. One measured unidirectional Cl^- flux into roots over a 10 min period. The other measured net Cl^- flux into roots of whole plants over a 3 h period. Rooted leaves were transferred into 100 mL of radioactive ^{36}Cl solution (0.1 µCi/mL) with roots lowered into the solution. The radioactive flux solution was rotated continuously on a rotating shaker (40 rpm).

Three 20 µL standards (radioactive solution) were collected at the start and end of every flux experiment. These standards were mixed with scintillation fluid, measured with the scintillation counter and were used along with its specific activity (radioactivity divided by total nmoles of nominal Cl^- in this 20 µL radioactive solution) in the conversion of $^{36}Cl^-$ content in plant tissues to nominal Cl^- content.

For the 3 h uptake experiment, every 30-60 min, the radioactive flux solutions were corrected for loss in volume due to evapotranspiration (about 1-1.5 mL h^{-1}). Evapotranspiration was accounted for by weighing the radioactive flux solution plus rooted leaves in it at the start of the experiment on a two-figure balance, and then re-weighing and correcting for any evapotranspiration by replacing the lost weight with deionised water. Prior to the removal of a rooted-leaf from the radioactive flux solution, the radioactive flux solution plus rooted leaves was weighed and corrected for by addition of deionised water. Following removal of the rooted-leaf, the new weight for the radioactive flux solution was recorded and used as a basis for subsequent checks for evapotranspiration losses.

At the end of the flux time, rooted leaves were carefully removed from the radioactive flux solution, rinsed in an ice-cold 5, 10 or 25 mM Cl^- rinse-solution for 2 min followed by another ice-cold 5, 10 or 25 mM Cl^- rinse for another 3 min. The rinsed roots were blotted dry, and each rooted-leaf was destructively sampled into separate parts of roots, callus, petiole and lamina. Each part was put into a scintillation vial, weighed and scintillation fluid added. The samples were shaken, placed in the dark and then analysed with a liquid scintillation counter at least one hour later.

To prevent the presence of chlorophyll within the lamina and petiole samples from increasing the counts measured by the scintillation counter, every sample was first bleached in sunlight for a week and then chemically bleached with 200 µL of domestic bleach (containing sodium hypochlorite 49.9 g/L, sodium hydroxide 12 g/L and alkaline salts 0.5 g/L) added to each vial. Samples were then kept in the dark for a week before being run on the scintillation counter.

5.5. Assessment of candidate Cl^- transporter genes (collaboration with Australian Centre for Plant Functional Genomics, ACPFG):

This component of the project involved assessment of a range of candidate Cl^- transporter genes. The study initially used Arabidopsis as a model system and involved screening knock out mutants of putative candidate genes for detecting variation in shoot Cl^- accumulation. The aim was to detect the most promising types, screen for their presence in grapevines and assess expression levels in relation to rootstock Cl^- exclusion capacity.

6. Results:

Results for each of the component sections are detailed as follows:

6.1 Yield, laminae, juice and wine ion concentrations, composition and sensory qualities of wine of Chardonnay and Shiraz on own roots and on a range of rootstocks in a saline environment:

Site 1: Own-rooted and grafted Chardonnay and Shiraz established in 1992:

Soil type and salinity: The soil type was uniform shallow brownish gravelly calcareous mottled clay (Walker et al. 2010). Soil $EC_{1:5}$ and $SAR_{1:5}$ values in the post harvest period were higher in 2009 and 2010 than in 2007 and 2008 (Table 1). Soil Ca^{2+} concentrations at the Chardonnay site decreased slightly in the latter two years and apart from an increase in 2008, were relatively stable at the Shiraz site. Soil Mg^{2+} and K^{+} concentrations were also relatively stable across the seasons. Soil Na^{+} and Cl^{-} concentrations were higher in 2009 and 2010 than in 2007 and 2008 (Table1).

The Na^{+} to Cl^{-} ratio values (Table 1) are presented on an ion basis. For example, for soil Na^{+} at the Chardonnay site in 2007, Na^{+} on an ion basis was 345/23 and for soil Cl^{-} at the same site in the same year, the value on an ion basis was 360/35.5 (Table 1). Hence the ratio of Na^{+}/Cl^{-} at that site in that year was 1.48. The ratio at the Chardonnay site varied within the range 1.16 to 1.72, while at the Shiraz site it varied within the range 1.47 to 1.83 (Table 1).

Laminae ion concentrations: Vines at this longer established site were characterised with respect to ion concentrations in laminae at flowering in each of seasons 2006-07, 2007-08 and 2008-09 (Table 2). Based on mean concentrations across the three seasons, Chardonnay on own roots and Shiraz on Ramsey had highest concentrations of Cl^{-} in laminae. Na^{+} concentrations in laminae of Chardonnay on own roots and all grafted vines were similar and relatively low (0.05 g/100 g dwt), while Na^{+} concentrations in Shiraz laminae were higher than in Chardonnay laminae and highest in vines on own roots and Schwarzmann rootstock (Table 2).

Laminae sampled from trial vines at three other time points throughout the season demonstrated increasing laminae Cl^{-} concentrations as the season progressed, with the exception of laminae of Shiraz on Schwarzmann, which showed no marked change throughout the season (Figure 1). Shiraz laminae Na^{+} concentrations increased as the season progressed, while in contrast, Chardonnay laminae Na^{+} concentrations decreased in February-March before increasing again near leaf fall (Figure 1). Laminae of Chardonnay on own roots had highest Cl^{-} concentrations at all sampling times. Laminae of Shiraz on own roots, Ramsey and 1103 Paulsen had similar Cl^{-} concentrations throughout, and at the latter sampling times, were higher than concentrations in laminae of 140 Ruggeri and Schwarzmann. Laminae Na^{+} concentrations were generally lower in Chardonnay than in Shiraz. Shiraz laminae Na^{+} concentrations became highest on 1103 Paulsen as the season progressed (Figure 1).

For Chardonnay, laminae Ca^{2+} concentrations were highest on 1103 Paulsen, while for Shiraz they were higher on Ramsey, 1103 Paulsen and 140 Ruggeri than on own roots and other grafted vines (Table 2). Laminae Mg^{2+} concentrations also tended to be higher for both Chardonnay and Shiraz on 1103 Paulsen (Table 2). Laminae K^{+} concentrations

increased between flowering and veraison (slightly for Shiraz, more steeply for Chardonnay) and then decreased towards leaf fall (Figure1).

Yield, berry weight and grape juice TSS, pH and titratable acidity: Yield of Chardonnay, based on four year means, was similar for vines on own roots or grafted on Ramsey and 1103 Paulsen (Table 3A). Yield of vines on Ramsey rootstock was significantly higher than on 140 Ruggeri, Schwarzmann and 101-14 rootstocks. However, yield of Chardonnay on 140 Ruggeri rootstock was not significantly different from yield of vines on own roots or on 1103 Paulsen rootstocks. Chardonnay on Schwarzmann had lower berry weight than Chardonnay on own roots or on each of the other rootstocks (Table 3A).

The target for total soluble solids in grape juice of Chardonnay at harvest was 22 ^{o}Brix. In general, this was met, with mean grape juice soluble solids of Chardonnay on 1103 Paulsen slightly lower than target (21.7 ^{o}Brix) and that of Chardonnay on 101-14 slightly above target (22.5 ^{o}Brix) (Table 3A). Rootstock had no effect on Chardonnay grape juice pH, while juice titratable acidity for Chardonnay on own roots was higher than for grafted vines (Table 3A).

Yield of Shiraz on 140 Ruggeri, based on 4 year means, was significantly higher than yield of Shiraz on own roots and on all other rootstocks, except for Shiraz on 1103 Paulsen (Table 3B). Berry weight was similar for Shiraz on 140 Ruggeri, Ramsey and 101-14, intermediate for Shiraz on own roots and 1103 Paulsen, and lowest for Shiraz on Schwarzmann (Table 3B).

The target for total soluble solids in grape juice of Shiraz at harvest was 24 ^{o}Brix. In general, this was met, with mean grape juice soluble solids of Shiraz on Ramsey, 140 Ruggeri and 101-14 slightly above target (24.4 – 24.5 ^{o}Brix) and Shiraz on 1103 Paulsen, Schwarzman and on own roots in the range of 25.0 to 25.3 ^{o}Brix (Table 3B).

Juice pH was similar for Shiraz on all rootstocks, but lower for Shiraz on own roots. Juice titratable acidity for Shiraz on 140 Ruggeri and Schwarzmann was lower than for Shiraz on own roots, which was similar to that on Ramsey, 1103 Paulsen and 101-14 (Table 3B).

Grape juice Cl^-, Na^+, K^+, Ca^{2+} and Mg^{2+} concentrations: Grape juice Cl^- concentrations were generally within acceptable limits for both Chardonnay and Shiraz, with highest values recorded for vines on own roots for Chardonnay (146 mg/L in 2009) and for vines on 1103 Paulsen for Shiraz (212 mg/L in 2009). Because of high residual variance in the repeated measures analysis of variance for grape juice Cl^- and Na^+ concentrations, the data were log transformed in order to minimise the residual variance. Based on the log transformed values across three seasons, Chardonnay on own roots had highest grape juice Cl^- concentrations, and Chardonnay on 101-14 rootstock had the lowest (Table 4A). There was no difference in grape juice Cl^- concentrations between Chardonnay on Ramsey, 1103 Paulsen, 140 Ruggeri and Schwarzmann rootstocks (Table 4A). Similarly, for Shiraz, grape juice Cl^- concentrations were similar between vines on own roots and on Ramsey and 1103 Paulsen rootstocks, and all were higher than for vines on 140 Ruggeri, Schwarzmann and 101-14 rootstocks (Table 4A).

Grape juice Na^+ concentrations were generally below 150 mg/L, with the exception of 2009, when concentrations reached 177 mg/L for vines on Ramsey, 188 mg/L for vines on 140 Ruggeri and 237 mg/L for vines on Schwarzmann. Again, based on log transformed values across three seasons, grape juice Na^+ concentrations for Chardonnay were highest for vines on Ramsey and Schwarzmann rootstocks and lowest for vines on own roots and 101-14 rootstock (Table 4A). Shiraz vines on 1103 Paulsen had highest grape juice Na^+ concentrations and vines on 101-14 rootstock the lowest, with vines on own roots and the other rootstocks intermediate (Table 4A).

Because residual variances in repeated measures analysis of variance for K^+, Ca^{2+} and Mg^{2+} were within acceptable limits, there was no requirement to log transform these data. With Chardonnay, grape juice K^+ concentrations were not significantly different between the rootstocks. However, Chardonnay on own roots had lower grape juice Ca^{2+} and Mg^{2+} concentrations than the grafted vines (Table 4B). With Shiraz, vines on own roots had lower grape juice K^+ and Mg^{2+} concentrations, whereas vines on 140 Ruggeri and 101-14 had lowest grape juice Ca^{2+} concentrations (Table 4B).

Wine Cl^-, Na^+ and K^+ concentrations, and pH: Based on mean data across three seasons (2007-2009), concentrations of Cl^- in wine of Chardonnay and Shiraz were below the upper limit of 607 mg/L (Table 5 A and B). For Chardonnay, Cl^- concentrations were highest in wine made from vines on own roots (127.5 mg/L) and lowest in wine made from vines on 101-14 rootstock (46.9 mg/L). Concentrations in wine from vines on Ramsey, 1103 Paulsen, 140 Ruggeri and Schwarzmann rootstocks were intermediate (range 72.7 to 84.4 mg/L) (Table 5A). For Shiraz, wine made from vines on own roots and on 1103 Paulsen had similar high Cl^- concentrations (383.0 and 350.8 mg/L, respectively). Cl^- concentrations in wines made from vines on Ramsey and 101-14 rootstocks were intermediate (270.9 and 260.8 mg/L, respectively), whereas wines made from vines on 140 Ruggeri and Schwarzmann rootstocks had lowest Cl^- concentrations (145.7 and 117.2 mg/L, respectively) (Table 5B).

Figure 2 shows the change in Cl^- and Na^+ concentrations during processing from juice, through must, to wine for Shiraz on own roots or grafted to Ramsey, 1103 Paulsen, 140 Ruggeri, Schwarzmann and 101-14 rootstocks. Fermentations were commenced on the day after crushing. The increase in must Cl^- and Na^+ concentrations is rootstock dependent, and in all cases was greatest during the first 1-2 days of fermentation (Figure 2). There was little difference between concentrations of Cl^- and Na^+ in must after 1-2 days and concentrations in final wine. The increase in Na^+ concentrations during fermentation was much less than the increase in Cl^- concentrations (Figure 2).

Relationship between juice and wine Cl^- and Na^+ concentrations: Figure 3 shows the relationship between juice and wine Cl^- and Na^+ concentrations. In each case there is a linear relationship. For Chardonnay, there was a small increase in Cl^- concentration in going from juice to wine, e.g. 80 mg/L to 115 mg/L, and an even smaller increase for Na^+, e.g. 80 mg/L to 85 mg/L (Figure 3A). For Shiraz, the increases were larger, e.g. for Cl^-, from 120 mg/L to 260 mg/L, and for Na^+, from 60 mg/L to 75 mg/L (Figure 3B). The bigger increases for Shiraz reflect extraction from skins during fermentation. The smaller increase in Na^+ may reflect, at least in part, lower Na^+ concentrations in skins (Gong et al. 2010a).

Wine spectral data: Spectral data for Shiraz wines is shown in Table 6. Colour density, total and ionised anthocyanins were all significantly higher for wines made from vines on own roots, and grafted to Schwarzmann and 101-14 rootstocks. Colour hue was lowest, with less brownish hue, in wines made from own roots and from 101-14 rootstock. Total phenolics were highest in wines made from Shiraz on Schwarzmann rootstock and lowest in wines made from Shiraz on 1103 Paulsen and 140 Ruggeri rootstocks, with wines from vines on own roots, Ramsey and 101-14 rootstocks intermediate (Table 6).

Table 1: EC and SAR of 1:5 soil water extracts, concentrations of Ca^{2+}, Mg^{2+}, K^{+}, Na^{+} and Cl^{-} in soil and soil Na^{+}:Cl^{-} ratio at (A) the Chardonnay site and (B) the Shiraz site. Values are means for top 60 cm, based on samples take at soil surface, 30 cm and 60 cm depths.

A.	Chardonnay site							
Year	$EC_{1:5}$ (dS/m)	$SAR_{1:5}$	Ca^{2+}	Mg^{2+}	K^{+} (mg/kg)	Na^{+}	Cl^{-}	Na/Cl
2007	0.37	4.18	70	19	7	345	360	1.48
2008	0.40	3.58	91	21	4	316	282	1.72
2009	0.57	5.93	63	16	13	459	613	1.16
2010	0.51	6.03	50	18	13	438	483	1.39

B.	Shiraz site							
Year	$EC_{1:5}$ (dS/m)	$SAR_{1:5}$	Ca^{2+}	Mg^{2+}	K^{+} (mg/kg)	Na^{+}	Cl^{-}	Na/Cl
2007	0.31	4.73	46	14	6	320	272	1.83
2008	0.38	3.90	77	18	4	336	337	1.54
2009	0.47	6.25	46	12	4	414	436	1.47
2010	0.42	5.40	42	16	10	363	350	1.60

Wine aroma and flavour profiling (2007): Aroma and flavour profiling of Chardonnay wines from the 2007 vintage revealed (a) general similarity in all attributes rated for all wines, (b) no significant differences in any aroma characteristics, and (c) a difference in the palate attribute smoothness, which was slightly higher in wine from rootstock 140 Ruggeri relative to wines from own roots or from other grafted vines.

Aroma and flavour profiling of the Shiraz wines from the 2007 vintage revealed (a) differences in 8 of the 27 wine attributes rated, (b) significant differences in dark berry, confection, savoury/brine, and vegetal aroma characteristics, with Schwarzmann having less intense dark berry aroma than the other five wines, 140 Ruggeri having more intense savoury/brine and vegetal odour characteristics than own rooted vines, and 1103 Paulsen and 140 Ruggeri having lower confection odours than Schwarzmann wines, (c) higher perceived acidity of Schwarzmann wines than wines from own roots, 1103 Paulsen and 101-14, and (d) least intense ripe berry flavours perceived for 101-14 wines (relative to all wines) and slightly less peppery flavour of 101-14 wines than wines from own roots, Ramsey, 1103 Paulsen and 140 Ruggeri rootstocks.

Wine difference testing and aroma and flavour profiling (2008): Difference testing of Chardonnay wines from the 2008 vintage revealed that Schwarzmann and Ramsey rootstock wines could not be clearly discriminated from the wine made from Chardonnay on own roots. This was in contrast to the 1103 Paulsen ($P<0.05$), 140 Ruggeri ($P< 0.01$) and 101-14 rootstock ($P< 0.01$) wines, which were perceived as different in aroma and flavour. The 1103 Paulsen rootstock wine seemed smoother with more fruit flavour persistence. The 140 Ruggeri rootstock wine seemed more aromatic and floral on the nose with more fruit flavour. The 101-14 rootstock wine was perceived as having more intense aroma with less acidity and a creamy mouth feel. The aroma and flavour profiling revealed that the sensory properties were all quite similar for the six Chardonnay wines. The key aroma attributes were fresh citrus, tropical and stone fruit aromas along with high intensity scores for stewed pear, confection and vanilla/caramel. The only significant aroma attribute was floral which was perceived highest in the Ramsey wine and lowest in the 140 Ruggeri wine. With regard to flavour, Schwarzmann was perceived as having most fresh citrus and confection flavour and Ramsey the least.

For Shiraz wine from 2008 vintage, all five rootstock wine comparisons were perceived as statistically different (Ramsey, 1103 Paulsen and 101-14, all $P<0.01$, and Schwarzmann and 140 Ruggeri, both $P<0.05$) from the own roots Shiraz wine. Specifically, (a) the Schwarzmann rootstock wine seemed to have more fruit flavour with softer mouth feel, (b) the Ramsey rootstock wine seemed more lifted in aroma with confection notes and less astringency, (c) the 1103 Paulsen rootstock wine seemed to have less sour and savoury flavours, (d) the 140 Ruggeri rootstock wine seemed smoother with fresher berry flavours, and (e) the 101-14 rootstock wine was perceived as having more intense confection and less bitterness.

The aroma and flavour profiling revealed that wines from vines on own roots and grafted to 140 Ruggeri shared similar characteristics such as high savoury/brine and licorice aroma, and low chocolate and vanilla aromas. Both wines had savoury flavours with the least ripe berry flavours. All wines showed high dried fruit aroma except the Ramsey and 1103 Paulsen wines. The most confection aroma was perceived in Ramsey and Schwarzmann wines. The Ramsey wine seemed slightly less acidic, with lower body and drying scores than the other wines. The Schwarzmann and 101-14 wines both had high confection, chocolate and vanilla aromas with the least savoury notes. These wines also had ripe berry flavours, with slightly fuller body and greater fruit length than the other wines. The 1103 Paulsen wines also showed higher confection-like characteristics and low savoury/brine notes, but with slightly less dried fruit aromas and ripe berry flavours.

Wine difference testing and aroma and flavour profiling (2009): For the 2009 vintage, there were no statistically significant differences perceived in overall aroma and flavour properties between the Chardonnay wine made from each rootstock and from the vines on own roots. The wines were all clean and intense with odour characteristics including citrus, confection, floral and tropical.

For Shiraz wine from the 2009 vintage, difference testing was not conducted, but instead, detailed wine aroma and flavour profiling was carried out. Wines from vines on own roots, 101-14 and Schwarzmann rootstock were all perceived with the highest dark berry aroma and higher stewed/dried fruit flavours. Wines from vines on own roots and 101-14 rootstock were perceived with more intense sweet spice and most vanilla notes; these wines also showed more persistent fruit flavours and were slightly more drying. Wine made from vines on 140 Ruggeri was also quite drying. Wines from 1103 Paulsen and 140 Ruggeri rootstocks had the least stewed/dried fruit flavours. 1103 Paulsen wines also seemed to have least sweet spicy notes on both aroma and palate. Wine from Ramsey rootstock had slightly less dried fruit aromas, but these flavours still came through on the palate as well as intense red berry flavours.

General observations across vintages: The between vintage effects on wine aroma and flavour profiles were generally greater than any rootstock effects. Rootstock effects on wine aroma and flavour profiles were more pronounced with Shiraz than with Chardonnay.

Salty taste in wine: Salty taste was not readily detected, but this may be related to the fact that mean wine Cl^- and Na^+ concentrations for Chardonnay wine were less than 130 and 102 mg/L, respectively, and for Shiraz wine they were less than 385 and 112 mg/L, respectively (Table 5). To further investigate salty taste, a Shiraz ‘base’ wine was obtained from the Riverland (South Australia) and spiked with NaCl to produce a range in salt concentrations. The base wine Cl^- concentration was 265 mg/L. After NaCl additions, the range in Cl^- concentrations was 265 to 854 mg/L. Sensory assessment by 10 trained assessors did not result in statistically significant detection of salty taste across the range (Figure 4). The wine containing 854 mg/L Cl^- was, however, perceived as more spicy (Figure 4).

Table 2: Three year average (2007-2009) of Cl^-, Na^+, K^+, Ca^{2+}, Mg^{2+} concentrations in laminae at flowering for (A) Chardonnay and (B) Shiraz vines on own roots, or grafted on Ramsey, 1103 Paulsen, 140 Ruggeri, Schwarzmann and 101-14 rootstocks. LSD = least significant difference.

A. Chardonnay/ Rootstock	Cl^-	Na^+	K^+	Ca^{2+}	Mg^{2+}
	(g/100g dwt)				
Own roots	0.44	0.05	1.01	1.92	0.34
Ramsey	0.26	0.05	1.05	2.15	0.32
1103 Paulsen	0.16	0.05	1.04	2.59	0.36
140 Ruggeri	0.23	0.05	1.10	1.99	0.30
Schwarzmann	0.25	0.05	1.14	1.79	0.27
101-14	0.19	0.05	1.18	1.70	0.28
$LSD_{Rootstock}$	0.08 ***	0.01 ns	0.09 *	0.20 ***	0.03 ***

B. Shiraz/ Rootstock	Cl^-	Na^+	K^+	Ca^{2+}	Mg^{2+}
	(g/100g dwt)				
Own roots	0.37	0.17	1.07	1.67	0.20
Ramsey	0.47	0.13	1.05	2.10	0.28
1103 Paulsen	0.30	0.13	1.07	2.24	0.30
140 Ruggeri	0.16	0.10	1.17	2.07	0.25
Schwarzmann	0.22	0.18	1.03	1.46	0.21
101-14	0.33	0.12	1.20	1.67	0.23
$LSD_{Rootstock}$	0.07 ***	0.03 ***	0.08 ***	0.15 ***	0.02 ***

(* = $P< 0.05$; ** = $P< 0.01$; *** = $P< 0.001$; ns = not significant)

Figure 1: Change in laminae Cl^-, Na^+ and K^+ concentrations throughout season 2006-2007 for Chardonnay (top) and Shiraz (bottom). Symbols are ● Own roots, ○ Ramsey, ▼ 1103 Paulsen, △ 140Ruggeri, ■ Schwarzmann, and □ 101-14. Bars represent least significant differences (P<0.05); ns = not significantly different.

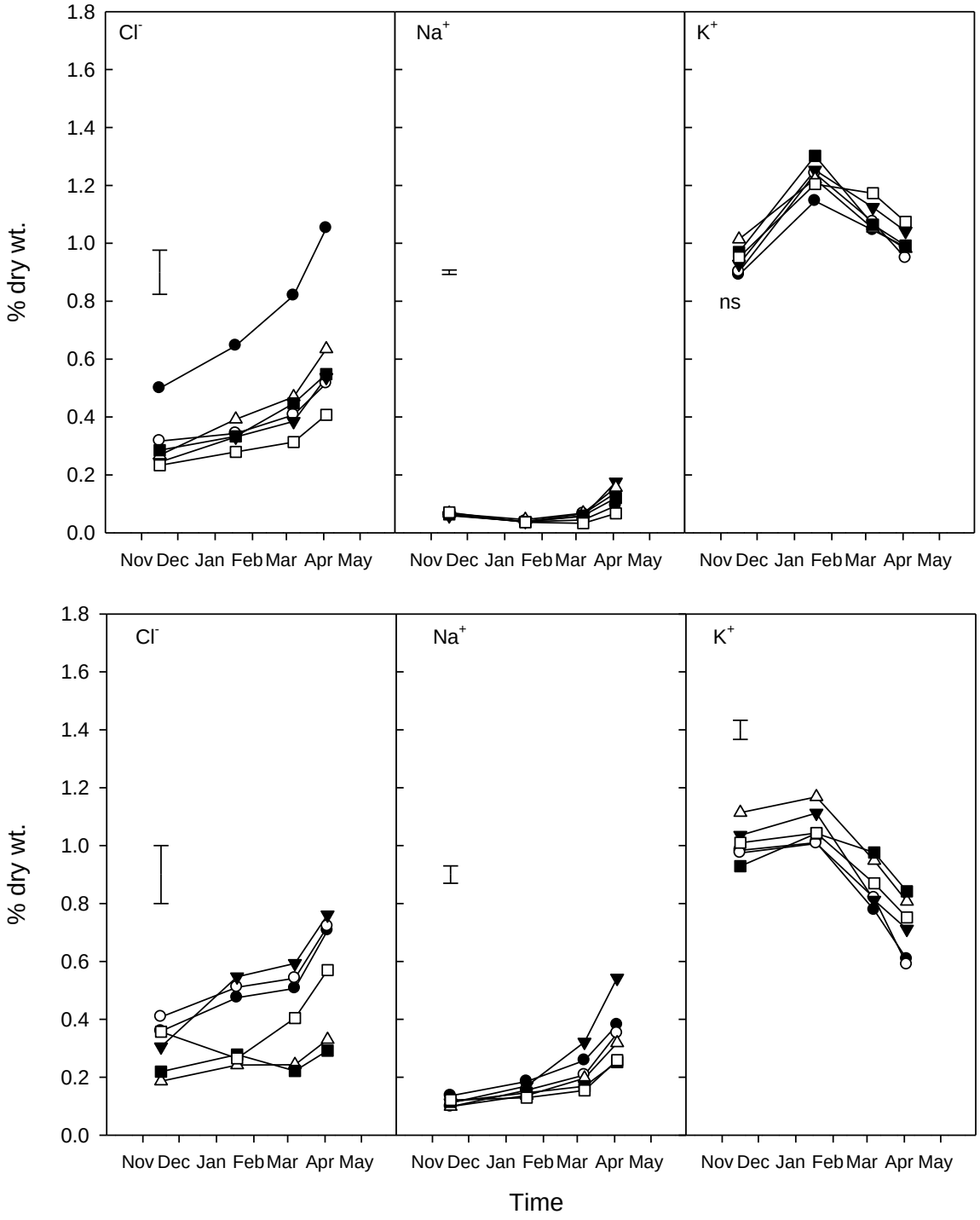


Table 3: Four year average (seasons 2006-07, 2007-08, 2008-09, 2009-10) of yield, berry weight, juice soluble solids, pH and titratable acidity of (A) Chardonnay and (B) Shiraz grapes grown on own roots, or on Ramsey, 1103 Paulsen, 140 Ruggeri, Schwarzmann and 101-14 rootstocks. LSD = least significant difference.

A. Chardonnay/ Rootstock	Yield (kg/vine)	Berry wt. (g)	Juice TSS (°Brix)	Juice pH	Juice TA (g/L)
Own roots	12.1	1.20	21.9	3.38	6.46
Ramsey	13.2	1.19	22.0	3.43	5.99
1103 Paulsen	11.7	1.18	21.7	3.46	6.15
140 Ruggeri	10.5	1.17	21.9	3.42	6.08
Schwarzmann	9.8	1.13	22.2	3.46	5.90
101-14	8.2	1.17	22.5	3.44	5.81
$LSD_{Rootstock}$	2.6 **	0.04 *	0.3 ***	0.06 ns	0.28 ***

B. Shiraz/ Rootstock	Yield (kg/vine)	Berry wt. (g)	Juice TSS (°Brix)	Juice pH	Juice TA (g/L)
Own roots	4.1	0.88	25.3	3.63	5.19
Ramsey	4.6	0.95	24.5	3.77	5.14
1103 Paulsen	6.2	0.89	25.0	3.82	5.03
140 Ruggeri	7.5	0.96	24.4	3.77	4.88
Schwarzmann	3.5	0.82	25.2	3.86	4.77
101-14	4.8	0.93	24.4	3.79	4.96
$LSD_{Rootstock}$	2.0 **	0.07 **	0.6 **	0.09 ***	0.26 *

(* = $P< 0.05$; ** = $P< 0.01$; *** = $P< 0.001$; ns = not significant)

Table 4: Three year average (2007-08, 2008-09 and 2009-10) of (A) Cl^- and Na^+ concentrations (log transformed) and (B) K^+, Ca^{2+}, Mg^{2+} concentrations (mg/L) of Chardonnay and Shiraz grape juice from vines grown on own roots, or grafted on Ramsey, 1103 Paulsen, 140 Ruggeri, Schwarzmann and 101-14 rootstocks. LSD = least significant difference.

A:	Chardonnay		Shiraz	
Rootstock	Cl^-	Na^+	Cl^-	Na^+
	(log_{10} transformed concentration , mg/L)			
Own roots	1.88^{a}	1.58^{cd}	2.04^{a}	1.67^{b}
Ramsey	1.62^{b}	1.96^{a}	1.94^{a}	1.64^{b}
1103 Paulsen	1.58^{b}	1.71^{bc}	2.04^{a}	1.88^{a}
Ruggeri 140	1.57^{b}	1.86^{ab}	1.64^{b}	1.52^{bc}
Schwarzmann	1.57^{b}	1.88^{a}	1.64^{b}	1.55^{b}
101-14	1.33^{c}	1.48^{d}	1.72^{b}	1.34^{c}
$LSD_{Rootstock}$	0.24	0.19	0.20	0.19

B:	Chardonnay			Shiraz		
Rootstock	K^+	Ca^{2+}	Mg^{2+}	K^+	Ca^{2+}	Mg^{2+}
Own roots	1477	50.8	75.5	1550	90.9	96.0
Ramsey	1471	54.2	80.7	1738	88.3	108.0
1103 Paulsen	1516	55.2	83.6	1855	85.0	121.5
Ruggeri 140	1476	56.1	86.7	1793	81.1	115.7
Schwarzmann	1393	54.5	80.9	1871	91.8	115.6
101-14	1425	54.3	86.0	1831	80.8	111.5
$LSD_{Rootstock}$	80.2 ns	3.1 *	3.2 ***	132.7 ***	4.4 ns	7.1 ***

(* = $P< 0.05$; ** = $P< 0.01$;*** = $P< 0.001$; ns = not significant)

Table 5: Three year average (2006-07, 2007-08 and 2008-09) of Cl^-, Na^+, K^+, concentrations and pH of (A) Chardonnay and (B) Shiraz wine made from grapevines grown on own roots, or grafted on Ramsey, 1103 Paulsen, 140 Ruggeri, Schwarzmann and 101-14 rootstocks. LSD = least significant difference.

A. Chardonnay/ Rootstock	Cl^-	Na^+	K^+	pH
		(mg/L)		
Own roots	127.5	46.4	647.4	3.18
Ramsey	72.7	101.6	538.9	3.25
1103 Paulsen	80.5	60.5	618.7	3.22
Ruggeri 140	84.4	90.2	521.9	3.21
Schwarzmann	79.2	100.4	525.3	3.24
101-14	46.9	46.7	551.0	3.20
$LSD_{Rootstock}$	22.5 **	20.6 **	90.2 ns	0.05 *

B. Shiraz/ Rootstock	Cl^-	Na^+	K^+	pH
		(mg/L)		
Own roots	383.0	91.0	1106.0	3.45
Ramsey	270.9	78.9	1029.1	3.48
1103 Paulsen	350.8	111.9	1086.7	3.44
Ruggeri 140	145.7	55.7	1003.6	3.47
Schwarzmann	117.2	63.4	974.8	3.46
101-14	260.8	56.5	1110.6	3.45
$LSD_{Rootstock}$	71.7 ***	21.9 **	39.9 ***	ns

(* = $P< 0.05$; ** = $P< 0.01$;*** = $P< 0.001$; ns = not significant)

Figure 2: Change in Cl^- and Na^+ concentrations during progression from juice, through must, to wine for Shiraz on own roots or grafted to Ramsey, 1103 Paulsen, 140 Ruggeri, Schwarzmann and 101-14 rootstocks. Grapes were processed on 9/3/2009. Bars represent least significant differences (P<0.05) for (a) juice, (b) must at 12/3/2009 and (c) wine.

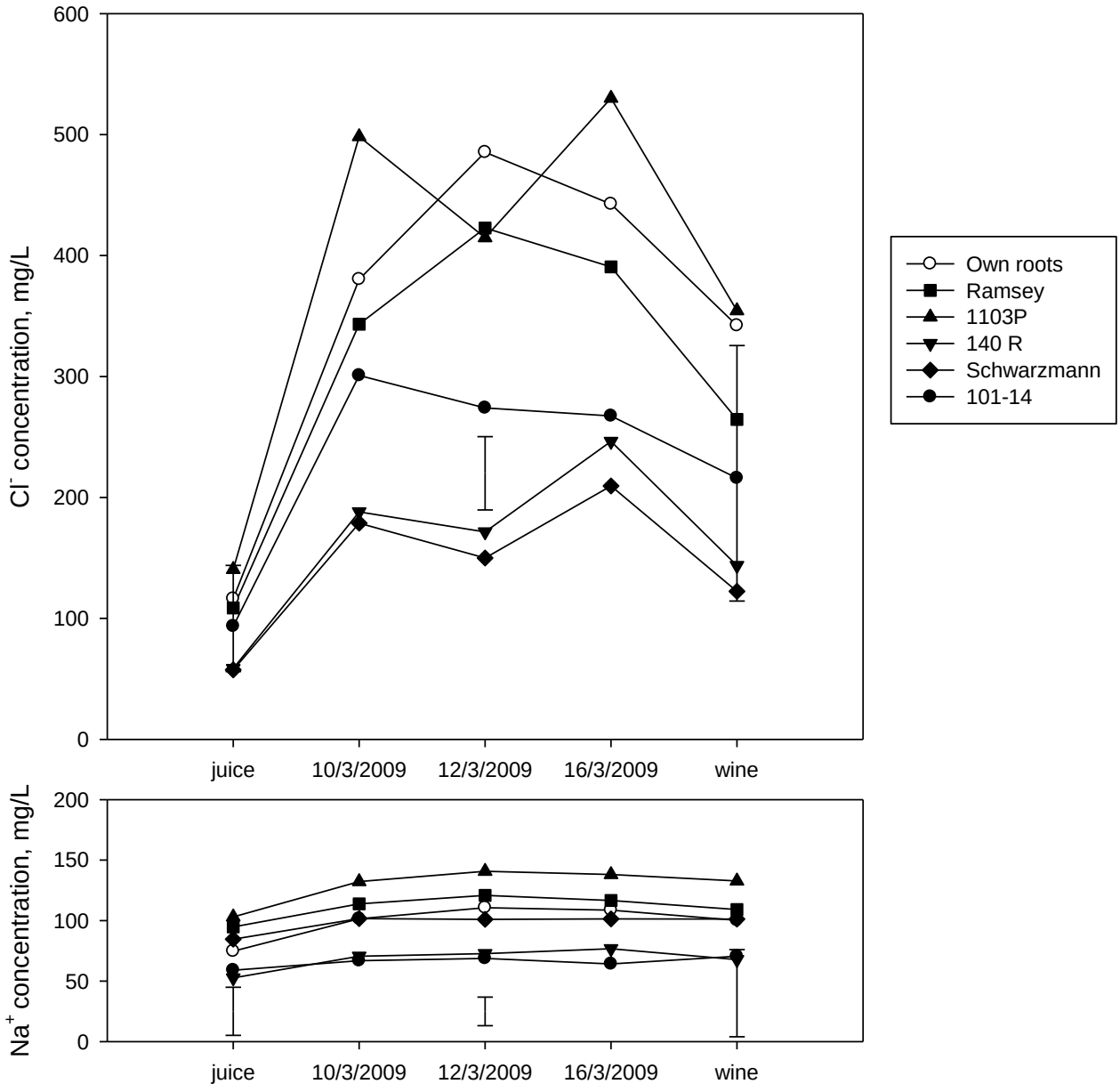


Figure 3: Relationship between grape juice and wine Cl^- and Na^+ concentrations for (A) Chardonnay and (B) Shiraz, based on data for vines on own roots or grafted to Ramsey, 1103 Paulsen, 140 Ruggeri, Schwarzmann and 101-14 rootstocks. Dotted lines provide an indication of wine ion concentration for a given grape juice ion concentration.

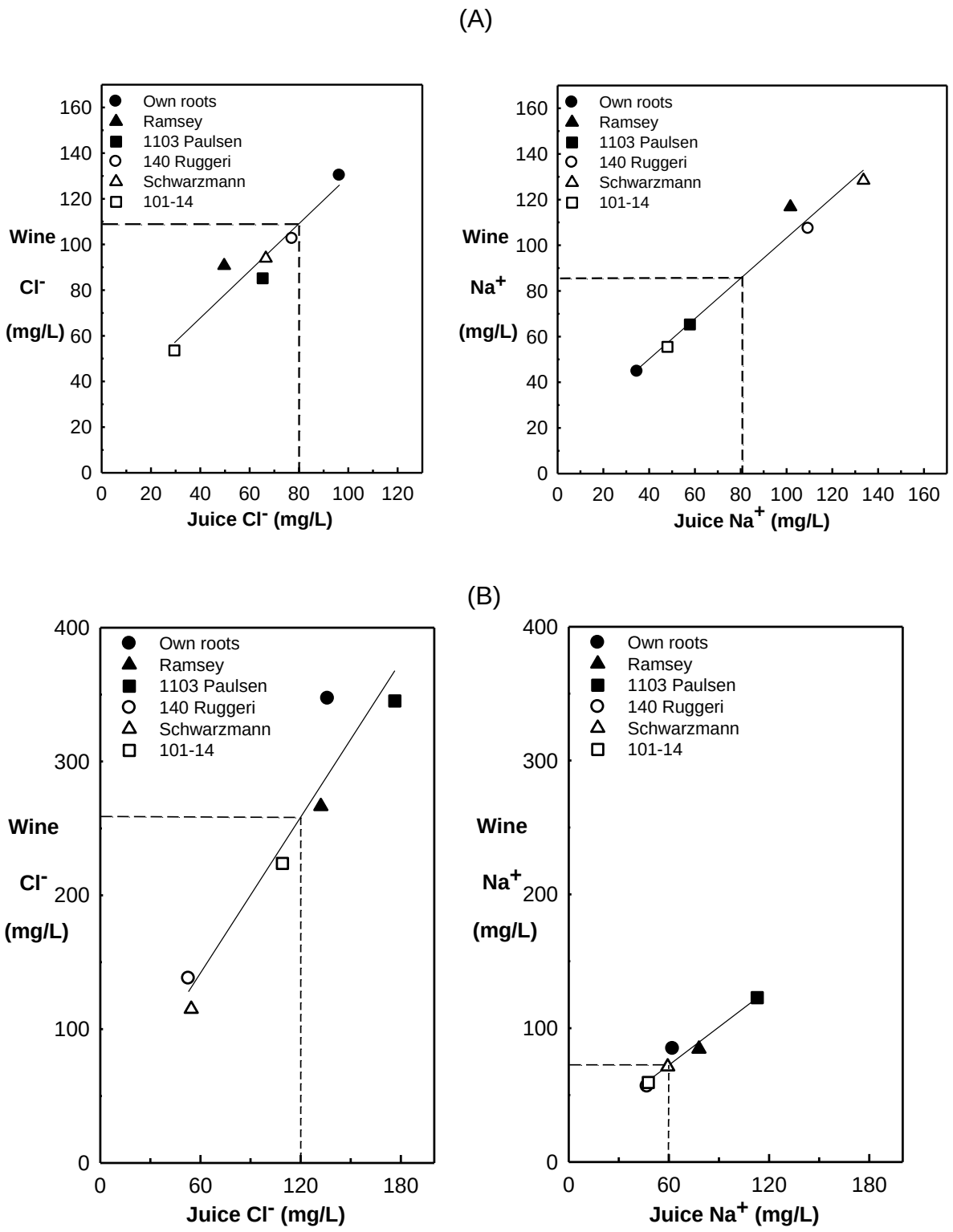
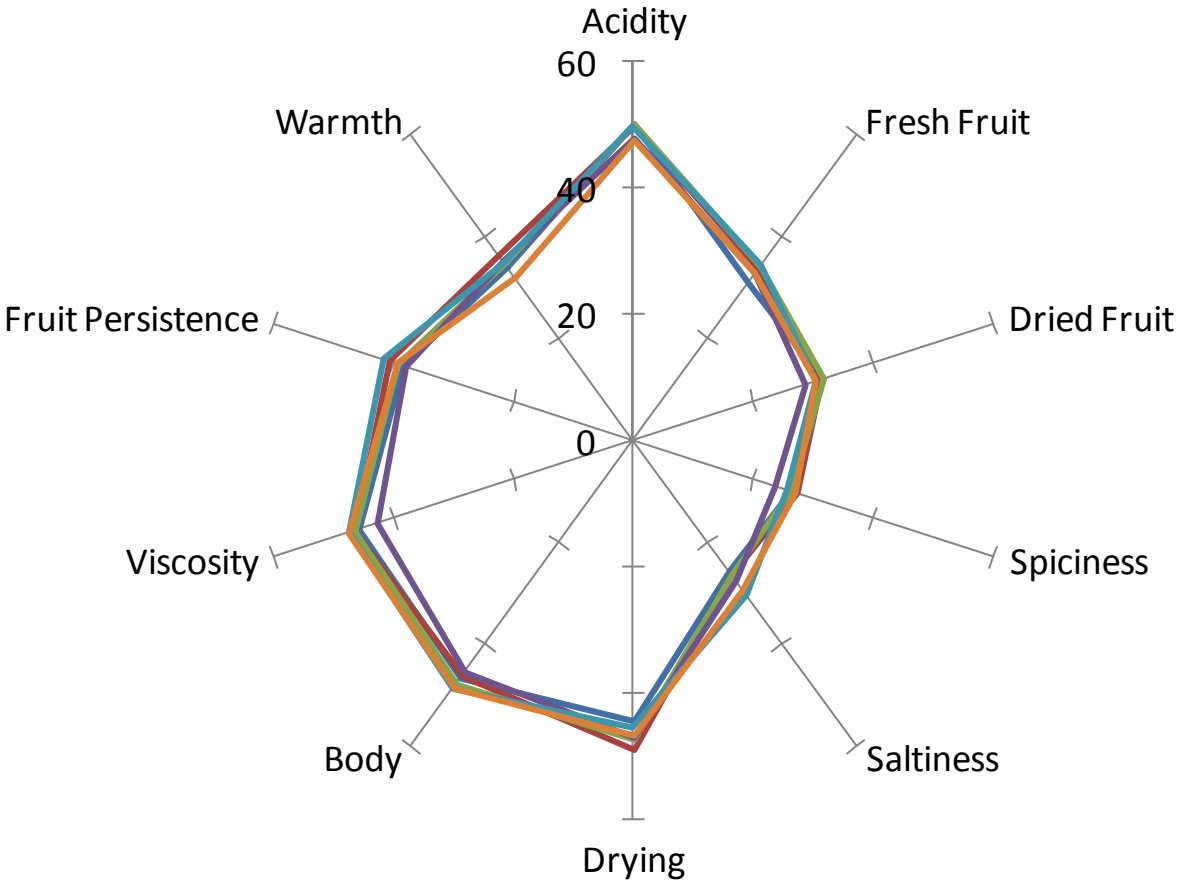


Figure 4: Sensory profile (palate) of Shiraz wines spiked with a range in Cl^- concentrations. Intensity scale: 0 = not present 100 = high intensity; ns = not significant



Attribute	Signficance[1]
Acidity	n.s.
Fresh Fruit	n.s.
Dried Fruit	n.s.
Spiciness	*P*<0.10
Saltiness	n.s.
Drying	*P*<0.20
Body	n.s.
Viscosity	n.s.
Fruit Persistence	n.s.
Warmth	n.s.

Triplicate rating from 10 assessors

Table 6: Three year average (2007-2009) of spectral properties colour density, colour hue, total anthocyanins, ionised anthocyanins and total phenolics of Shiraz wine made from grapes grown on own roots, or on Ramsey, 1103 Paulsen, 140 Ruggeri, Schwarzmann and 101-14 rootstocks.

Rootstock	Colour density (abs. units)	Colour hue (abs. units)	Total Antho. (mg/L)	Ionised Antho. (mg/L)	Total Phenolics (abs. units)
Own roots	14.75	0.53	502.4	126.6	48.2
Ramsey	12.83	0.57	432.4	106.2	48.2
1103 Paulsen	12.55	0.59	412.1	100.2	46.1
Ruggeri 140	12.55	0.57	426.5	99.2	45.0
Schwarzmann	14.67	0.58	484.0	118.8	52.8
101-14	14.51	0.53	486.7	127.0	49.9
LSDRootstock	0.80 ***	0.02 ***	49.8 **	6.4 ***	1.7 ***

(* = $P< 0.05$; ** = $P< 0.01$; *** = $P< 0.001$; ns = not significant)

Site 2: Wood versus laminae Cl^- and Na^+ concentrations in long established Shiraz vines:

This experiment was established to determine whether there was significant change in wood Cl^- and Na^+ concentrations between pre- and post-dormancy (i.e. pre-leaf fall and post-bud burst). Two locations in the same vineyard were sampled (Locations A and B). Laminae Cl^- and Na^+ concentrations were about 10-fold higher than wood concentrations (Figure 5). There was an increase in Cl^- and Na^+ concentrations in the wood pre-leaf fall. Concentrations then remained relatively stable in the period from post-bud burst to veraison, with concentrations at Location B slightly higher than at Location A (Figure 5). Pre-leaf fall Cl^- and Na^+ concentrations in laminae were higher than concentrations measured just after bud burst. At Location A, laminae Cl^- concentrations increased significantly in the period post-bud burst to veraison (Figure 5).

Figure 5: Cl^- and Na^+ concentration in laminae and wood of Shiraz vines, measured at two time points pre-leaf fall and 2-3 time points post-leaf fall. Values are means ± se.

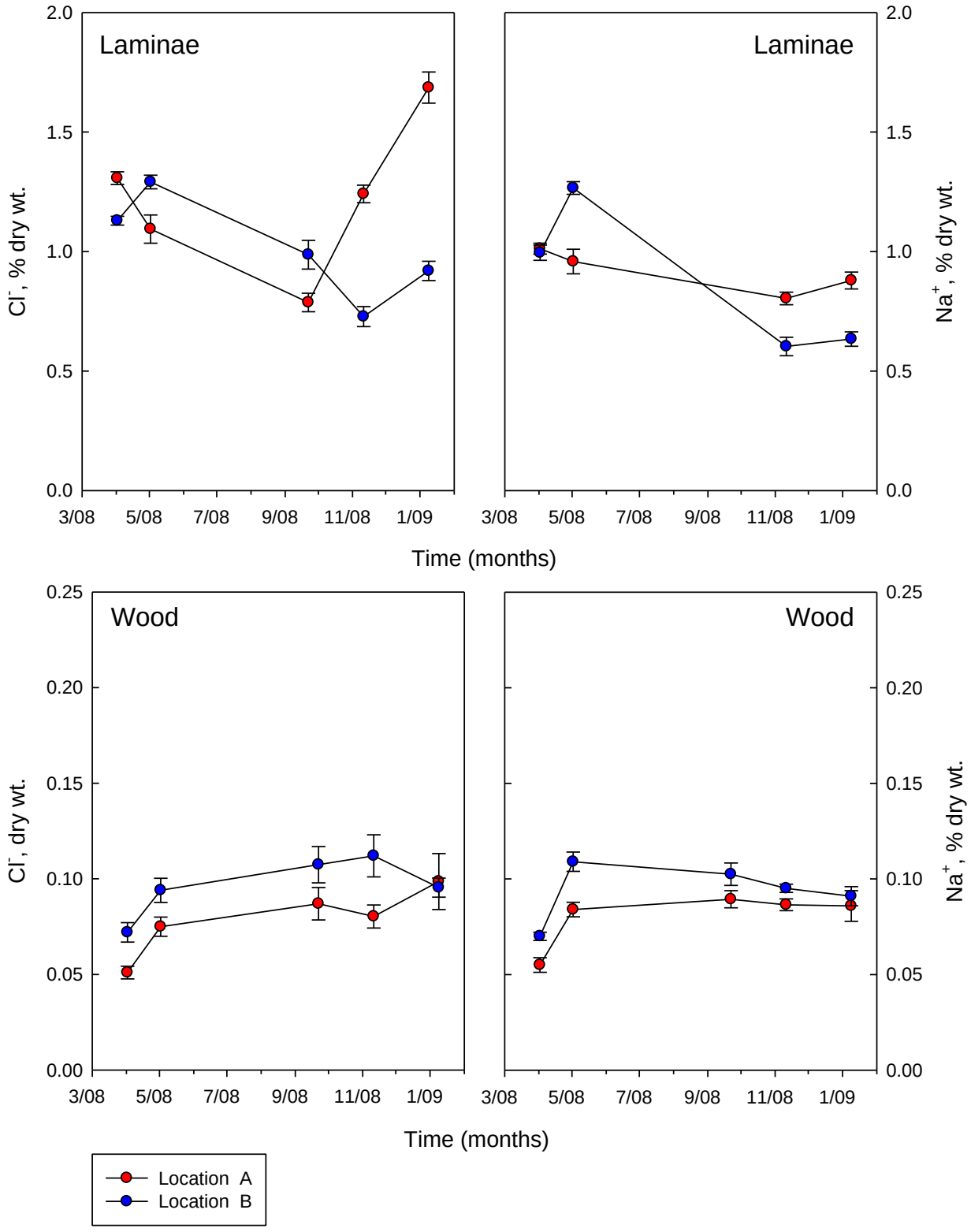
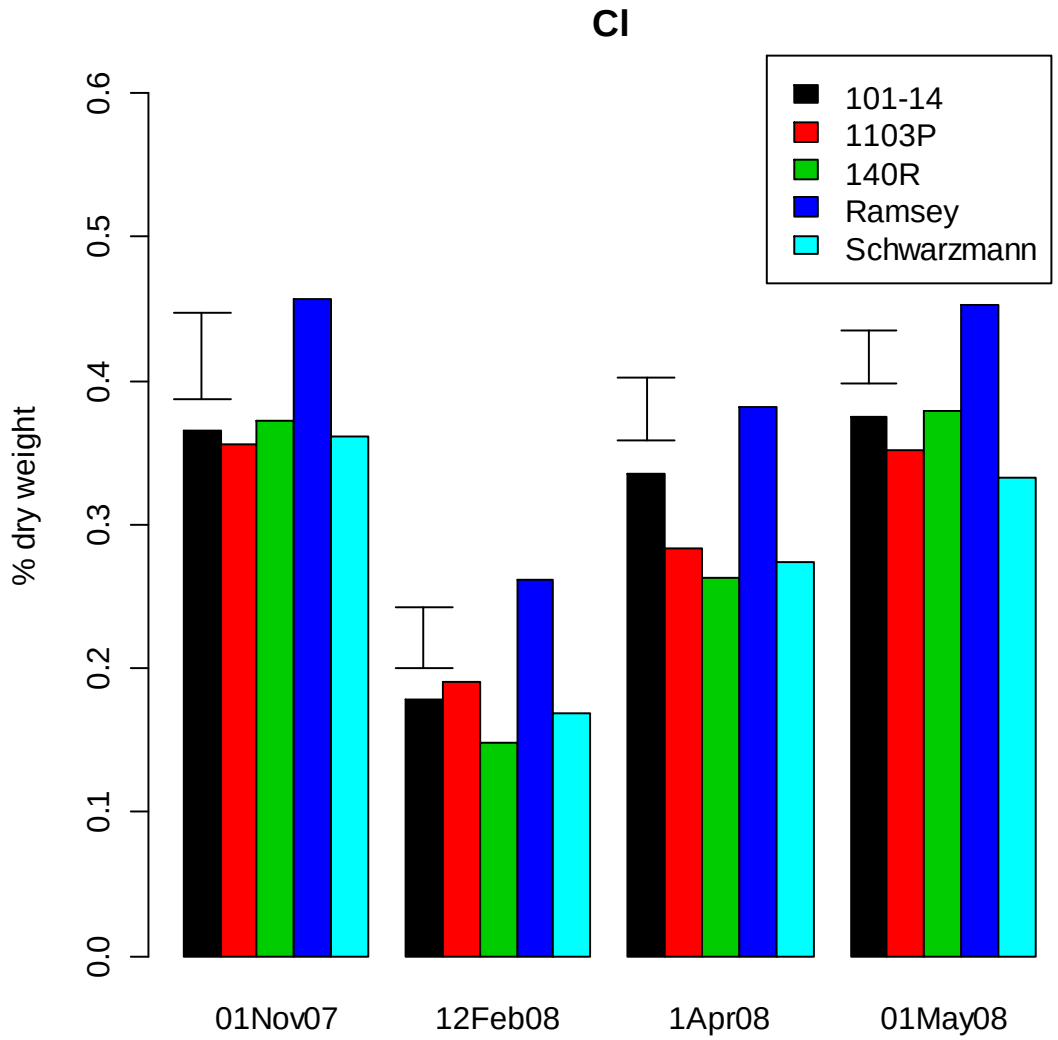


Figure 6: Comparison of Cl^- concentrations in laminae of Chardonnay grafted on Ramsey, 1103 Paulsen, 140 Ruggeri, Schwarzmann and 101-14 rootstocks at 4 time points throughout the course of the 2007-2008 season. Laminae were sampled opposite basal bunches.

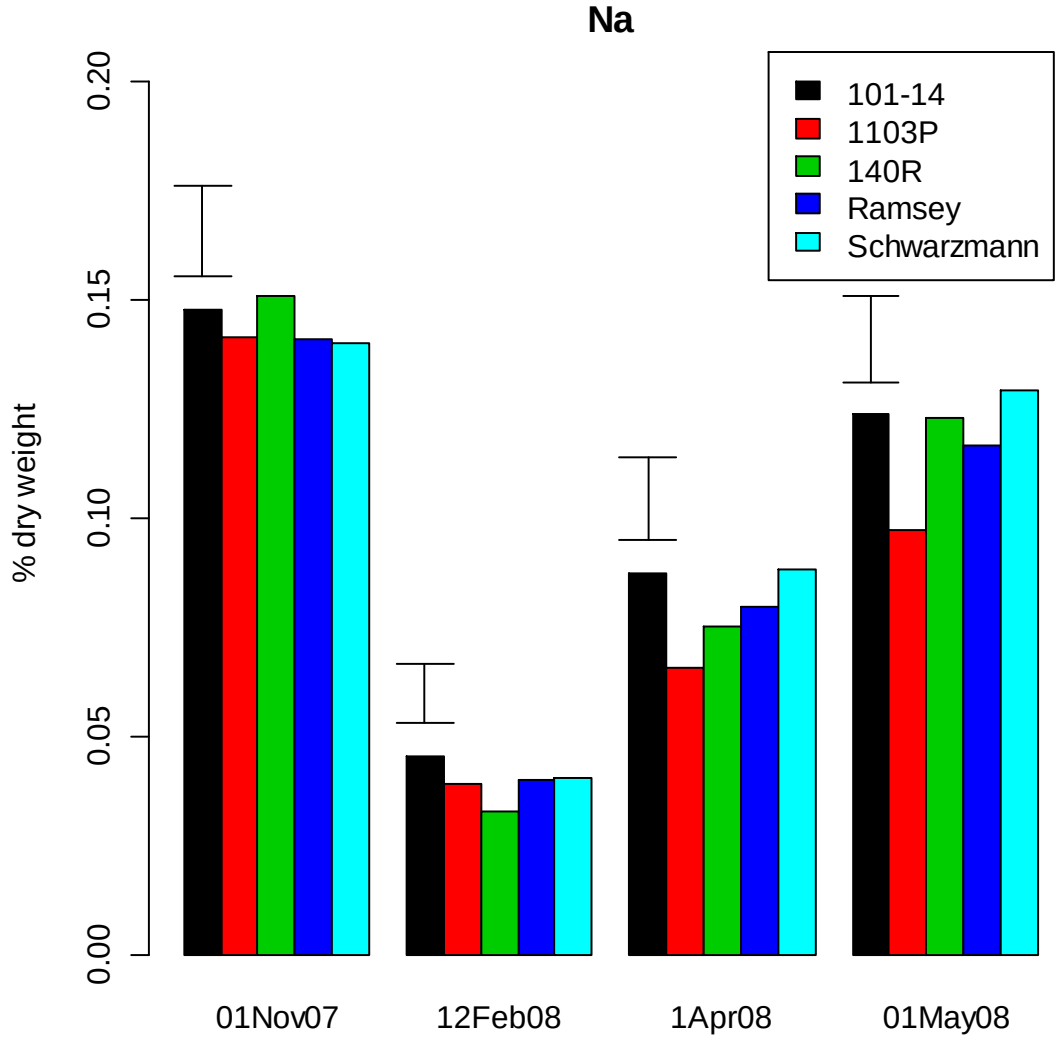


Site 2: Chardonnay on a range of rootstocks established in 2005:

Laminae Cl^- and Na^+ concentrations: Laminae sampled at four times throughout the season showed a reduction in both Cl^- (Figure 6) and Na^+ (Figure 7) concentrations (expressed on a dry weight basis) between November (flowering time) and early February. Concentrations then increased as the season progressed towards leaf fall. Chardonnay on Ramsey had highest concentrations of Cl^- in laminae at the November, February and May sampling times. At the April sampling time, laminae concentrations were again highest on Ramsey rootstock, but not significantly different from concentrations on 101-14 rootstock (Figures 6 and 7).

Soil electrical conductivity: Solusampler data for 25 cm, 50 cm and 75 cm depths showed a similar annual pattern with concentrations rising from December through to late March-late April before declining to reach winter-spring lows around August-September (Figure 8). Correlations were evident between Solusampler EC and lamina Cl^- (Figure 9A) and Na^+ (Figure 9B) concentrations, as evidenced from data obtained on 12 February 2008.

Figure 7: Comparison of Na^+ concentrations in laminae of Chardonnay grafted on Ramsey, 1103 Paulsen, 140 Ruggeri, Schwarzmann and 101-14 rootstocks at 4 time points throughout the course of the 2007-2008 season. Laminae were sampled opposite basal bunches.



There were no differences between the rootstocks in mean yield over the two seasons, nor were there any differences in mean grape juice TSS at harvest (Table 7). Berry weight was however, significantly higher for vines on Ramsey and 140 Ruggeri rootstocks (Table 7).

Mean grape juice Cl^- concentrations for the two seasons were all relatively low (range 20.7 to 24.9 mg/L) (Table 7) compared with those measured for Chardonnay vines from the longer established site (range 47.3 to 90.8 mg/L for vines on Ramsey, 1103 Paulsen, 140 Ruggeri and Schwarzmann). Only vines on 101-14 rootstock were similar (21.9 mg/L at the younger site compared with 26.0 at the longer established site). Similarly, mean grape juice Na^+ concentrations for the two seasons were all relatively low (range 16.8 to 21.9 mg/L) (Table 7) compared with those measured for Chardonnay vines at the longer established site (range 40.0 to 127.2 mg/L). Mean grape juice K^+, Ca^{2+} and Mg^{2+} concentrations for Chardonnay for the two seasons (Table 7) were all slightly higher at the younger site relative to those at the longer established site, but site factors as well as vine age may have contributed to this difference.

Figure 8: Changes in soil (Solusampler) EC under 5 rootstocks at 25, 50 and 75 cm depth.

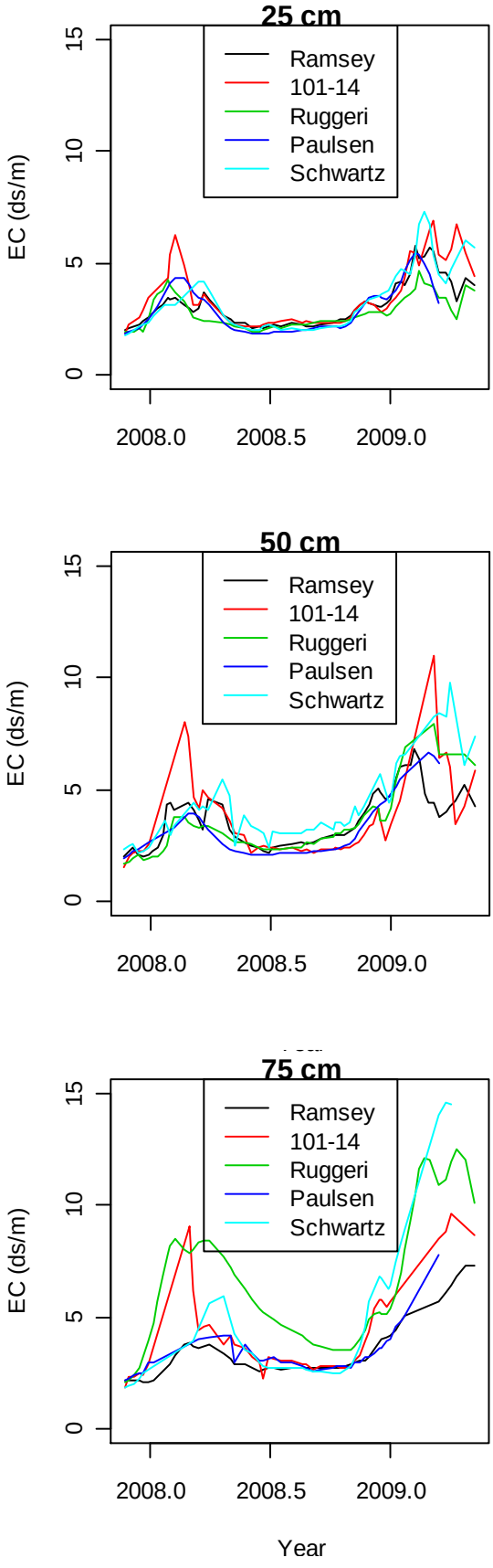
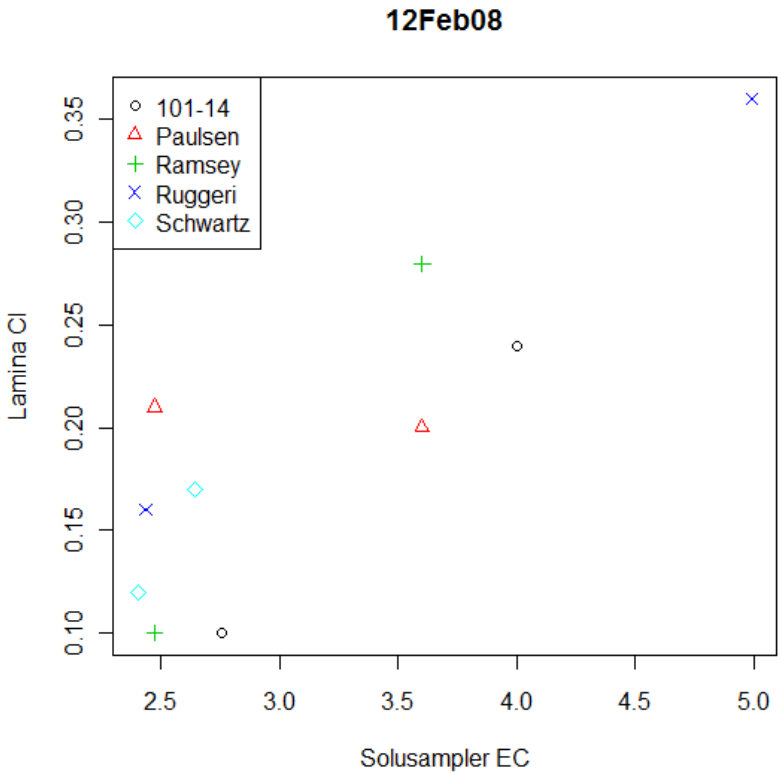


Figure 9: Relationship between Solusampler EC and laminae Cl^- (A) and Na^+ (B) concentrations (% dry weight). Solusampler and laminae samples were taken on 12 Feb. 2008.

(A)



(B)

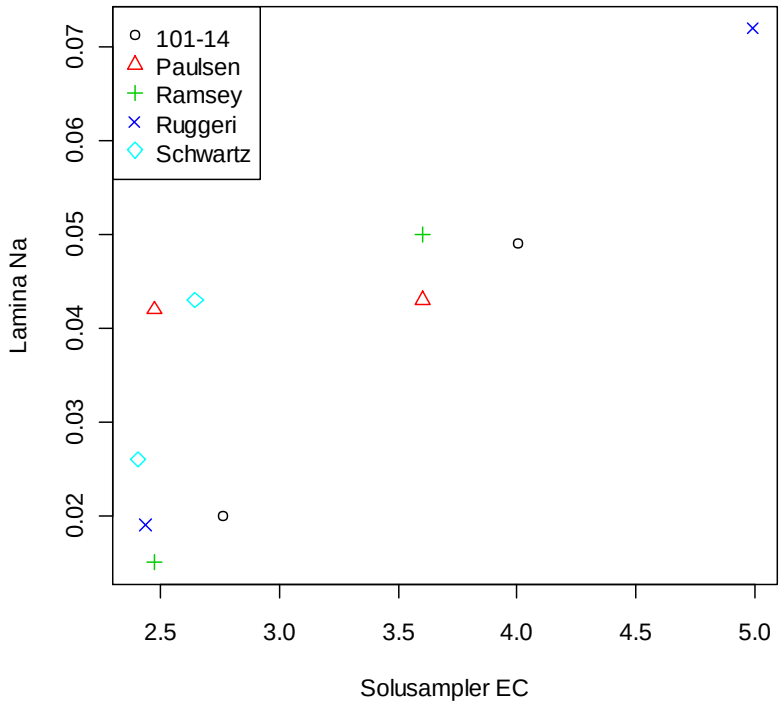


Table 7: Two year means (2009-2010) for (A) yield, berry weight, juice soluble solids, pH, titratable acidity and pruning weight, and (B) grape juice Cl^-, Na^+, K^+, Ca^{2+} and Mg^{2+} concentrations of Chardonnay grapes grown on Ramsey, 1103 Paulsen, 140 Ruggeri, Schwarzmann and 101-14 rootstocks at Padthaway SA.

A. Rootstock	Yield (kg/vine)	Berry wt. (g)	Juice TSS (°Brix)	pH	TA (g/L)	Pruning wt. (kg/vine)
Ramsey	8.8	1.29	21.9	3.60	5.69	1.10
1103 Paulsen	9.7	1.23	22.0	3.56	5.75	1.05
140 Ruggeri	9.6	1.27	21.9	3.57	5.65	0.93
Schwarzmann	8.9	1.20	21.5	3.59	5.32	0.65
101-14	8.7	1.20	21.9	3.59	5.19	0.82
$LSD_{Rootstock}$	1.2 ns	0.04 ***	0.4 ns	0.03 ns	0.25 ***	0.13 ***

B. Rootstock	Cl^-	Na^+	K^+ (mg/L)	Ca^{2+}	Mg^{2+}
Ramsey	24.9	18.4	1635	60.3	96.6
1103 Paulsen	21.3	17.3	1632	62.0	101.7
140 Ruggeri	20.7	21.9	1610	58.7	101.6
Schwarzmann	20.8	21.9	1595	62.9	91.4
101-14	21.9	16.8	1595	58.8	97.0
$LSD_{Rootstock}$	2.8 *	1.8 ***	57.5 ns	3.0 *	4.9 ***

(* = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$; ns = not significant)

6.2 Effects of salinity on inorganic/organic anion inter-relationships in grape berries:

6.2.1 Partitioning of inorganic ions and organic acids in Shiraz and Chardonnay grape berries:

Chloride, Na^+, K^+, malic and tartaric acid concentrations were higher in almost all cases in skin than in pulp (Tables 8, 9, 10 and 11). For example, K^+ was 4.0 (1103 Paulsen at Padthaway) to 5.5 (Shiraz on own roots at Padthaway) times higher in the skin than in the pulp, while Cl^- was 3.8 (Merbein Shiraz on 1103 Paulsen) to 26.3 (Merbein Chardonnay on 1103 Paulsen) times that in pulp, and Na^+ was 1.6 (Chardonnay on 1103 Paulsen and 140 Ruggeri at Merbein) to 2.5 (Shiraz on own roots at Merbein) times that in pulp. Significant positive correlations existed between Cl^- and Na^+ concentrations in both pulp and skin of grapes of both varieties at both locations (not shown).

Tartaric acid concentration was lower in wine from grafted vines of the three rootstocks (not shown). Addition of tartaric acid for pH adjustment of wine from own-rooted vines, but not for wine of grafted vines, would have contributed. As a result, the ratio of tartaric/malic acid in wine was lower when made from Ramsey and 1103 Paulsen grafted vines than from own-rooted vines (not shown).

The average malic acid concentrations in both pulp and skin between the two sites were obviously different, being higher at Padthaway (Tables 8 and 9) than Merbein (Tables 10 and 11). At both sites, Chardonnay and Shiraz grafted to the three rootstocks had lower skin tartaric/malic acid ratios compared with those on their own roots, except for Chardonnay on 140 Ruggeri at Padthaway, which did not show a statistically significant difference compared with own-rooted vines (Tables 9 and 11). A significant negative linear regression existed between malic acid and both Cl^- and Na^+ concentrations in skin of Chardonnay berries (not shown). No regression relationship between malic acid and Cl^- or Na^+ in the berry pulp was observed.

6.2.2 Inorganic ions and organic acids in wines:

Positive linear correlations existed for Cl^- concentrations between wine and pulp in Chardonnay, and between wine and both pulp and skin Cl^- concentrations in Shiraz (Figure 10). There were also linear correlations between wine Na^+ concentration and both pulp and skin Na^+ concentrations in Shiraz, while there was no correlation between wine and pulp Na^+ in Chardonnay (Figure 10). It is notable that there was a 2.5-fold increase in Cl^- concentrations between pulp and wine, but little if any increase in Na^+ concentrations (Figure 10).

6.2.3 Further detail:

Further detail can be found in the following paper:

Gong, H., Blackmore D.H. and Walker R.R. (2010a) Organic and inorganic anions in Shiraz and Chardonnay grape berries and wine as affected by rootstock under saline conditions. Australian Journal of Grape and Wine Research 16, 227-236.

Table 8: Rootstock effects on inorganic ions (µmol g^{-1}FW) in Chardonnay and Shiraz grape berries at Padthaway.

Padthaway Scion	Rootstock	Cl^-		K^+		Na^+	
		Pulp	Skin	Pulp	Skin	Pulp	Skin
Chardonnay	Own roots	2.48	25.8	28.6	115.1	1.7	3.3
	Ramsey	0.61	7.9	28.9	116.2	1.5	2.6
	1103 Paulsen	1.40	10.2	29.1	115.0	1.3	2.5
	140 Ruggeri	1.35	13.3	27.0	112.2	2.0	3.7
	LSD	0.79	6.3	ns	ns	ns	ns
Shiraz	Own roots	2.21	25.2	22.4	123.3	1.8	4.3
	Ramsey	1.31	14.3	26.2	121.1	1.2	2.6
	1103 Paulsen	1.75	18.5	25.5	137.1	1.4	3.4
	140 Ruggeri	0.64	8.5	26.7	131.2	1.1	2.5
	LSD	0.59	5.6	2.2	ns	0.4	0.8

FW, fresh weight; LSD, least significant difference between rootstocks (*P*<0.05); ns, not significantly different.

Table 9: Rootstock effects on organic acids (mg g^{-1}FW) in Chardonnay and Shiraz grape berries at Padthaway.

Padthaway Scion	Rootstock	Malic acid		Tartaric acid		Tartaric/malic acid	
		Pulp	Skin	Pulp	Skin	Pulp	Skin
Chardonnay	Own roots	2.14	5.19	2.55	5.94	1.20	1.16
	Ramsey	1.84	6.10	2.35	5.89	1.31	0.99
	1103 Paulsen	2.18	5.94	2.24	5.89	1.08	1.01
	140 Ruggeri	1.95	5.52	2.52	6.05	1.36	1.11
	LSD	0.25	0.68	ns	ns	0.19	0.11
Shiraz	Own roots	1.36	2.93	2.11	4.74	1.57	1.71
	Ramsey	1.58	3.43	2.25	4.13	1.44	1.24
	1103 Paulsen	1.64	3.63	2.13	4.55	1.31	1.28
	140 Ruggeri	1.71	3.85	2.32	4.52	1.41	1.19
	LSD	0.23	0.56	ns	0.40	ns	0.17

FW, fresh weight; LSD, least significant difference between rootstocks (*P*<0.05); ns, not significantly different.

Table 10: Rootstock effects on inorganic ions (µmol g^{-1}FW) in Chardonnay and Shiraz grape berries at Merbein.

Merbein Scion	Rootstock	Cl^-		K^+		Na^+	
		Pulp	Skin	Pulp	Skin	Pulp	Skin
Chardonnay	Own roots	18.06	98.0	25.3	102.6	23.5	54.1
	Ramsey	4.00	41.7	29.6	123.6	10.3	17.4
	1103 Paulsen	0.65	17.1	28.1	127.3	2.3	3.8
	140 Ruggeri	0.73	15.8	29.6	120.6	3.7	6.1
	LSD	4.16	16.8	ns	15.3	6.8	14.5
Shiraz	Own roots	12.68	72.3	22.9	103.2	14.0	35.1
	Ramsey	9.05	54.5	30.6	142.1	13.1	28.3
	1103 Paulsen	11.71	44.4	30.9	122.9	12.2	27.2
	140 Ruggeri	2.39	17.7	26.8	114.6	4.2	8.1
	LSD	7.07	25.7	3.1	20.7	ns[#]	19.1

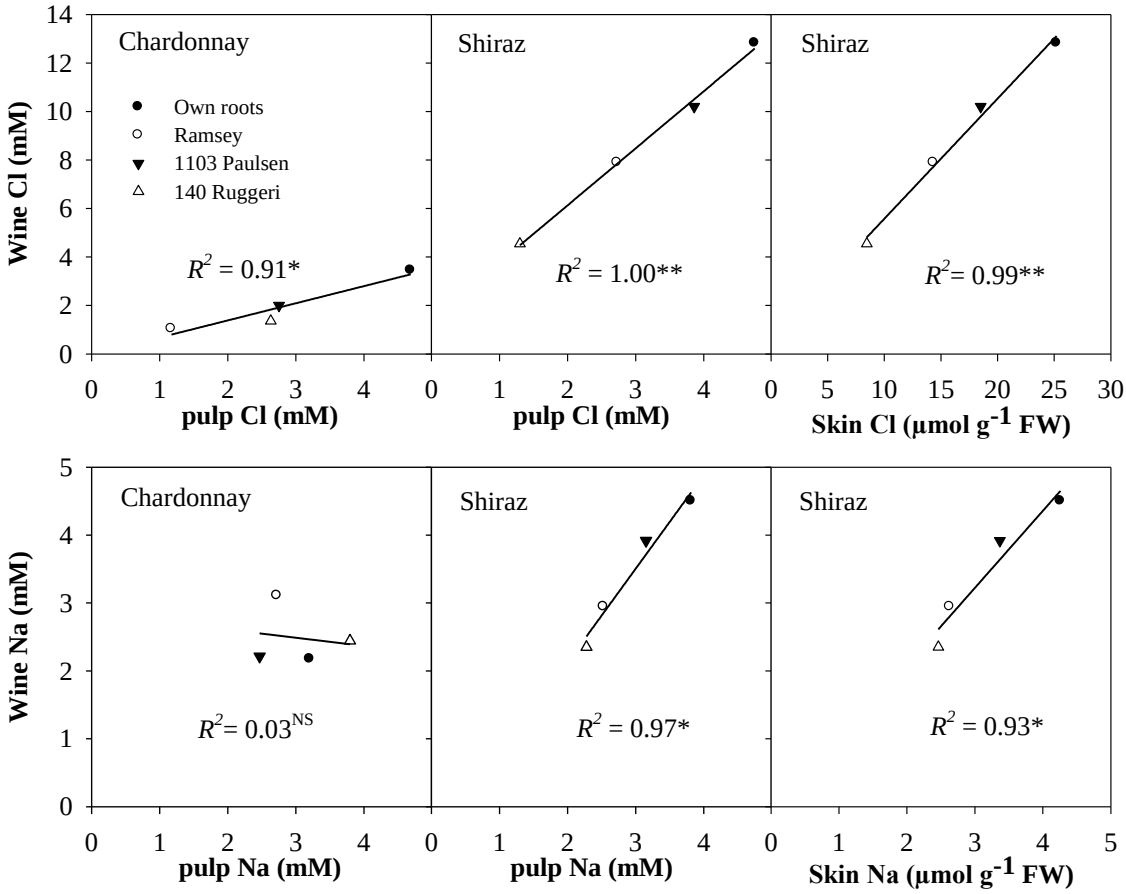
FW, fresh weight; LSD, least significant difference between rootstocks (P<0.05); ns, not significantly different. [#] P<0.053.

Table11: Rootstock effects on organic acids (mg g^{-1}FW) in Chardonnay and Shiraz grape berries at Merbein.

Merbein Scion	Rootstock	Malic acid		Tartaric acid		Tartaric/malic acid	
		Pulp	Skin	Pulp	Skin	Pulp	Skin
Chardonnay	Own roots	1.06	0.89	2.92	3.22	2.86	4.03
	Ramsey	1.37	3.35	2.80	4.25	2.07	1.35
	1103 Paulsen	1.24	3.67	2.48	4.48	2.01	1.22
	140 Ruggeri	1.05	3.45	2.62	4.32	2.50	1.31
	LSD	0.18	0.83	0.22	0.67	0.47	0.72
Shiraz	Own roots	0.85	1.14	2.08	4.93	2.46	4.83
	Ramsey	1.34	2.54	2.24	5.31	1.76	2.22
	1103 Paulsen	1.04	2.01	1.74	5.55	1.71	3.20
	140 Ruggeri	0.89	2.48	1.79	6.18	2.03	3.06
	LSD	0.33	0.99	ns	ns	0.46	1.53

FW, fresh weight; LSD, least significant difference between rootstocks (P<0.05); ns, not significantly different.

Figure 10: Correlation between wine and berry Cl^- and Na^+ concentrations at Padthaway.



6.3 Variation for Cl^- and Na^+ exclusion in a family from a cross between grapevine rootstocks K 51-40 and 140 Ruggeri:

6.3.1 Chloride concentrations for vines in the pot trial:

There were significant differences (P<0.001) between hybrids for mean Cl^- concentrations in laminae (Figure 11A), and this variation was continuous and normally distributed (Figure 15A). Hybrid 'HB63' showed lowest laminae Cl^- concentration while 'HB18' had the highest. There was a significant difference (P<0.002) in laminae Cl^- concentrations between the two parents, with 140 Ruggeri having half that of K 51-40. The male parent 140 Ruggeri sat at the low Cl^- concentration range and the female parent K 51-40 sat at the high concentration range (Figure 11A). The average lamina Cl^- concentration for the hybrid family was 22.16 mg/g dw, which was higher than 140 Ruggeri and lower than K 51-40. There was no evidence of transgressive segregation, there being no significant differences between the parent cultivars and the hybrids that fell at the end of the distributions presented in Figure 11A. Similar results were observed for petiole Cl^- concentrations (Figure 11B). There was a strong correlation between laminae and petiole Cl^- concentrations, with r^2 being 0.76 (P<0.001).

In the root, there were also significant differences (P<0.001) between hybrids for mean Cl^- concentrations, with 'HB49' showing the highest and 'HB 20' the lowest Cl^- concentrations in this hybrid family (Figure 11C). There was no significant difference for mean root Cl^- concentration between K 51-40 and 140 Ruggeri vines (Figure 11C). Three hybrids (HB10, HB25 and HB49) showed apparent transgressive segregation and had higher root Cl^- concentrations compared to their parents (Figure 11C). There was no relationship between root Cl^- concentrations and either laminae or petiole chloride concentrations.

6.3.2 Chloride concentrations for vines in the solution culture trial:

There were significant differences (P<0.001) between hybrids for laminae Cl^- concentrations and the variation was continuous (Figure 12A). There was a seven-fold difference between the parent cultivars for mean laminae Cl^- concentrations. Hybrid MI39 had the highest mean lamina Cl^- concentration in the family, which was significantly greater than that for K 51-40 (Figure 12A). K 51-40 vines had a 3.5 fold higher mean petiole Cl^- concentration than those of 140 Ruggeri (Figure 12B). Two hybrids, HB21 and HB76 had significantly higher mean petiole Cl^- concentrations than K 51-40 (Figure 12B). A strong correlation existed between laminae and petiole Cl^- concentrations, with an r^2 value of 0.71 (P<0.001). Roots of K 51-40 had significantly higher mean Cl^- concentrations than those of 140 Ruggeri vines (Figure 12C). The distribution of mean root Cl^-concentrations remained continuous in the hybrid family in solution culture conditions (Figure 12C), but the variation was less than that in the pot trial. There were no correlations between mean root Cl^- concentrations and either mean laminae or petiole Cl^- concentrations.

6.3.3 Sodium concentrations for vines in the pot trial:

There was no significant difference for mean laminae Na^+ concentrations between K 51-40 and 140 Ruggeri vines, but there were significant differences between hybrids (Figure 13). The mean concentration for the hybrids was 2.32 mg/g dw, which was higher than that for either parent. There were 13 hybrids which showed significantly higher laminae Na^+ concentration than their parents. These were HB4, HB18, HB25, HB30, HB43, HB49, HB61, HB63, HB78, HB79, MI33, MI36 and MI47 (Figure 13). The distribution of Na^+ accumulation was skewed (Figure 15 B) and different from that of Cl^- (Figure 15 A).

6.3.4. Sodium concentrations for vines in the solution culture trial:

There were significant differences in mean laminae Na^+ concentrations between 'HB' and 'MI' hybrids and, thus, the data were analysed separately (Figure 14A and 14B). There were significant differences (P<0.001) in Na^+ concentrations between 'HB' hybrids (Figure 14A). Significant differences (P<0.001) in Na^+ exclusion also existed among 'MI' hybrids (Figure 14B). Both sets of hybrids showed skewed frequency distributions, with skewness being 1.42 and 1.96 for 'HB' and 'MI' hybrids, respectively.

6.3.5 Correlation between Na^+ and Cl^- in vines:

Na^+ and Cl^- concentrations in laminae were correlated significantly for vines in the pot trial (r^2 = 0.13; P< 0.005). In the solution culture trial, r^2 was 0.01 (P< 0.588) and 0.40 (P< 0.035) for 'HB' hybrids and 'MI' hybrids, respectively (not shown).

Figure 11: Mean (n=6) Cl^- concentrations in laminae, petioles and roots of vines propagated clonally from K 51-40 × 140 Ruggeri hybrids, their parents and two reference rootstocks grown in pots under glasshouse conditions and irrigated daily with a complete nutrient solution containing 50 mM Cl^- for 27 days.

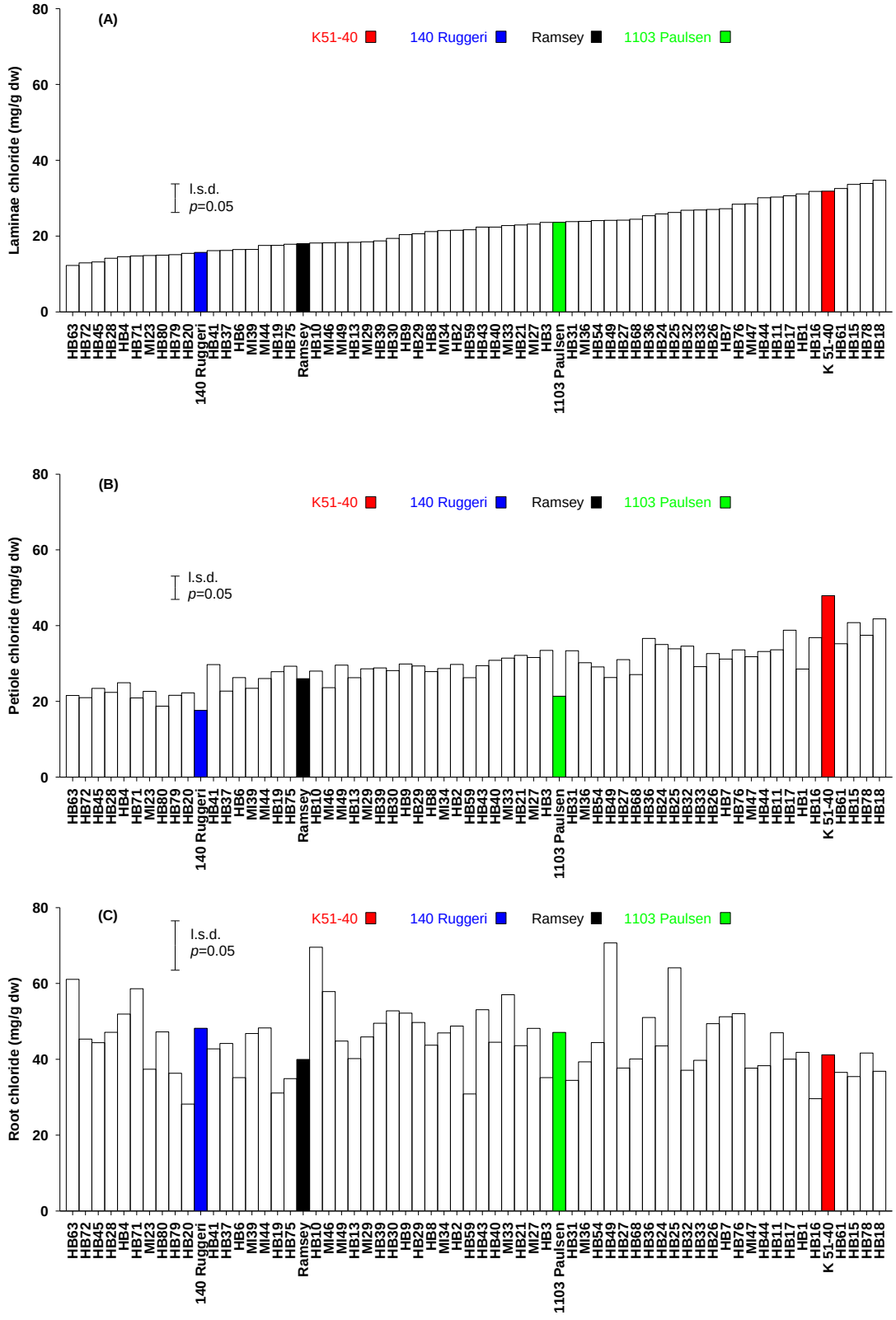


Figure 12: Mean (n=6) Cl^- concentrations in laminae, petioles and roots of vines propagated clonally from K 51-40 × 140 Ruggeri hybrids, their parents and two reference rootstocks grown in aerated nutrient solution cultures under glasshouse conditions containing 25 mM Cl^- for 27 days.

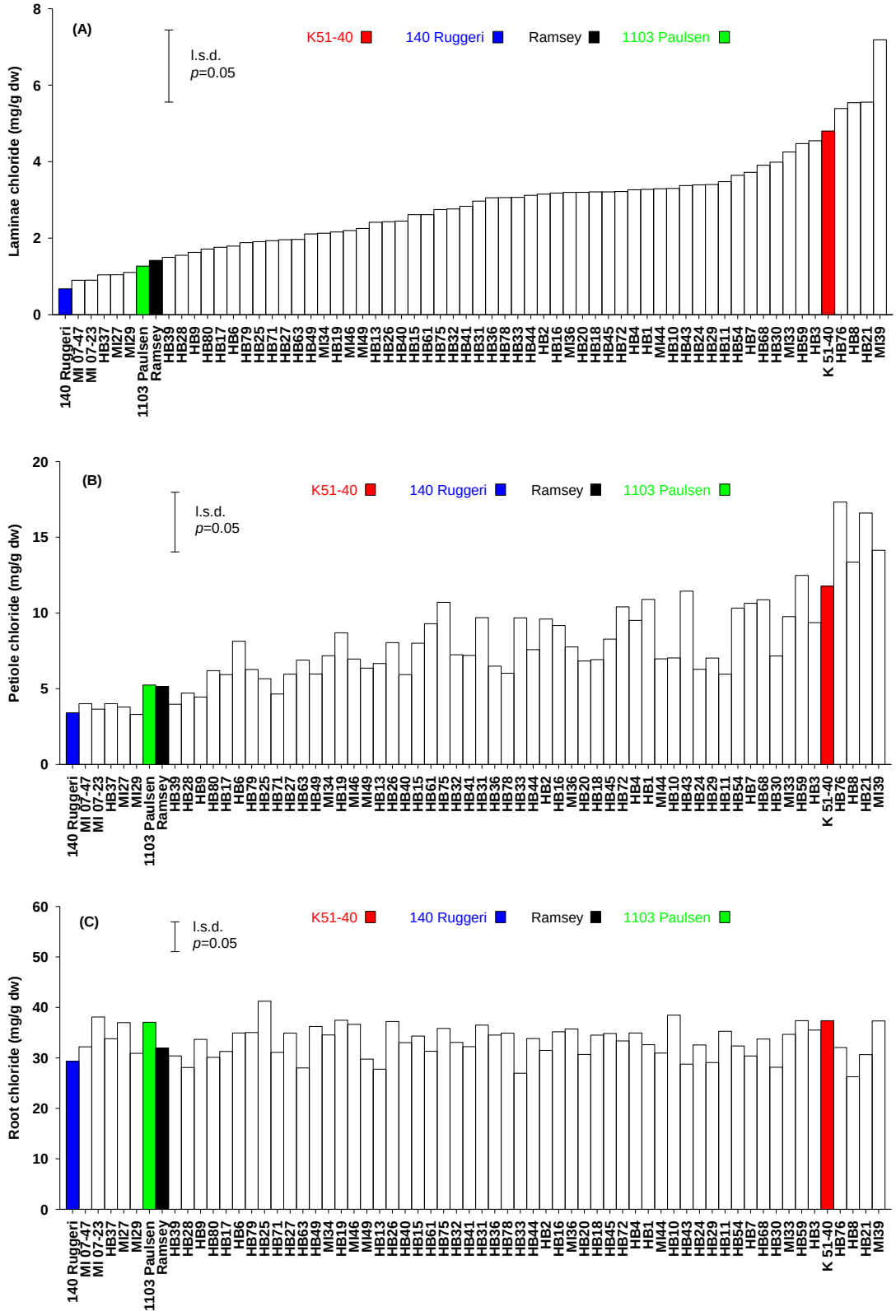
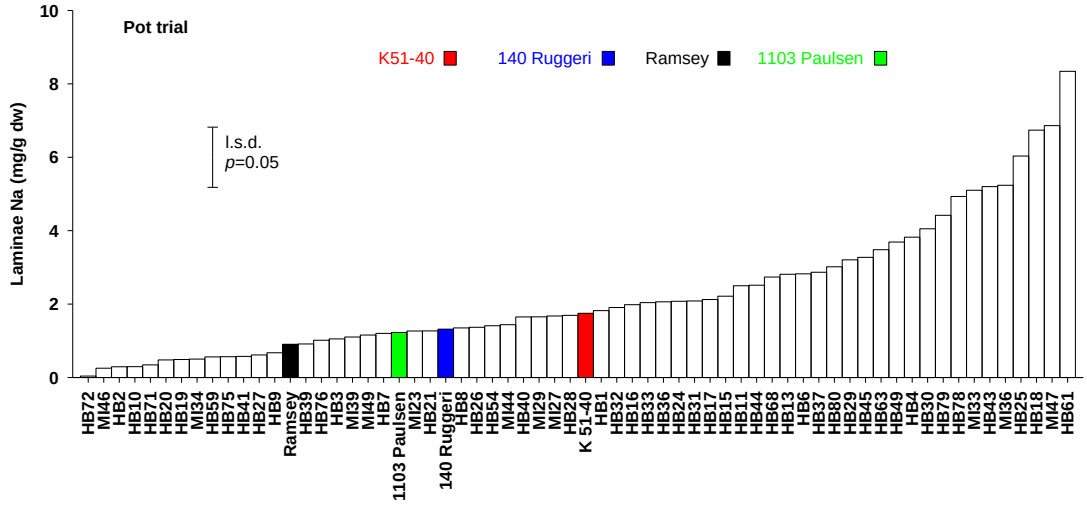


Figure 13: Mean (n=6) Na^+ concentrations in laminae of vines propagated clonally from K 51-40 × 140 Ruggeri hybrids, their parents and two reference rootstocks grown under glasshouse conditions in pots irrigated with nutrient solution containing 30 mM Na^+ for 27 days.



6.3.6. Comparison of data obtained in pot and solution culture trials:

Mean Cl^- concentrations of vines grown in potting mix (Figure 11) showed no overall relationship with those from the solution culture trial (Figure 12) (data not shown). Mean Cl^- concentrations for some hybrids, however, were similar between the two trials (Figures 11 and 12). For example, HB28, HB37, HB63, HB71, HB79, HB80 and MI23 had low lamina Cl^- concentrations in both experiments and HB76 had high leaf Cl^- concentrations regardless of root medium. Conversely, the mean leaf Cl^- concentrations of HB17, MI39 and MI47 were not correlated between experiments. HB17 and MI47 had high lamina Cl^- concentrations in the pot trial (Figure 11 A), whereas in the solution culture trial (Figure 12A), their concentrations were low. MI39 had low lamina Cl^- concentrations in the pot trial, however it was one of the poorest Cl^- excluders in solution culture trial – this was possibly due to its relatively poor vigour at the commencement of salt treatment in the solution culture trial. Although there was no significant correlation for leaf Cl^- concentrations between the two experiments, variation for leaf Cl^- concentrations among hybrids remained continuous in both trials (Figures 11 and 12).

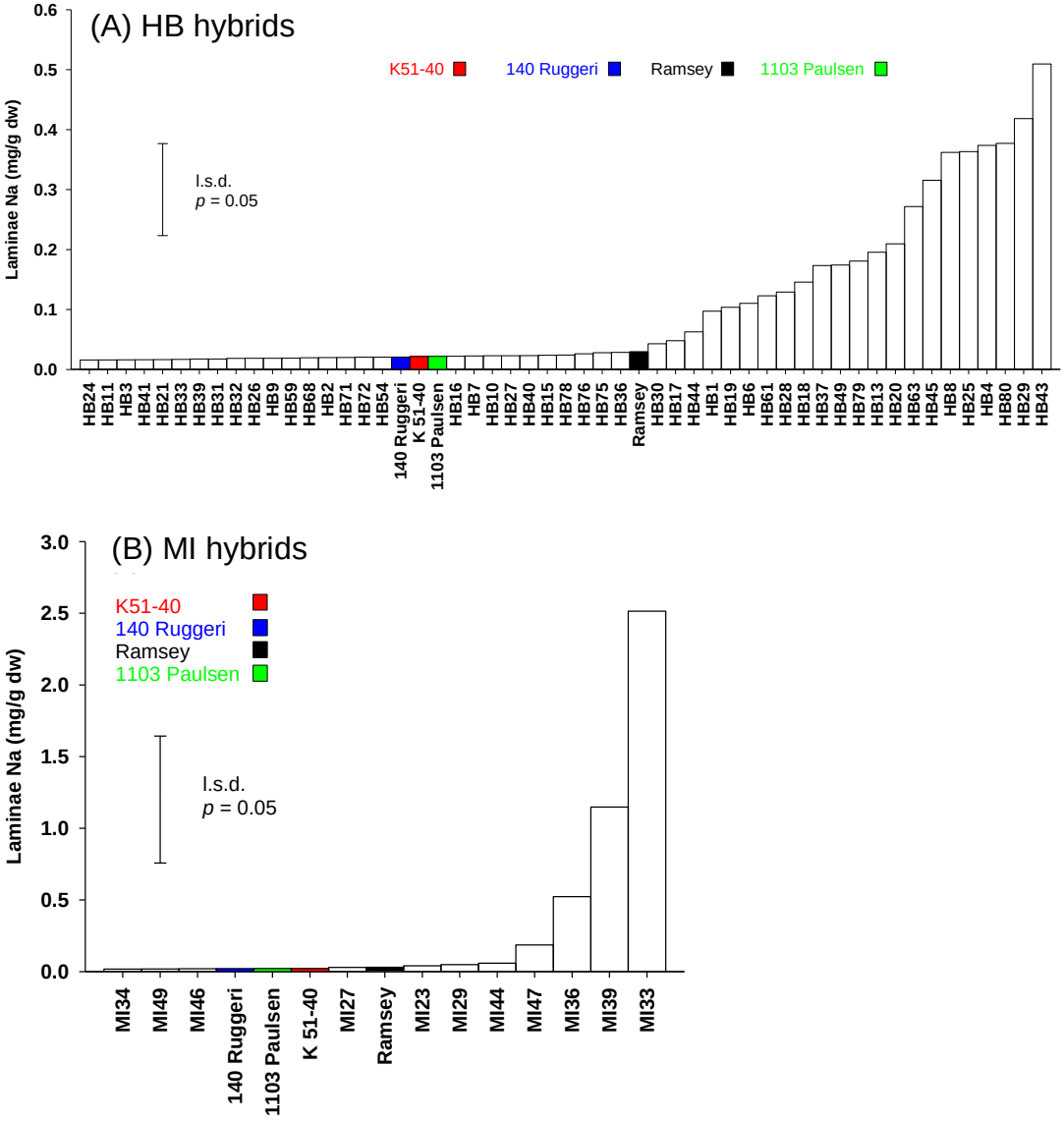
Only a very small number of hybrids had lower end concentrations of both Cl^- and Na^+ in both pot and solution culture trials.

6.4 Characterisation of the Cl^- exclusion mechanism:

6.4.1 Chloride uptake and transport in rooted leaves:

In the culture solution containing 25 mM Cl^-, 140 Ruggeri accumulated less Cl^- than K 51-40 in the lamina and petiole, while this was the opposite in the roots (Figure 16). These differences were evident at the start of the experiment and were maintained throughout its duration.

Figure 14: Mean (n=6) Na^+ concentrations in laminae of (A) HB and (B) MI hybrids (from K 51-40 × 140 Ruggeri), their parents and two reference rootstocks grown under glasshouse conditions in aerated nutrient cultures containing 15 mM Na^+ for 27 days.



6.4.2 Chloride transport in the apoplast:

Addition of 8-hydroxy-1,3,6-pyrenetrisulphonic acid (PTS), an apoplastic tracer (Yeo et al. 1999), significantly increased (P<0.001) the fluorescence in the roots of both K 51-40 and 140 Ruggeri rooted leaves under Cl^- treatment. No significant PTS fluorescence was observed above background readings in leaves of either K 51-40 or 140 Ruggeri (Figure 17). However, during the 24h PTS uptake period, Cl^- concentration in the shoot (lamina + petiole) of K 51-40 increased from a background value of approximately 80 umol g^{-1} dw to 135.8 ± 16.5 umol g^{-1} dw for the (-) PTS and 113.5 ± 7.5 umol g^{-1} dw for the (+) PTS treatment, whereas there was no significant increase in Cl^- concentration in shoots of 140 Ruggeri in (+) and (-) PTS treatments. The difference in Cl^- concentration in shoots of the two genotypes after the 24 h period was 6.5 fold (Figure 17). This indicates that Cl^- transport through the symplastic pathway contributed to the difference of Cl^- accumulation in the leaves of both rootstocks.

Figure 15: Distribution of mean (n=6) concentrations of (A) Cl^- and (B) Na^+ in laminae of grapevines propagated clonally from K51-40 x 140 Ruggeri hybrids grown in potting mix and irrigated daily with 50mM Cl^- under glasshouse conditions.

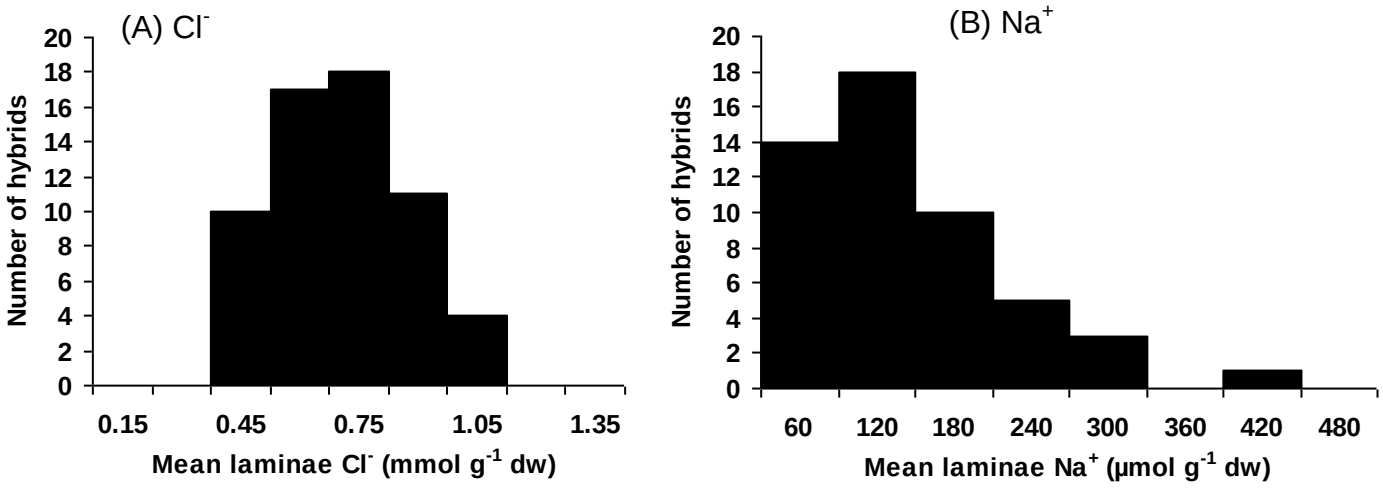


Figure 16: Chloride concentration in the lamina, petiole and root of rooted leaves of K 51-40 and 140 Ruggeri in modified Hoagland solution containing 25 mM Cl^- with Na^+:Mg^{2+}:Ca^{2+} of 6:1:1.

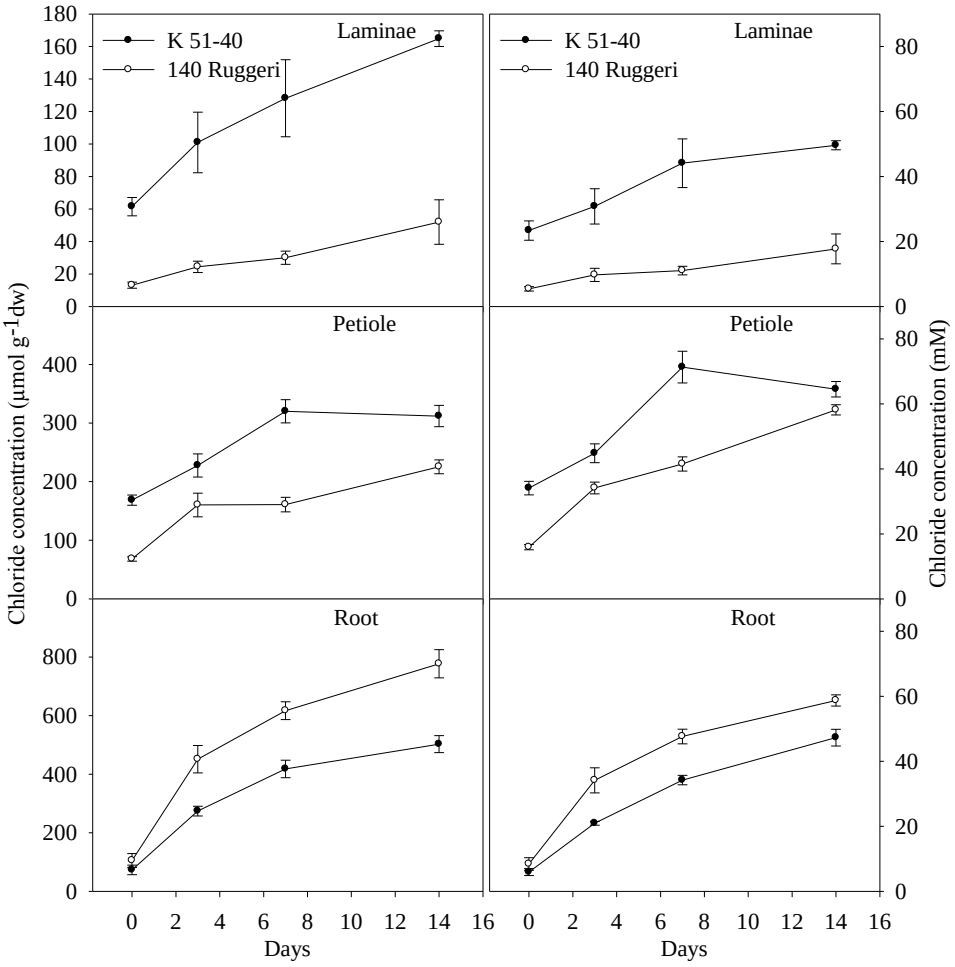
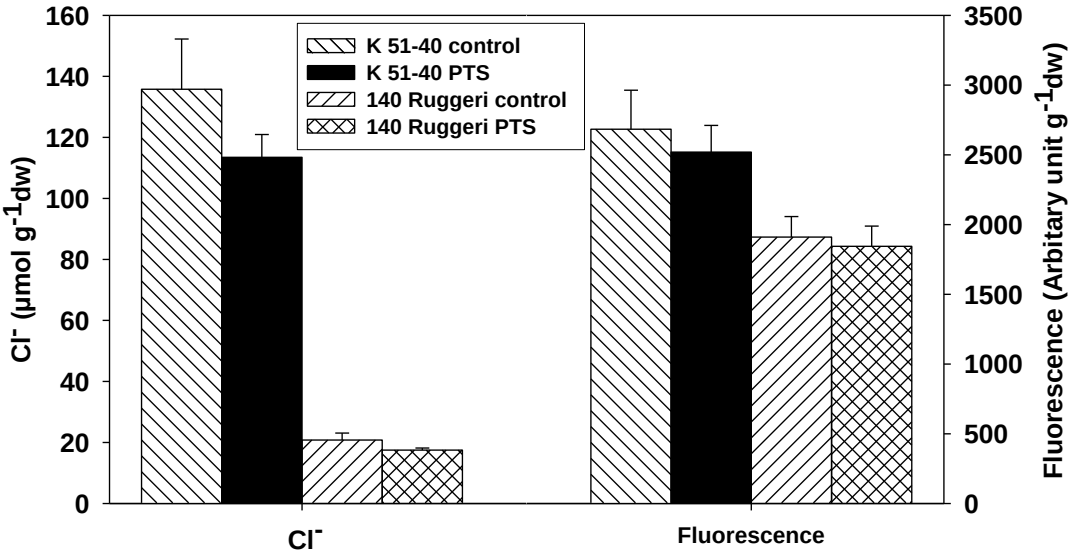


Figure 17: Fluorescence and Cl^- concentrations of leaves (lamina + petiole) in the rooted leaves of K51-40 and 140 Ruggeri as affected by salt and 8-hydroxy-1,3,6-pyrenetrisulphonic acid (PTS) treatment. Two weeks after being transferred to modified Hoagland nutrient solution, the rooted leaves were subjected to 25 mM Cl^- (in the form of NaCl, $MgCl_2$ and $CaCl_2$ with Na^+, Mg^{2+} and Ca^{2+} in the ratio of 6:1:1) without or with 100 mg/L of PTS for 24 h, after which the plants were harvested for Cl^- and fluorescence analysis. Bars represent se of means (n=6 for control and 12 for PTS treatment).



6.4.3 Chloride uptake fluxes into roots:

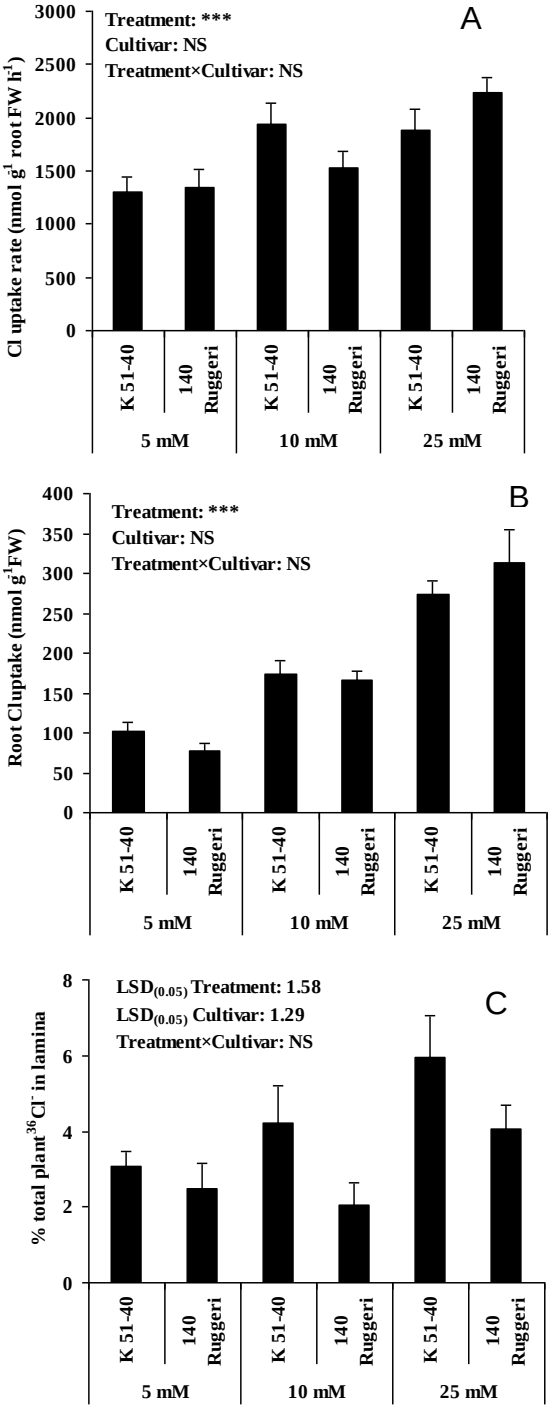
$^{36}Cl^-$ uptake for 10 minutes in flux solution was regarded as unidirectional flux into the root cell cytoplasm (Tregeagle et al. 2007). There was no significant difference in the unidirectional Cl^- flux into the root of rooted-leaves between the two genotypes (Figure 18 B). $^{36}Cl^-$ uptake over three hours also did not show a significant difference between rooted-leaves of the two genotypes (Figure 18 A).

With the increase of Cl^- concentration in external solution, the root Cl^- uptake significantly increased (Figure 18 A and B). The percentage of $^{36}Cl^-$ transported to the lamina was significantly lower in 140 Ruggeri than K 51-40 (Figure 18 C), based on an analysis of variance across all three concentrations of Cl^- in the external solution. The difference between the genotypes was clearly evident at 10 and 25 mM Cl^- in the external solution, but not at 5 mM (Figure 18 C).

6.5 Assessment of candidate Cl^- transporter genes (collaboration with Australian Centre for Plant Functional Genomics, ACPFG):

The model system studies narrowed the range of candidates to a family of genes with four accessions of the gene family as putative candidates. One of the accessions was chosen for examination in grapevines based on its high expression levels in roots, negligible expression in shoot and its down regulation in roots by salt stress. Transcripts of this accession were identified in grapevine. However expression levels in roots of 140 Ruggeri and K 51-40 grown with and without salt treatment did not show a strong correlation with Cl^- exclusion.

Figure 18: Chloride uptake measured by $^{36}Cl^-$ influx over 3 h (A), unidirectional influx of Cl^- measured by $^{36}Cl^-$ over 10 min (B) and percentage of $^{36}Cl^-$ transport into the lamina as compared to the total $^{36}Cl^-$ uptake in rooted leaves of K 51-40 and 140 Ruggeri (C). Three days after transfer to modified Hoagland solution, the rooted leaves were subjected to 10 mM Cl^- in the form of NaCl and $CaCl_2$ for 6 days. The rooted leaves were then subjected to 5, 10 and 25 mM Cl^- treatment without nutrients for 24 h, after which the whole intact plants were used for $^{36}Cl^-$ flux experiment. The Ca^{2+} activity was always kept at 0.69 mM during the experiment. Bars represent ± standard error of means; LSD, least significant difference; ****P*<0.001; NS, not significant.



7. Discussion:

7.1 Yield, laminae, juice and wine ion concentrations, composition and sensory qualities of wine of Chardonnay and Shiraz on own roots and on a range of rootstocks in a saline environment:

Laminae versus juice Cl^- and Na^+ concentrations: In general terms, at the site established in 1992, and particularly at the March lamina sampling time, there was a correlation between laminae Cl^- concentrations and grape juice Cl^- concentrations. A correlation was also apparent between Shiraz lamina Na^+ concentrations and grape juice Na^+ concentrations. However, because of the low lamina Na^+ concentrations, a positive correlation with grape juice Na^+ concentrations was not obtained for Chardonnay.

Of interest at the younger (2005) vineyard, there appeared to be a reduction in both Cl^- and Na^+ concentations (on a dry weight basis) in laminae between the November sampling and the February sampling, before increasing again between the February and late season (1 April and 1May) samplings. Reasons for this reduction are unknown, especially since in February soil solution EC values were at a seasonal high, and there was also evidence of a general correlation in February between laminae Cl^- and Na^+ concentrations and soil EC values. The results may indicate a change in Cl^- and Na^+ partitioning between key organs in the period November to February. Previous research has shown that Cl^- concentrations can decrease in grapevine laminae (Walker et al. 1981), but that observation was made for grapevines under conditions of salinity 'stress relief', whereas in this case, soil solution salinity was at a seasonally high level. The same effect was evident for Na^+ concentrations in laminae of Chardonnay at the longer established vineyard site, but not for Cl^- in laminae of Chardonnay at that site, indicating that it may not be a general phenomenon and that other factors appear to be involved. The effect was not evident for Cl^- and Na^+ in laminae of Shiraz at the longer established site, with the possible exception of Cl^- in laminae of Shiraz on 101-14 rootstock.

Yield: The study at the site established in 1992 has shown that in relation to the range of specific desired traits, and using yield as an example, the better performing rootstocks with Chardonnay as scion were not necessarily the better performing rootstocks with Shiraz as scion. For example, with regard to yield, Ramsey, own roots and 1103 Paulsen performed equally well with Chardonnay, whereas 140 Ruggeri and 1103 Paulsen performed equally well with Shiraz. It is notable also that at this site Ramsey (with Chardonnay) and 140 Ruggeri (with Shiraz) were the highest yielding rootstocks in seasons 1996 and 1997 (Walker et al. 2010), which indicates a level of sustainability in yield performance of these two rootstocks over time under saline conditions (irrigation water EC of 2.5 dS/m in 1996-97 and 1.8 dS/m in 2007-10). Furthermore, the yield performance of Ramsey and 1103 Paulsen with Chardonnay and 140 Ruggeri and 1103 Paulsen at Padthaway compared reasonably well with rootstock performance in a hot inland region such as Sunraysia (Merbein, Victoria), where Ramsey and 1103 Paulsen performed equally with Chardonnay, and Ramsey and 140 Ruggeri performed equally well with Shiraz (Walker et al. 2010; and unpublished data).

With regard to Chardonnay at Padthaway, while Ramsey, own roots and 1103 Paulsen performed equally well with respect to yield in the longer established (1992) vineyard, there were no differences between the rootstocks (Ramsey, 1103 Paulsen, 140 Ruggeri, Schwarzmann and 101-14) in yield performance at the younger (2005) vineyard. This was despite large differences in pruning wood weights between the rootstocks, with Ramsey and 1103 Paulsen leading to pruning wood weights that were about 1.6-fold higher than

Schwarzmann. Assuming a direct relationship between pruning wood weights and total leaf area, this suggests that Schwarzmann would have a higher irrigation water use index (yield/irrigation water applied) than Ramsey and 1103 Paulsen (Walker et al. 2005).

Grape juice Cl^- and Na^+ concentrations: As for laminae Cl^- concentrations, grape juice Cl^- concentrations in Chardonnay were significantly higher from vines on own roots than from grafted vines. This was in contrast with Shiraz, where grape juice Cl^- concentrations were equally highest from vines on own roots, Ramsey and 1103 Paulsen. Grape juice Cl^- concentrations on these rootstocks were all significantly higher than concentrations in Shiraz juice from vines on 140 Ruggeri, Schwarzmann and 101-14 rootstocks. The higher Cl^- concentration measured in juice of Chardonnay on own roots at this site was similar to the response measured in seasons 1996 and 1997 (Walker et al. 2010). However, the response for Shiraz was different in that whereas previously, Shiraz on own roots had significantly higher grape juice Cl^- concentrations than vines on Ramsey and 1103 Paulsen rootstocks (Walker et al. 2010), by seasons 2008 to 2010, there was no difference between vines on own roots and vines on Ramsey and 1103 Paulsen. This indicates a diminished capacity of these rootstocks to exclude Cl^- over time as reported by Tregeagle et al. (2006).

As found in previous work (Walker et al. 2010), rootstock effects on grape juice Na^+ concentrations were different to the effects on grape juice Cl^- concentrations, indicative of the different mechanisms that regulate uptake and transport of these ions. For Chardonnay, concentrations of Na^+ in grape juice (mean values over three seasons) varied over a wide range, from 40 mg/L (own roots and 101-14) to 112-117 mg/L (140 Ruggeri and Ramsey) and to 127 mg/L (Schwarzmann). Log transformed data across the three seasons confirmed higher Na^+ concentrations in grape juice from 140 Ruggeri, Ramsey and Schwarzmann (range in actual values for these rootstocks was 112 to 127 mg/L) relative to vines on own roots and 101-14 rootstock (approximately 40 mg/L) . For Shiraz, Na^+ concentrations overall were lower than for Chardonnay, with 1103 Paulsen having the highest concentration (94 mg/L), and with the other rootstocks in the range 37 to 62 mg/L. Log transformed data confirmed the higher Na^+ concentrations in grape juice of Shiraz on 1103 Paulsen rootstock.

For Chardonnay at the younger (2005) vineyard, grape juice Na^+ concentrations were all significantly lower than concentrations in the longer established (1992) vineyard.

Wine Cl^- and Na^+ concentrations: Three season means for Cl^- concentrations in Chardonnay wine were below 130 mg/L, and for Shiraz wine they were below 384 mg/L. These concentrations are well below the limit for Cl^- concentrations in wine of 607 mg/L (Australian Food Standards Code, 2010). Three season means for Na^+ concentrations in Chardonnay wine reached 101.6 and 100.4 mg/L for Ramsey and Schwarzmann, respectively. For Shiraz wine, they reached 111.9 mg/L (1103 Paulsen). These concentrations would exceed the limit for wine import to South Africa (100 mg/L) and Switzerland (60 mg/kg) (Stockley and Lloyd-Davies 2001). For Chardonnay, only wine from vines on own roots (46.4 mg/L) and 101-14 (46.7 mg/L), and for Shiraz, only vines on 140 Ruggeri (55.7 mg/L) and 101-14 (56.5 mg/L) were below the 60 mg/kg value for importation of wine into Switzerland, assuming a limit of 60 mg/kg equates to 60 mg/L.

Virtually all the Cl^- and Na^+ from Shiraz grape berries that ends up in wine is extracted during the initial 1-2 days of fermentation. Linear relationships existed between grape juice and wine Cl^- concentrations, and between grape juice and wine Na^+ concentrations. For Chardonnay, wine Na^+ concentrations were virtually identical to grape juice Na^+ concentrations. For Cl^-, there was slight increase in going from Chardonnay grape juice

(e.g. 80 mg/L) to wine (e.g. 110 mg/L). This was slightly higher than that observed in an earlier study (Walker et al. 2010), where there was virtually a 1:1 relationship. In both studies there was no skin contact during the Chardonnay winemaking process, but possibly in the current study, breakage of some berries between picking and the commencement of winemaking may have contributed to the higher Cl^- concentrations in wine than in juice. With Shiraz, Na^+ concentrations in wine (e.g. 75 mg/L) were only slightly higher than concentrations in grape juice (e.g. 60 mg/L). However, for Cl^-, concentrations in wine (e.g. 260 mg/L) were over 2-fold higher than concentrations in grape juice (e.g. 120 mg/L). In a previous study, the average increase in Cl^- between grape juice and wine was 1.7-fold (Walker et al. 2010). It is of interest that the increase in Na^+ concentration in going from grape juice to wine was significantly less than the increase in Cl^- concentration. We have also demonstrated similarity between grape berry pulp Na^+ concentration and wine Na^+ concentration, but an increase of 2.5-fold between Cl^- concentration in grape berry pulp and Cl^- concentration in wine (Gong et al. 2010a). A possible contributing factor in the case of Shiraz, where skin contact occurs during fermentation, is an apparent difference between the concentrations of Cl^- and Na^+ that accumulate in skins, as shown by Gong et al. (2010a). For example, skin Cl^- concentrations were on average 24.2 and 28.2 mg/g fresh weight for Chardonnay and Shiraz respectively, whereas sodium concentrations were just 9.3 and 11.1 mg/g fresh weight respectively.

Wine aroma and flavour profiles: Difference testing revealed differences between Shiraz wine made from vines on own roots and from all grafted wines. However, with Chardonnay, difference testing in two of the three seasons revealed no significant differences between wines from grafted and own-rooted vines. The season or year of vintage had a bigger impact than rootstock on aroma and flavour profiles, with profile differences being more pronounced with Shiraz than with Chardonnay.

7.2 Effects of salinity on inorganic/organic anion inter-relationships in grape berries:

As outlined by Gong et al. (2010a), K makes up approximately 70% of the mineral cations in ripe grape berries (Hale 1977, Walker et al. 1998). Although skin weight is a small part (8-22%, Hamatschek 1991, Walker et al. 1998) of the whole grape berry, it contains much K^+ because of its high concentration in the skin e.g. concentrations 4.0-5.5 times higher than in pulp (Gong et al. 2010a). This contrasts with Cl^-, where concentrations in skin were 3.8 (Merbein Shiraz on 1103 Paulsen) to 26.3 (Merbein Chardonnay on 1103 Paulsen) times that in the pulp and with Na^+, where concentrations were about 1.6-2.5 times that in the pulp. In this case, the combined concentrations of K^+ and Na^+ in skins were 1.6- (Merbein Chardonnay on own roots) to 15.8-fold (Padthaway Shiraz on 140 Ruggeri) higher than concentrations of Cl^-. This indicates that other anions in skin, most likely tartrate and malate (Storey 1987) balanced the excess of K^+ and Na^+ over Cl^-. Similarly in the pulp, the combined concentrations of K^+ and Na^+ were 2.7- (Merbein Chardonnay on own roots) to 49.6-fold (Padthaway Chardonnay on Ramsey) higher than concentrations of Cl^-, with tartrate the most likely counter ion (Ruffner 1982).

Malic and tartaric acids account for more than 90% of acids present in grape berries (Terrier and Romieu 2001) and influence grape and wine acidity. The concentration of malic acid at berry maturity was higher in the skin than pulp (Tables 9, 11), which is consistent with other studies (Iland and Coombe 1988, Gutierrez-Granda and Morrison 1992). There was also a significant negative linear regression between malic acid and Cl^- concentration in the skin. The higher malic acid and lower Cl^- concentrations in skin of most grafted Chardonnay and Shiraz vines, and vice versa for own rooted vines, may indicate competition for similar

transport proteins involved in loading into skins. Alternatively, higher salt concentrations in skins may be associated with accelerated malic acid catabolism. For example, Cramer et al. (2007) found in a glasshouse experiment that although malic acid in the shoot tip of grapevine increased dramatically after the initial osmotic shock by water stress and salinity, the concentration decreased with time as Cl^- accumulated.

Also, the concentration of malic acid in grapes from the warm irrigated (Merbein) site was generally lower than that from the cooler Padthaway site. During grape ripening, high temperature can result in a lower acid content at maturity, mostly because of increasing degradation of malic acid during respiration (Coombe 1987, Soyer et al. 2003).

Overall, grape berry skins were shown to be an important repository of ions with K^+, Cl^- and Na^+ concentrations on average 4.4, 10.8 and 2.0 times the concentration in pulp and implicated Cl^- in particular, and possibly also malate and tartrate as counter ions balancing Na^+ and K^+ concentrations in skin cells. Cl^- and Na^+ concentrations in pulp and skin were highly correlated. Wine Cl^- and Na^+ concentrations were similarly highly correlated with pulp and skin Cl^- and Na^+ concentrations (data not shown).

7.3 Variation for Cl^- and Na^+ exclusion in a family from a cross between grapevine rootstocks K 51-40 and 140 Ruggeri:

In both pot and solution culture trials, 140 Ruggeri showed a better capacity than K 51-40 for Cl^- exclusion in laminae and petioles, and the hybrids of K 51-40 × 140 Ruggeri showed a continuous variation in Cl^- accumulation in both leaves (laminae and petioles) and roots.

In this study, two groups of hybrids from crosses made 20 years apart were used. There were no differences between the two groups with respect their overall capacity for Cl^- exclusion. The only significant difference between the groups was with respect to laminae Na^+ concentrations in solution culture, for which each group was analysed separately.

There were significant negative correlations between lamina Cl^- concentrations and shoot length at the start of salt treatment in solution culture, yet the r^2 values were quite low (≤ 0.033). Therefore, differences among the hybrids for Cl^- accumulation were not strongly associated with plant vigour. Determination of a vine vigour and salt tolerance index was not an objective of the study, mainly because the large size of the experiments (384 vines in each case) precluded inclusion of a nutrient solution only control.

Chloride concentrations in laminae, petioles and roots were lower in the solution culture trial than in the pot trial. The reasons for this might be related to the salinity level and growth conditions. In the solution culture trial, Cl^- concentration in the nutrient solution was 25 mM Cl^-, which was half that used in the pot trial. The mean Cl^- concentration in the roots for the hybrids in the solution culture trial was 74% of that in the pot trial, showing that Cl^- accumulation in roots is less affected by the type of growing medium relative to Cl^- accumulation in laminae and petioles. For example, in the lamina and petiole, the average Cl^- concentrations for the hybrids in the solution culture trial were 13% and 27% of those in the pot trial, respectively. Besides the difference in nutrient solution Cl^- concentration (50 mM in the pot trial and 25 mM in the solution culture trial), lower Cl^- transport from the root to the shoot in the solution culture trial may have been due to differences in aerial and rootzone temperatures. Higher rootzone temperature can increase ion accumulation in plants (Zelleke and Kliewer 1980) and accordingly the higher maximum rootzone temperatures in the pot trial may have enhanced Cl^- uptake in the roots. Higher air

temperature is known to stimulate leaf transpiration (Reddy et al. 1995), which may have increased total Cl^- flux in xylem and therefore its accumulation in the leaves for the pot trial.

The present results suggest involvement of more than one gene in Cl^- exclusion, which is consistent with the conclusion by Sykes (1985), who observed a continuous variation in Cl^- accumulation in shoots in the hybrid family of Ramsey × Sultana. Studies by Newman and Antcliff (1984) and Sykes (1987) observed that hybrids between *V. berlandieri* and *V. vinifera* had low petiole Cl^- concentrations and discontinuous distributions within backcrosses to *V. vinifera* under vineyard conditions, suggesting a single dominant gene for Cl^- exclusion in *V. berlandieri*. It might be speculated that genetic control of Cl^- exclusion is dependent on the grapevine species involved.

To our knowledge, there is little information with regard to variation in Na^+ accumulation in shoot tissues by hybrids of grapevine. While the distribution of laminae Na^+ concentrations in the hybrids was skewed in both trials, there was no obvious sign of major gene segregation. In citrus, Na^+ exclusion in hybrids between Rangpur lime and Trifoliate orange was shown to be continuous (Sykes 1992), suggesting a polygenic trait. To some extent, our results were in agreement with this.

The variability for Cl^- and Na^+ concentrations in leaves and roots reported here indicated an extensive genetic variation for the ability by the hybrids to exclude these ions from their leaves. The values obtained for the intra-class correlation coefficient, a statistic which estimates the degree of genotypic determination (Falconer and Mackay 1996) and, thus, an upper estimate of the broad sense heritability, supported this argument. As the vines grown in the experiments were clonal, estimates of broad sense heritability from intra-class correlation coefficients indicated that upper limits of about 40% and 35% of the total phenotypic variation in Cl^- exclusion of this hybrid family could be attributed to heritable sources in the pot trial and solution culture trial, respectively. For Na^+ variation, the upper limits due to genotypic difference were approximately 60% and 40% in the pot and solution culture trials, respectively.

Low correlations between Na^+ and Cl^- concentrations in laminae observed in both trials suggest that the mechanisms for excluding these two ions are different. This is consistent with previous work in citrus (Walker 1986, Sykes 1992).

In summary, the variation in Cl^- exclusion within the hybrid family from a cross between K 51-40 and 140 Ruggeri appeared to be controlled by more than one gene. Further work and analysis would involve studying quantitative trait loci for Cl^- exclusion using a larger family.

7.4 Characterisation of the Cl^- exclusion mechanism:

Previous studies between 140 Ruggeri and K 51-40 have demonstrated that the better Cl^- excluding capacity of 140 Ruggeri (Tregeagle et al. 2006; Walker et al. 2010) cannot be explained by differences in water use by the genotypes (Tregeagle et al. 2010). This contrasts with previous studies involving citrus rootstocks where genotype capacity for Cl^- exclusion was linked to lower water use (Moya et al. 2003).

One of the questions to arise from the work of Tregeagle et al. (2010) was whether there was a difference in Cl^- transport through the apoplast of the two genotypes, or more specifically whether the roots of K 51-40 had higher transport through this pathway that might explain the higher concentrations of Cl^- accumulated in petioles and laminae of this genotype. This question was addressed using the fluorescent apoplast tracer PTS (Yeo et

al. 1999) in studies with rooted leaves of the two genotypes. No obvious PTS fluorescence was observed in the leaves of either genotype, despite a significant increase in concentration of Cl^- accumulated in the lamina and petiole of the rooted leaves during the PTS treatment period. These results demonstrated that the apoplast pathway did not contribute significantly to the difference in root to shoot transport of Cl^- between the two genotypes. The results suggest that the symplast is the primary pathway for Cl^- transport through roots to the stele.

The previous study by Tregeagle et al. (2010) also demonstrated no significant difference in the unidirectional Cl^- flux into excised roots of 140 Ruggeri and K 51-40. The results reported here have extended that observation and further demonstrated no significant difference in the unidirectional Cl^- influx into roots of intact rooted leaves. In addition, Cl^- influx studies involving three concentrations of Cl^- in solution culture (5, 10 and 25 mM Cl^-) demonstrated no significant difference between the genotypes in net Cl^- influx into roots of whole plants. However, as demonstrated by Tregeagle et al. (2010), the percentage of Cl^- transported to the lamina of rooted leaves was significantly lower in 140 Ruggeri than in K 51-40. This is consistent with lower xylem Cl^- concentrations and lower root to shoot transport of Cl^- in 140 Ruggeri relative to K 51-40 (Tregeagle et al. 2010). In addition, while we have shown here that rooted leaves of 140 Ruggeri accumulate significantly lower concentrations of Cl^- in lamina and petiole, the roots of intact rooted leaves of 140 Ruggeri accumulate higher concentrations of Cl^- than roots of K 51-40. This may be a consequence of similar unidirectional Cl^- influx (10 min) and Cl^- uptake (3 hr) of rooted leaves of the two genotypes, but lower release of Cl^- into the xylem of 140 Ruggeri.

The results point to the root symplast and potentially to the root stele as the site of xylem loading as being critical factors in the Cl^- exclusion mechanism. Accordingly, further emphasis was placed on the role of Cl^- transporters in root membranes, and in particular Cl^- transporters that might be located in the root stele.

7.5 Candidate Cl^- transporter genes involved in Cl^- exclusion:

The study used a reverse genetics approach involving a model plant system (Arabidopsis) to reduce from a range of candidates to a family of genes, with four accessions of the gene family as putative candidates. Two of the four accessions showed a high level of root specific expression, while the expression level in the shoot was found to be high for the other two accessions. When exposed to salt stress at 150 mM NaCl, one of the accessions exhibited down-regulation of root expression. This candidate was chosen for further examination in grapevines as a potential candidate for involvement in the Cl^- loading process into the xylem. This was based on its high expression levels in roots, negligible expression in shoot, its down regulation by salt stress, and when mutated, it consistently led to lower concentrations of Cl^- in the shoot.

Using RNA extracts from Cabernet Sauvignon (*V. vinifera*), 140 Ruggeri (*V. berlandieri x V. rupestris*) and K 51-40 (*V. champini x V. riparia*), transcripts of the putative Cl^- transporter were identified in grapevine, and expression levels in various tissues including roots were investigated in K51-40 and 140 Ruggeri plants grown with and without salt treatment. Encouraging results were obtained, but further work needs to be done. Identification of the Cl^- transporter or transporters may be the first step towards development of a marker for use in rapid screening of hybrid progeny for Cl^- exclusion capacity. Development of genetic and management strategies for minimising the uptake of Cl^- under saline conditions will be greatly facilitated once the mechanism for Cl^- exclusion capacity in grapevines is established.

7.6 General:

The project combined field-based studies to investigate the performance of specific rootstocks with Chardonnay and Shiraz as scions under saline conditions with laboratory-based studies to advance our understanding of salt exclusion mechanisms in grapevine.

Given that the sites chosen for the study included sites investigated in the mid 1990s, this study enabled insights into the sustainable performance of specific rootstocks under saline conditions. While there was consistency in yield performance of some rootstocks between seasons 1996 and 1997 and seasons 2007 to 2010, it was clear that with Shiraz, the Cl^- exclusion capacity of Ramsey and 1103 Paulsen had diminished over time. The study also showed that the increase in Cl^- in must during fermentation from grape juice to wine was significantly higher for Cl^- than for Na^+.

Wine sensory studies demonstrated few differences between wine made from Chardonnay on own roots and that from Chardonnay on the range of rootstocks. However, significant differences were demonstrated between Shiraz wine made from own rooted vines and wine from each of the grafted vines. Means over the three seasons of winemaking revealed significantly lower colour density, total and ionised anthocyanins for Ramsey, 140 Ruggeri and 1103 Paulsen rootstocks relative to that from own roots, 101-14 and Schwarzmann rootstocks. In 2009, this corresponded with significantly lower dark berry aroma and lower stewed/dried fruit flavours in wine from Ramsey, 140 Ruggeri and 1103 Paulsen grafted vines.

Mean wine Cl^- concentrations were below 385 mg/L (Shiraz) and 128 mg/L (Chardonnay), while mean wine Na^+ concentrations were below 112 mg/L. Sensory panels were unable to consistently identify salty taste in these wines. A study with Shiraz wine spiked with NaCl to develop a range in Cl^- concentrations from 265 mg/L to 854 mg/L also did not consistently identify salty taste. This was in contrast to a study by Bastion et al. (2010) which was able to consistently detect salty taste in Shiraz wines within this range of Cl^- concentrations, indicating the need for further research in this area.

The study significantly advanced understanding of the Cl^- and Na^+ exclusion mechanisms in grapevine. More than one gene appears to be involved in both cases, based on results from the hybrid family obtained from the cross between 140 Ruggeri and K 51-40. Thirty to forty percent of the variation in Cl^- exclusion within the hybrid family was heritable. Forty to sixty percent of the variation in Na^+ exclusion within the hybrid family was heritable.

Strong evidence was obtained to suggest that the Cl^- exclusion mechanism in 140 Ruggeri resides at the level of xylem loading, leading to significantly lower concentrations of Cl^- in xylem of 140 Ruggeri relative to K 51-40. The results indicated that Cl^- transport in the grapevine root is primarily through the symplast, rather than through the apoplast. These data pointed to a role for root and more likely root stele Cl^- transporters in the Cl^- exclusion mechanism. Initial assessment of a range of putative Cl^- transporters identified a promising family of candidates, but while these genes appear to be active in grapevines, preliminary data has not yet confirmed a role for these genes in the Cl^- exclusion process.

8. Outcomes/Conclusions:

- The project achieved all objectives as set out in the original application
- In terms of yield performance with Chardonnay as scion, Ramsey and 1103 Paulsen were equivalent to own-rooted vines, whereas with Shiraz as scion, the better rootstocks were 140 Ruggeri and 1103 Paulsen.
- Chardonnay wine from grafted vines generally was perceived as having few consistent differences in aroma and flavour relative to wine from vines grafted to Ramsey, 1103 Paulsen, 140 Ruggeri, Schwarzmann and 101-14 rootstocks.
- Shiraz wine from each of the grafted vines was consistently perceived as having differences in aroma and flavour relative to wine from vines on own roots. The principal aroma attributes that discriminated the 2009 wines were dark berry, dried fruit, floral, sweet spice and vanilla; the principal differences in wine flavour were perceived in red berry, stewed/dried fruit, sweet spice, bitterness and fruit flavour length.
- Wines of Shiraz from vines on own roots, and from vines on 101-14 and Schwarzmann rootstocks in 2009 were all perceived with the highest dark berry aroma and stewed/dried fruit flavours.
- Evidence of diminished capacity for Cl^- exclusion over time with Shiraz as scion was demonstrated for Ramsey and 1103 Paulsen rootstocks.
- For Chardonnay, the average increase in Cl^- in must from juice to wine was 1.4-fold, but the average increase for Na^+ was significantly less at 1.1-fold.
- For Shiraz, the average increase in Cl^- in must from juice to wine was 2.2-fold, but the average for Na^+ was significantly less at just 1.25-fold.
- Wines in all cases were less than the 607 mg/L limit for Cl^-, but in some years reached relatively high Na^+ concentrations. Mean wine Na^+ concentrations across the three seasons were less than 112 mg/L. Not all countries have limits for Na^+ in wine, and there is a wide range in listed limits. The concentrations of Na^+ recorded in wine of Chardonnay and Shiraz with some rootstocks would have exceeded the limit for import into some countries.
- Multiple genes were demonstrated to be responsible for both Cl^- and Na^+ exclusion in a family of hybrids obtained by crossing a good Cl^- excluder (140 Ruggeri) with a poor Cl^- excluder (K 51-40). The heritability of ion exclusion in this family was 30-40% for Cl^- and 40-60% for Na^+.
- No evidence was obtained to link the greater Cl^- accumulation by K 51-40 with higher transport through the root apoplast. The data suggest transport of ions through the grapevine root occurs largely through the root symplast.
- Flux studies with $^{36}Cl^-$ provided further evidence that Cl^- exclusion in 140 Ruggeri is regulated at the level of loading into the root xylem, leading to lower concentrations of Cl^- in xylem and subsequent lower concentrations of accumulated Cl^- in petioles and laminae.
- Reverse genetics approaches identified a putative gene family from a range of candidates selected for a possible role in Cl^- transport.
- Transcripts of the putative Cl^- transporter genes were identified in grapevine and preliminary assessment has yielded encouraging results, but further work needs to be done.
- The field component of the study has delivered information of immediate practical value to grape growers and winemakers in regions affected by salinity. The information will permit more informed choices to be made with regard to rootstock selection for such conditions, taking into account yield, grape juice and wine ion concentrations, sensory assessment and longer term vine

performance. Winemakers will have improved knowledge of juice to wine increases for Cl^- and Na^+ for specific scion varieties to aid harvest and winemaking decisions.

- Economic benefits of this research are clear, in that wine exceeding limits for Cl^- and Na^+ for either the domestic market or specific export destinations, is in danger of being rejected with consequent financial losses to the wine company, the wine exporter or the grower. There are supply chain consequences in particular for growers producing grapes that have higher than acceptable limits for Cl^- and Na^+ which may put them in danger of having their fruit rejected by winemakers. Over the long term, it seems likely that salt excluding rootstocks may be important for survival of regions that have no alternative but to use poorer quality water for irrigation purposes.
- The apparent diminished capacity of some rootstocks, e.g. 1103 Paulsen and Ramsey, for salt exclusion when grown under saline conditions over a number of years (Tregeagle et al. 2006 and this study) highlights the importance of rootstock choice when re-planting, in order to reduce the likelihood (and cost) of having to re-plant earlier than normally would be required.
- With regard to the environment, there is now a considerable body of knowledge relating to how best to manage soils in regions affected by salinity. The importance of supplementary rainfall for leaching purposes is well known. So too is the importance of guarding against fine particle dispersion where soils have become sodic. Rootstocks are a component of the solution, but for sustainable soil management, application of best available knowledge in this area is crucial.

9. Recommendations:

- Continue research to identify rootstocks with even better Cl^- and Na^+ exclusion capacity than those currently available. The highly heterozygous nature of grapevines together with data that indicate potential to achieve high capacity for both Cl^- and Na^+ exclusion through breeding and selection indicate that further investment in this area will potentially be worthwhile.

- With regard to the above, while considerable advances have been made in characterizing the Cl^- exclusion mechanism in grapevines, there is further work to be done. It will be much easier to develop plant-based and vineyard-based solutions to excessive Cl^- and Na^+ uptake by grapevines when the mechanisms for Cl^- and Na^+ exclusion have been fully characterised.

- Continue studies on salty taste in wine. This study revealed that salty taste was not consistently detectable in the range 265 to 854 mg/L, and while this study was in contrast with other studies which were able to detect salty taste within this range, the need for further work to establish the reasons for the inconsistency are needed.

- Extend information on rootstock choice for saline conditions to industry and encourage regular monitoring of vine salt status within vineyards and the adoption of best practice in relation to maintaining low vine salt loads under challenging conditions.

10. Appendix 1: Communication:

10.1 Presentations

Australian Wine Industry Technical Conference: Workshop on 'Irrigated rootzone salinity'. Presentation title 'Sustainable salt exclusion by salt tolerant rootstocks', July 2007.

Invited 'Guest Lecture' (R. Walker) to La Trobe University viticulture students, Bundoora, Victoria, August 2007

Australian Society of Viticulture and Oenology Seminar: 'Water, Friend or Foe?'. Mildura Arts Centre Theatre, November 2007. Presentation title 'Sustainable use of grapevine rootstocks for salt exclusion in commercial viticulture'.

National Grapevine Rootstock Forum, held at DPI Victoria, Irymple, on 21 May 2008. The title of the presentation was 'Rootstocks for saline conditions'.

Invited 'Guest Lecture' (R. Walker) to La Trobe University viticulture students, Bundoora, Victoria, August 2008.

Seminar (H. Gong) titled 'Grapevine chloride exclusion', CSIRO Plant Industry, Merbein, Friday 8 May 2009.

Seminar (H. Gong) titled 'Grapevine chloride exclusion', CSIRO Plant Industry, Adelaide, Friday 26 June 2009.

American Society of Enology and Viticulture Symposium on 'Rootstock characteristics and selection', NapaValley, California, June 2009, Presentation titled "Rootstock attributes and selection for Australian conditions'.

Workshop presentation (R.Walker) to Limestone Coast WICTSC on 'Salt tolerant rootstocks for long term sustainability', Padthaway, South Australia, 17 June 2010.

Workshop presentation (R. Walker) to Australian Wine Industry Technical Conference on 'Sustainable salt tolerance of salt excluding rootstocks, Adelaide, South Australia, 8 July 2010.

Workshop presentation (R. Walker) to Australian Wine Industry Technical Conference on 'Rootstock impacts on chloride, sodium and potassium accumulation by grapevines', Adelaide, South Australia, 4 July 2010.

10.2 Conference Proceedings

Tregeagle, J. M., Walker, R.R., Tisdall, J.M. and Tester, M. (2007) Sustainable use of grapevine rootstocks for salt exclusion in commercial viticulture. In: 'Water, Friend or Foe?', Eds. W. Cameron, K. DeGaris, C. Dundon, G. McCorkelle and P.Pattison, Proceedings of Australian Society of Viticulture and Oenology Seminar, Mildura, Victoria, November 2006, Australian Society of Viticulture and Oenology, Adelaide, SA, pp. 42-47.

10.3 Conference Posters

Gong, H., Walker, R., Blackmore, D., Clingeleffer, P., Sykes, S. and Tester, M. (2009) Variation for chloride exclusion in a family from a cross between rootstocks K 51-40 and 140 Ruggeri. Abstract in the Australian Journal of Grape and Wine Research, for the Eighth International Symposium on Grapevine Physiology and Biotechnology, Adelaide, SA, November 2008.

Gong, H., Blackmore, D. and Walker, R. (2009) Rootstock and rootzone salinity effects on partitioning of inorganic ions and organic acids in Shiraz and Chardonnay grape berries. Abstract in the Australian Journal of Grape and Wine Research, for the Eighth International Symposium on Grapevine Physiology and Biotechnology, Adelaide, SA, November 2008.

Gong, H., Jha, D., Tester, M. and Walker, R. (2010) Rootstocks with different Cl^- exclusion capacity exhibit similar net Cl^- uptake but differ in root to shoot Cl^- transport. Abstract in Proceedings of the Australian Wine Industry Technical Conference, Adelaide, South Australia, July 2010.

10.4 Industry Journal Articles

Walker, R.R. and Clingeleffer, P.R. (2009) Rootstocks attributes and selection for Australian conditions. Australian Viticulture, July-August Edition, 2009.

10.5 Peer Reviewed Journal Papers

Gong, H., Blackmore, D.H. and Walker, R.R. (2010a) Organic and inorganic anions in Shiraz and Chardonnay grape berries and wine as affected by rootstock under saline conditions. Australian Journal of Grape and Wine Research, 16, 227-236.

Walker, R.R., Blackmore, D.H. and Clingeleffer, P.R. (2010) Impact of rootstock on yield and ion concentrations in petioles, juice and wine of Shiraz and Chardonnay in different viticultural environments with different irrigation water salinity. Australian Journal of Grape and Wine Research, 16, 243-257.

Tregeagle, J.M., Tisdall, J.M., Tester, M. and Walker, R.R. (2010) Cl^- uptake, transport and accumulation in grapevine rootstocks of differing capacity for Cl^- exclusion. Functional Plant Biology, 37, 665-673.

Gong, H., Blackmore, D.H., Clingeleffer, P.R., Sykes, S.R., Jha, D., Tester, M. and Walker, R.R. (2010b) Contrast in chloride exclusion between two grapevine genotypes and its variation in their hybrid progeny. Submitted.

10.6 General Communications

GWRDC ‘R&D at Work’, article on ‘Salinity impacts on vines’.

11. Appendix 2: Intellectual Property and/or valuable information arising from the research:

The work on candidate Cl^- transporter genes that was undertaken in this project in collaboration with the Australian Centre for Plant Functional Genomics (ACPFG) has resulted in discovery of a gene family that may be involved in controlling the accumulation of Cl^- in shoots of plants. Pending the results of further work by a PhD student at ACPFG (a project separate to the work undertaken in this project), a patent application to protect this discovery may be submitted.

Other data obtained in this project are in the public domain and have either has been published or will be published in international peer reviewed journals. Much of the public domain material has been presented at industry meetings and symposia and will continue to be extended as opportunities arise.

12. Appendix 3: References cited in the report

Bastion, S.E.P., de Loryn, L.C., Collins, C., Petrie, P.R. and Johnson, T.E. (2010) Determination of NaCl detection and recognition thresholds in grape juice and wine and sensory perception of salt in white wine. Proceedings 14th Australian Wine Industry Technical Conference, Adelaide, South Australia, July 2010.

Bravdo, B. and Shoseyov, O. (2000) Aroma studies of fruits and wine in Israel. Acta Horticulturae 526, 399-405.

Commonwealth of Australia (2009) Wine production Requirements (Australia only). http://www.foodstandards.gov.au/thecode/foodstandardscode/standard451wineprodu4297.cfm (accessed 27/9/09).

Coombe, B.G. (1987) Distribution of solutes within the developing grape berry in relation to its morphology. American Journal of Enology and Viticulture 38, 120-127.

Cramer, G.R., Ergul, A., Grimplet, J., Tillett, R.L., Tattersall, E.A.R., Bohlman, M.C., Vincent, D., Sonderegger, J., Evans, J., Osborne, C., Quilici, D., Sclauch, K.A., Schooley, D.A. and Cushman, J.C. (2007) Water and salinity stress in grapevines: early and late changes in transcript and metabolite profiles. Functional & Integrative Genomics 7, 111-134.

Downton, W.J.S. (1977) American Journal of Enology and Viticulture 28, 210-214.

Falconer, D.S. and Mackay, T.F.C. (1996) Introduction to quantitative genetics. 4th edn. Lonman, New York.

Gong, H., Blackmore, D.H. and Clingeleffer, P.R. (2010a) Organic and inorganic anions in Shiraz and Chardonnay grape berries and wine as affected by rootstock under saline conditions. Australian Journal of Grape and Wine Research 16, 227-236.

Gutierrez-Granda, M. J. and Morrison, J.C. (1992) Solute distribution and malic enzyme activity in developing grape berries. American Journal of Enology and Viticulture 43, 323-328.

Hale, C.R. (1977) Relation between potassium and the malate and tartrate contents of grape berries. Vitis 16, 9-19.

Hamatschek, J. (1991) Structure of the grape berry – basis for winemaking. Viticulture and Enological Sciences 46, 58-68.

Iland, P.G. and Coombe, B.G. (1988) Malate, tartrate, potassium, and sodium in flesh and skin of Shiraz grapes during ripening: concentration and compartmentation. American Journal of Enology and Viticulture 39, 71-76.

Leske, P.A., Sas, A. N., Coulter, A. D., Stockley, C.S. and Lee, T.N. (1997) The composition of Australian grape juice: chloride, sodium and sulphate ions. Australian Journal of Grape and Wine Research 3, 26-30.

Moya, J.L., Primo-millo, E. and Talon, M. (1999) Morphological factors determining salt tolerance in citrus seedlings: the shoot to root ratio modulates root uptake of chloride ions and their accumulation in leaves. Plant, Cell and Environment 22, 1425-1433.

Newman, H.P. and Antcliff, A.J. (1984) Vitis 23, 106-112.

Reddy, V.R., Reddy, K.R. and Hodges, H.F. (1995) Carbon dioxide enrichment and temperature effects on cotton canopy photosynthesis, transpiration, and water use efficiency. Field Crops Research 41, 13-23.

Ruffner, H.P. (1982) Metabolism of tartaric and malic acids in Vitis: a review – part A. Vitis 21, 247-259.

Snedecor and Cochran (1967) Statistical Methods, 6th Edn. Iowa State University Press, Iowa, USA.

Soyer, Y., Koca, N. and Karadeniz, F. (2003) Organic acid profile of Turkish white grapes and grape juices. Journal of Food Composition and Analysis 16, 629-636.

Stockley, C. and Lloyd-Davies, S. (2001) Analytical specifications for the export of Australian wine. Australian Wine Research Institute: Glen Osmond, South Australia.

Storey, R. (1987) Potassium localization in the grape berry pericarp by energy-dispersive X-ray microanalysis. American Journal of Enology and Viticulture 38, 301-309.

Sykes, S. R. (1985) Variation in chloride accumulation by hybrid vines from crosses involving the cultivars Ramsey, Villard Blanc and Sultana. American Journal of Enology and Viticulture 36, 30-37.

Sykes, S.R. (1987) Variation in chloride accumulation in hybrids and backcrosses of *Vitis berlandieri* and *Vitis vinifera* under glasshouse conditions. American Journal of Enology and Viticulture 38(4), 313-320.

Sykes, S.R. (1992) The inheritance of salt exclusion in woody perennial fruit species. Plant and Soil 146, 123-129.

Terrier, N. and Romieu, C. (2001) Grape berry acidity. In: Molecular biology and biotechnology of the grapevine. Ed. K.A. Roubelakis-Angelakis (Kluwer Academic Publishers: Dordrecht, Netherlands) pp. 35-57

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

Tregeagle, J.M., Tisdall, J.M., Tester, M. and Walker, R.R.(2010) Cl^- uptake, transport and accumulation in grapevine rootstocks of differing capacity for Cl^- exclusion. Functional Plant Biology 37, 665-673.

Walker, R.R., Torokfalvy, E., Scott, N.S., and Kriedemann, P.E. (1981) An analysis of photosynthetic response to salt treatment in *Vitis vinifera*. Australian Journal of Plant Physiology 8, 359-374.

Walker, R.R. (1986) Sodium exclusion and potassium-sodium selectivity in salt-treated Trifoliate orange (*Poncirus trifoliata*) and Cleopatra mandarin (*Citrus reticulata*) plants. Australian Journal of Plant Physiology 13, 293-303.

Walker, R.R., Clingeleffer, P.R., Kerridge, G.H., Ruhl, E.H., Nicholas, P.R. and Blackmore, D.H. (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 6, 227-239.

Walker, R.R., Gibberd, M.R. and Stevens, R.M. (2005) Improving vineyard water use efficiency. In Proceedings Twelfth Australian Wine Industry Technical Conference, Eds. R. Blair, P.Williams and S. Pretorius, Australian Wine Industry Technical Conference Inc., Urrbrae, South Australia, Australia. pp 152-158.

Walker, R.R., Blackmore, D.H. and Clingeleffer, P.R. (2010) Impact of rootstock on yield and ion concentrations in petioles, juice and wine of Shiraz and Chardonnay in different viticultural environments with different irrigation water salinity. Australian Journal of Grape and Wine Research 16, 243-257.

Yeo, A.R., Flowers, S.A., Rao, G., Welfare, K., Sennanayake, N. and Flowers, T.J. (1999) Silicon reduces sodium uptake in rice (*Oryza sativa* L.) in saline conditions and this is accounted for by a reduction in the transpirational bypass flow. Plant, Cell and Environment 22 (5), 559-565.

Zelleke, A. and Kliewer, W.M. (1980) Effect of root temperature, rootstock and fertilization on bud-break, shoot growth and composition of ‘Cabernet Sauvignon’ grapevines. Scientia Horticulturae 13, 339-347.

13. Appendix 4: Staff (A list of all staff that have been engaged on the Project).

Dr Rob Walker, CSIRO Plant Industry (Project Leader)
Dr Haijun Gong, CSIRO Plant Industry (Postdoctoral Fellow)
Mr Peter Clingeleffer, CSIRO Plant Industry (Collaborating Research Scientist)
Ms Deidre Blackmore, CSIRO Plant Industry (Project Research Support Scientist)
Professor Mark Tester, Australian Centre for Plant Functional Genomics
Dr Deepa Jha, Australian Centre for Plant Functional Genomics

14. Appendix 5: Budget reconciliation