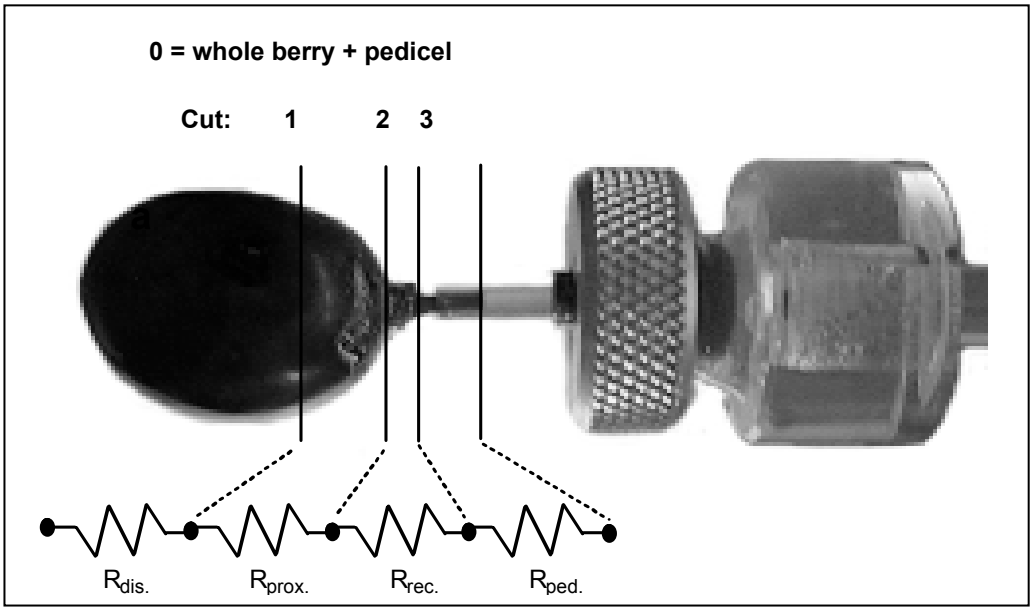
Cooperative Research Centre for Viticulture

Australian Government
Grape and Wine Research and Development Corporation

Shiraz berry weight loss at sub-optimal maturity – defining the problem.



FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **CRV 01/02a**

Principal Investigator: **Professor Steve Tyerman**

Research Organisation: Cooperative Research Centre for Viticulture

Date: **June 8th 2007**

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The Cooperative Research Centre for Viticulture is a joint venture between the following core participants, working with a wide range of supporting participants.

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1. Abstract

Berries of *Vitis vinifera* L. cv Shiraz can undergo weight loss during later stages of ripening while other varieties such as Chardonnay and Sultana are less prone to this. Hydraulic characteristics of the xylem connections into developing berries were examined in Shiraz, Chardonnay and Sultana. Chardonnay tended to prevent back-flow by having a 10 fold lower conductance for flow out compared to flow in, while for Shiraz both directions of flow gave equal conductance. Cell vitality and the selectivity of cell membranes of the berry play a role in these phenomena so we examined the changes in cell vitality at key stages in development. There was a loss of cell membrane integrity during late ripening stages. The techniques developed may show differences in berry quality based on the pattern of cell death in the pericarp.

2. Executive Summary

In contrast to other grape varieties, berries of *Vitis vinfera* L. cv Shiraz reach a maximum weight around 90 days after flowering then lose up to 25% of that weight prior to harvest. It has generally been accepted that water flow into and out of berries via the xylem ceases at veraison and water continues to enter only via the phloem. The first direct measurements of pedicel xylem water flow by us suggest otherwise.

Using a modified pressure probe for single berries and a flow meter for bunch measurements, water flow into berries via the xylem was found to gradually decrease after veraison. In 2005 Shiraz showed an eight fold and Chardonnay a four fold reduction in hydraulic conductance into berries from veraison to harvest. Around day 90, water flow into Shiraz berries plateaued rather than continue to decrease, coinciding with the commencement of weight loss. Chardonnay berries were found to hydraulically isolate more effectively from the vine than Shiraz. Systematic excision of the berries located the regions where hydraulic conductance changed from veraison to harvest. Distinct differences were found between Shiraz and Chardonnay. We also determined the hydraulic conductance for flow out of the berries for Shiraz and Chardonnay. Here we observed a ten fold higher hydraulic conductance for Shiraz that was similar to hydraulic conductance for flow in to the berry at the equivalent stage of development. This indicates that Shiraz and Chardonnay have contrasting hydraulics. Chardonnay is able to rectify flow in some way so that the system acts like a valve preventing flow out of the berry. For Sultana this comparison could not be done because very negative pressures had to be applied which caused cavitation. These measurements strongly suggest that a degree of xylem connection is maintained during the ripening of Shiraz berries. It also leaves open the possibility of backlow of water from Shiraz berries via the xylem to the vine during the period of berry weight loss. Dye tracer studies with Lucifer yellow were performed on intact bunches on potted vines. These clearly showed backflow from Shiraz berries back to the vine but not from Chardonnay berries at similar development stages.

The cell membranes of the berry are likely to play a role in these phenomena so we examined the changes in cell leakiness using vital stains at key stages in development. Two stains were used, fluorescein diacetate (FDA) and propidium iodide (PI). Both stains showed loss of cell membrane integrity during later stages, but PI showed a sudden accessibility of DNA that then subsided just after the peak in berry weight. This technique shows promise in berry quality assessment.

During the 2004-05 season we also conducted a preliminary trial with a standard molybdenum foliar spray on Shiraz. This was based on a casual observation by Mardi Longbottom that berry weight loss was not observed in Merlot that had been treated with molybdenum. The 2004-05 season preliminary experiment on Shiraz vines in the Coombe vineyard showed that peak berry weight was delayed with molybdenum treatment, so a full factorial treatment was carried out on SARDI vines at Nuriootpa during 2005-06 season. ABA was measured in berries during ripening in this experiment because ABA synthesis involves an enzyme step which requires a molybdenum cofactor. Wines were also made from this trial and sensory analysis performed. Although peak berry weight was also delayed there was no significant difference observed between molybdenum treated and control vines in berry weight loss or in wine properties and sensory scores. There was however a large increase in the molybdenum content of the wines.

3. Background

Please refer to the Introduction in the attached publications:

Tyerman, S.D., Tilbrook, J., Pardo, C., Kotula, L., Sullivan, W., Steudle, E. (2004) Direct measurement of hydraulic properties in developing berries of *Vitis vinifera* L. cv Shiraz and Chardonnay. Aust. J. Grape and Wine Research 10: 170-181.

Tilbrook J. and Tyerman SD (2006) Water, sugar and acid: how and where they come and go during berry ripening. *In* Finishing the job : optimal ripening of Cabernet Sauvignon and Shiraz : Proceedings, ASVO Seminar, Mildura Arts Centre, Mildura, Vic, Friday, 21 July 2006 / edited by David Oag, Kerry De Garis, Steve Partridge, Christopher Dundon, Melissa Francis, Russell Johnstone & Richard Hamilton. pp 1-7

4. Project Aims and Performance Targets

- Objectives

- To gain an understanding of the extent, degree and possible causes of the phenomenon known as Shiraz berry weight loss
- Investigate the physiological & anatomical reasons for shrivel
- Investigate the synchrony of the annual onset of berry weight loss.
- Determine the influence of some vineyard management practices and climatic influence on berry weight loss
- Assess the influence of genotype on the variation in weight loss of ripening Shiraz berries
- In collaboration with industry assess the impact of berry shrivel on wine quality/style

Milestone	**Year**							**Progress**	**Revised Date for Achievement of Milestone**
	1	**2**	**3**	**4**	**5**	**6**	**7**		
Appoint PhD Student	---							PhD student appointed	
Develop PhD Research Proposal	---							Completed	
Initiate glasshouse & field experiments on synchrony	---------							Completed	
Develop experimental techniques	---------							Completed	
Sampling from existing CRCV sites	--------							Completed	
Commence genotype studies	----------							Completed	
Annual reporting to funding bodies	----------							As required	
Prepare articles & reports for industry	----------							Partly completed	
Apply for funding extension for PhD student	-							Not relevant	

5. Methods

Please refer to the Methods in the attached publications:

Tyerman, S.D., Tilbrook, J., Pardo, C., Kotula, L., Sullivan, W., Steudle, E. (2004) Direct measurement of hydraulic properties in developing berries of *Vitis vinifera* L. cv Shiraz and Chardonnay. Aust. J. Grape and Wine Research 10: 170-181.

Tilbrook J. and Tyerman SD (2006) Water, sugar and acid: how and where they come and go during berry ripening. *In* Finishing the job : optimal ripening of Cabernet Sauvignon and Shiraz : Proceedings, ASVO Seminar, Mildura Arts Centre, Mildura, Vic, Friday, 21 July 2006 / edited by David Oag, Kerry De Garis, Steve Partridge, Christopher Dundon, Melissa Francis, Russell Johnstone & Richard Hamilton. pp 1-7.

6. Results/Discussion

Please refer to the Results and Discussions in the attached publications:

Tyerman, S.D., Tilbrook, J., Pardo, C., Kotula, L., Sullivan, W., Steudle, E. (2004) Direct measurement of hydraulic properties in developing berries of *Vitis vinifera* L. cv Shiraz and Chardonnay. Aust. J. Grape and Wine Research 10: 170-181.

Tilbrook J. and Tyerman SD (2006) Water, sugar and acid: how and where they come and go during berry ripening. *In* Finishing the job : optimal ripening of Cabernet Sauvignon and Shiraz : Proceedings, ASVO Seminar, Mildura Arts Centre, Mildura, Vic, Friday, 21 July 2006 / edited by David Oag, Kerry De Garis, Steve Partridge, Christopher Dundon, Melissa Francis, Russell Johnstone & Richard Hamilton. pp 1-7

The following powerpoint slides from Jo Tilbrook's final seminar are presented as a summary that includes some of the work in the references above and new work that is not yet published.

Shiraz harvest 2006

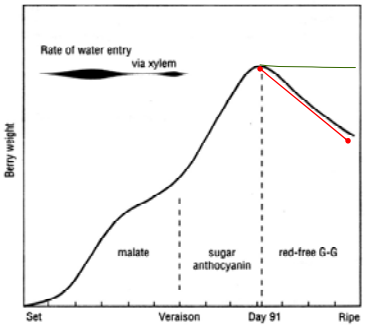
- 440,000 tonnes
- ~1/4 of total wine grape crush in Australia. 2006 WFA Vintage Report
- Grower return based on weight.



Shiraz weight loss

- Field grown Shiraz lose 10-30 % weight ~ 90 DAF with little regard to season, climate or plant water status

McCarthy (1997), Rogiers et al. (2004) Tyerman et al. (2004)



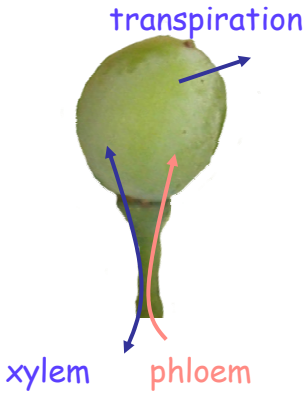
Modified from Coombe and McCarthy (2000)

Does it matter?

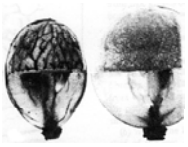
- Premature weight loss may impact on
  - Sugar metabolism in berries. Coombe and McCarthy (2000)
  - Mineral nutrient concentrations in berries. Rogiers et al. (2000)
  - Estimation of vineyard yields. McCarthy (1999)
  - High berry sugar → high % alcohol in wine.
  - Grower income vs wine company.

Water budget of a berry

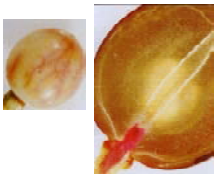
- Xylem dominant inflow pre-veraison, phloem dominant inflow post-veraison. Greenspan et al.(1994)
- Anatomical and physiological evidence supports this. Findlay et al. (1987), Düring et al. (1987)
- Xylem backflow may occur. Lang & Thorpe (1989), Greenspan et al. (1996)



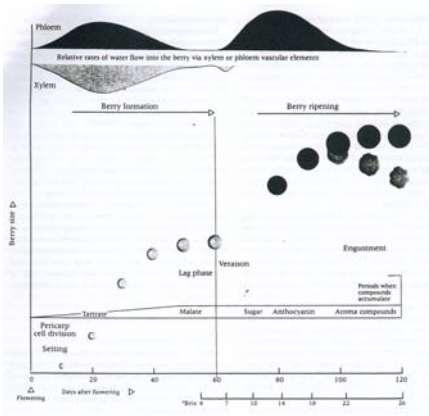
Xylem function



Muscat Gordo Blanco perfused with eosin
(Findlay et al. 1987)



Shiraz perfused with fuchsin
(Rogiers et al. 2001)



Coombe, B. and Iland, P. (2004)

Viticultural practices & Shiraz final berry weight

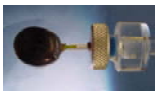
- Rootstocks, N application and irrigation treatments
  - Can alter time course of post veraison expansion or weight $_{max}$
  - **NO** effect on weight $_{final}$
  - Larger berries lose more volume than smaller berries.
- Transpiration rates decrease in shrivelling berries.

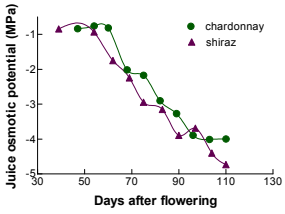
  Rogiers et al. (2004a 2004b)

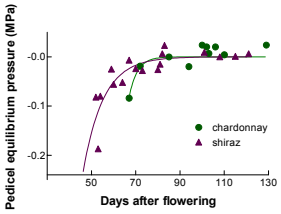
Approaches

- Xylem function
  - Direct hydraulic measurement water flow in & out of berry xylem
- Vitality of pericarp cells in the berry
  - Pericarp cell membrane competence
  - Membranes intact or "leaky"?
  - Vitality
- Viticultural
  - Application of foliar Mo
  - Effect on kinetics of berry weight accumulation

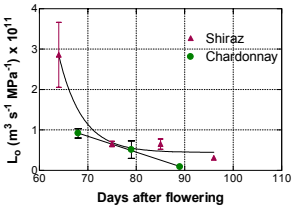
Hydraulic conductance





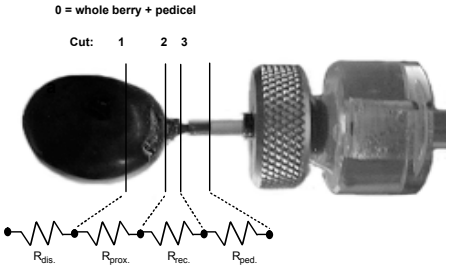


- Juice osmotic potential reduces to ~ -4 MPa
- Equilibration P changes -ve → +ve from veraison to around 90 daf
- Result of hydraulic isolation OR loss of semi-permeable osmotic barrier

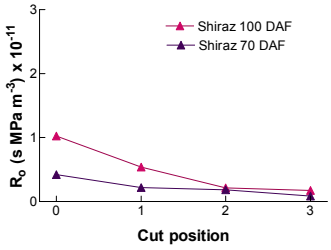


HPFM – transient measures

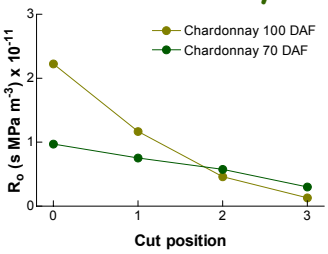
Pressure clamp to locate changes in conductance



Shiraz



Chardonnay



1 / conductance = resistance

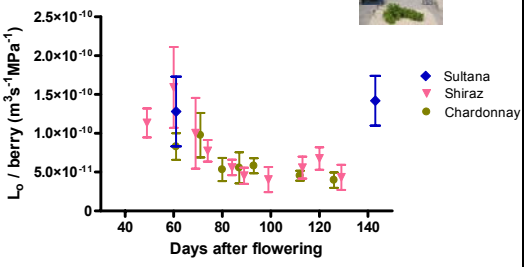
Flow into berries

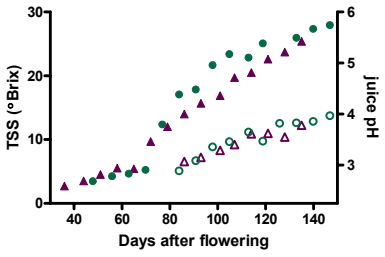


Flow in



- Conductance (L_o) in is similar in Shiraz and Chardonnay (steady state vs transient data)
- Their L_o reduces *gradually* post-veraison
- Sultana L_o is 3 x greater at maturity

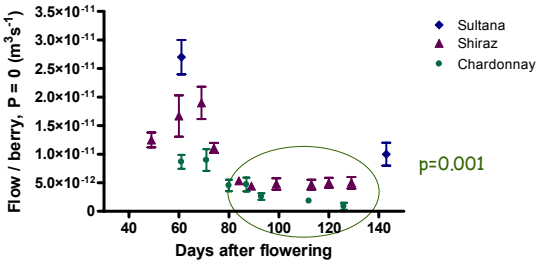


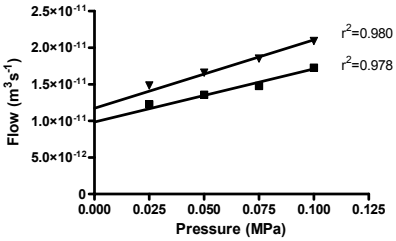


Flow into berries

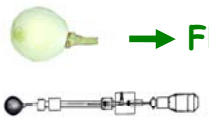


- At P=0, Shiraz maintains a higher flow rate than Chardonnay post 90 daf (p=0.001).
- Shiraz maintains xylem hydraulic connection
- Backflow is more likely in Shiraz
- Sultana has 2 x flow in than Shiraz at maturity

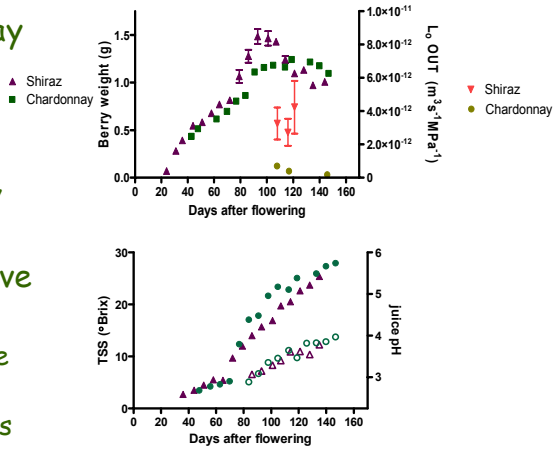




Flow out of berries



- L_o <u>out</u> 10x larger in Shiraz than Chardonnay at maturity
- L_o <u>out</u> of Chardonnay 10x smaller than L_o <u>in</u>
  - berries seem to rectify
- Sultana berries generate highly negative P in xylem at maturity
  - too negative to measure with probe
  - pericarp cell membranes still osmotically competent



Is backflow possible?

- Shiraz berries maintain a hydraulic connection
- Chardonnay berries appear to isolate from the vine
- Literature says no, xylem tracheids stretch and break at veraison
- Test?
  - Lucifer yellow
  - Non-toxic, low pKa,
  - dissociated at low pH of *Vitis vinifera* xylem sap
  - doesn't bind to or cross cell walls

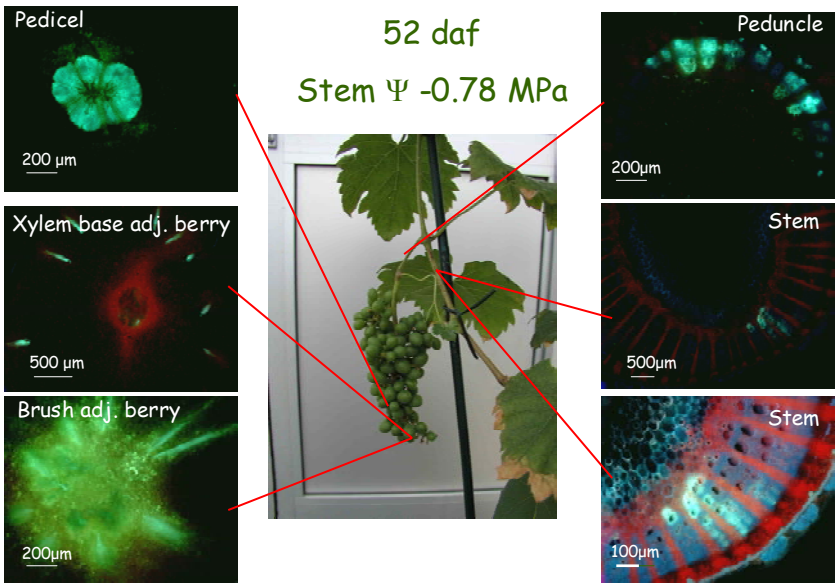
Visualise backflow



Shiraz 4.1 ° Brix

52 daf

Stem Ψ -0.78 MPa



Xylem backflow – presence or absence of Lucifer yellow

	Shiraz			Chardonnay	
Days after flowering	52	101	118	52	97
Cut berry torus	+	+	+	+	-
Cut berry pedicel	+	+	+	+	-
Distal tip rachis	+	+	-	+	-
Distal 1/4 rachis	+	+	-	+	-
Mid rachis	+	-	-	+	-
Mesial 1/4 rachis	+	-	-	+	-
Peduncle	+	-	-	+	-
Stem below node	+	-	-	-	-
Stem above node	-	-	-	-	-
Stem Ψ (Mpa)	-0.75	-0.49	-0.61	-0.68	-0.65
° Brix	4.1	22.3	27.7	6.6	22.7

Questions?

- What happens to membrane competence within the pericarp during development?
- Is cell vitality maintained until harvest?
- Do membranes become leaky?
- Why do table grapes maintain turgidity while wine grapes don't?



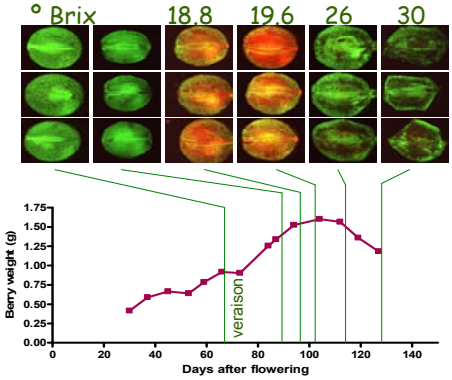


Pericarp vitality

- Fluorescein diacetate
  - Membrane permeable
  - Non polar, not fluorescent
  - Esters cleaved → polar, fluorescent green
- Propidium iodide
  - Membrane impermeable
  - Binds to DNA & RNA in cell nuclei
  - Fluoresces red when bound
  - → membranes NOT intact

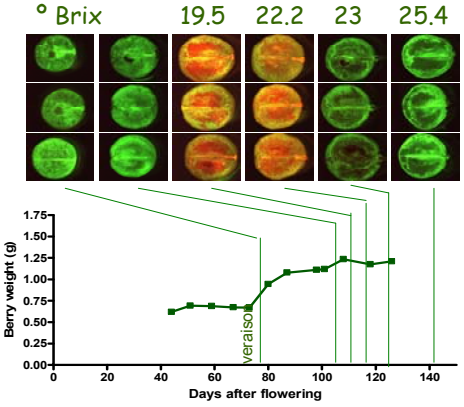
Shiraz pericarp vitality through development

- Pericarp cell membranes permeable at ~98 daf
- Occurs close to $weight_{max}$
- Nucleic acids accessible to PI
- Red correlates with regions showing loss of cell vitality

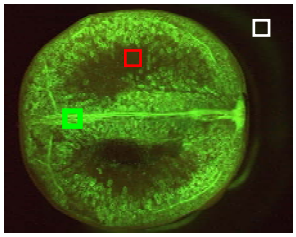


Chardonnay pericarp vitality through berry development

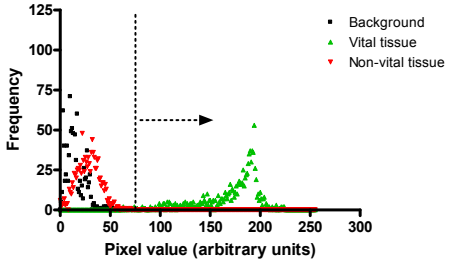
- Pericarp cell membranes permeable 110 daf
- Occurs after weight weight$_{max}$
- Contemporaneous with Shiraz
- Red correlates with regions showing loss of cell vitality



Pericarp cell vitality



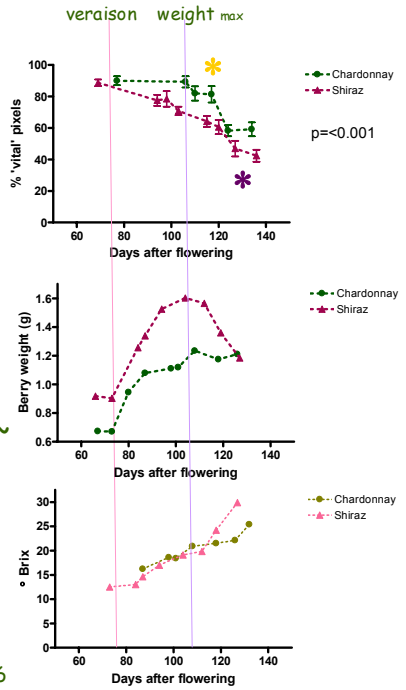
Vitality: Cells with living cytoplasm



- Total area in mm & pixels
- Pixels with arbitrary value >75 are "vital"
- Vital pixels/total pixels x 100 = % "vitality"

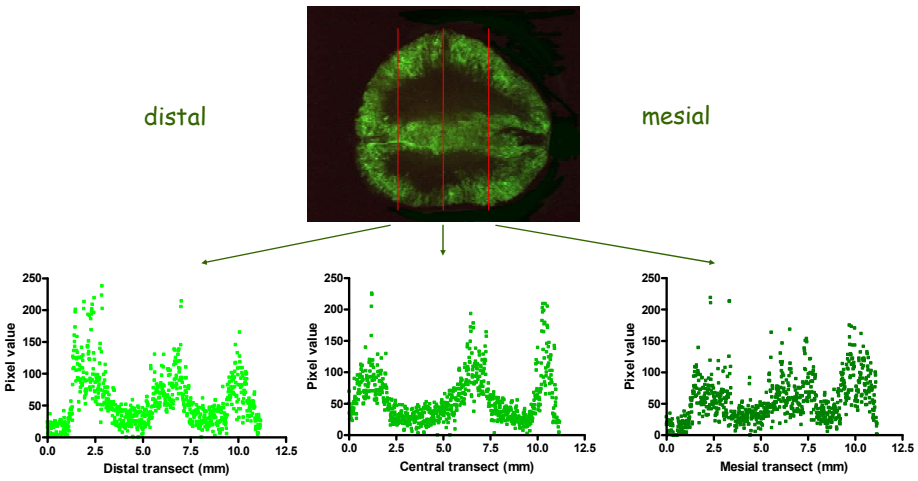
Berry vitality

- Vitality curve of Chardonnay & Shiraz significantly different
- Chardonnay (*) harvested at ~80 % vitality
- Shiraz (*)harvested at ~ 50% vitality
- What are flavour implications?



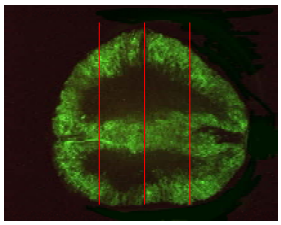
Data from Coombe vineyard 2005-2006

Shiraz vitality – premium fruit

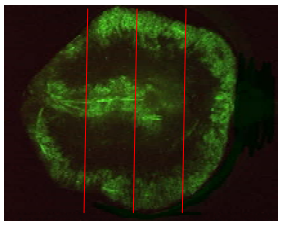


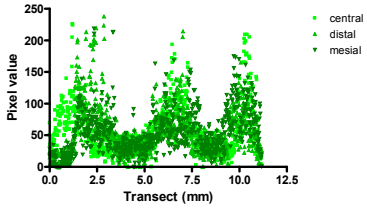
Fruit supplied by Hardys on day of harvest

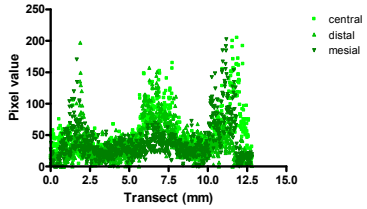
Shiraz vitality – premium fruit



distal mesial

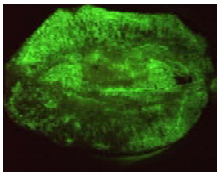




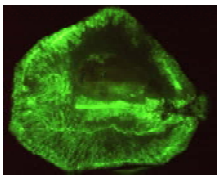


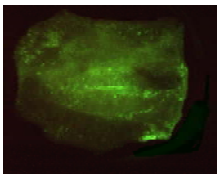
30 ° Brix

Shiraz vitality – A ←→C grade

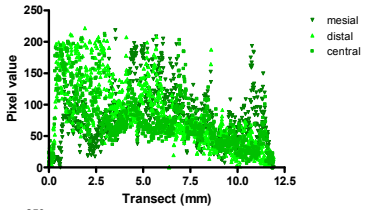


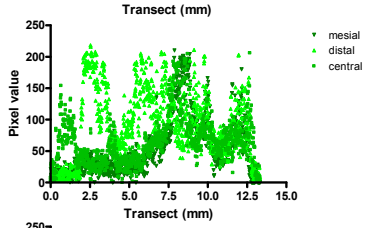
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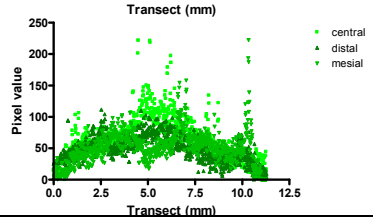




29.6 ° Brix







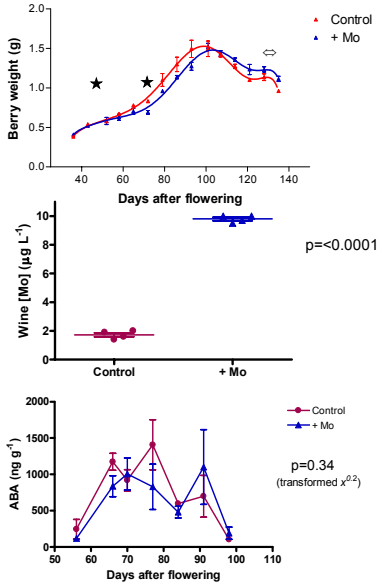
Viticulture: Foliar Mo

- Information that Mo treated Merlot fruit maintains weight at maturity

  Longbottom 2004
- Mo cofactor involved in ABA synthesis
- 2 seasons, 2 vineyards
- Berry weight accumulation
- ABA analysis
- Sensory analysis of wine

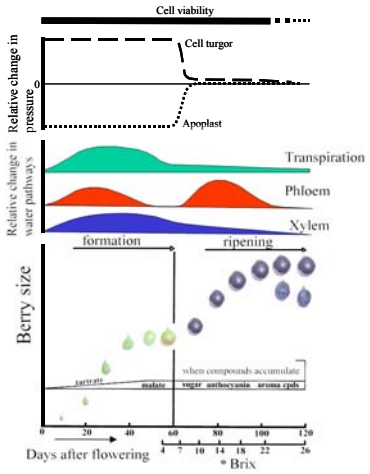
Foliar Mo

- Significant difference in
  - Kinetics of berry weight accumulation
  - Pruning weights
  - Mo content in wine
- No significant difference in
  - Yield
  - ABA in berries
  - Bunch numbers
  - Sensory analysis



Summary

- L_o into berries tapers from veraison to maturity in wine grapes.
- Varietal differences in how efficiently berries isolate from the vine
- Table grapes maintain osmotically competent pericarp cells and do not isolate hydraulically from vine
- Pericarp cell vitality through berry development differs significantly in varieties.
- Backflow is likely to be why Shiraz lose weight before wine making maturity is reached
- Natural senescence process has commenced as berry $weight_{max}$ approaches



Shiraz berry weight loss at sub-optimal maturity – defining the problem

- **Shiraz berries do not isolate hydraulically from the vine in maturity**
- **Backflow is possible and highly likely**
- **Significantly more cell senescence within the pericarp post veraison**
- **All exacerbated by the extended time between veraison and harvest while the 'flavour develops' for winemaking**

7. Outcomes and Conclusions

The outcomes of the project are listed against each of the original objectives.

To gain an understanding of the extent, degree and possible causes of the phenomenon known as Shiraz berry weight loss:

We have applied a new technique using a pressure probe to determine the water relations parameters of whole berries. This work was published in 2004. Our approach has been to compare varieties that are more or less prone to berry shrinkage during ripening. From this comparative approach there is compelling evidence that Shiraz berries have quite different hydraulic properties compared to varieties that normally do not show berry weight loss. The main feature of Shiraz berries is an inability of the xylem in the berry to seal off completely after veraison and in particular the display of a high outward directed (to the vine) hydraulic conductance.

Investigate the physiological & anatomical reasons for shrivel:

Our conclusions from the 2004 publication was that there is quantitative evidence for berry shrinkage to be partially accounted for by backflow into the vine, due to continued hydraulic connection in Shiraz berries. Anatomical studies have proceeded during 05-06 and we have evidence for different rates of cell senescence in the berry (comparing Chardonnay, Shiraz and Sultana) that may included changes in vascular cell viability. The latter is likely to affect the hydraulic properties. Dye tracer studies have shown the presence of backflow in Shiraz berries after veraison.

Investigate the synchrony of the annual onset of berry weight loss:

This component of the project was not continued due to higher priorities for time in the berry physiology components of the project.

Determine the influence of some vineyard management practices and climatic influence on berry weight loss:

We have examined the effect of molybdenum spraying which is being used to treat poor fruit set in some varieties. Two seasons have been examined and we have found that the treatment delays berry shrinkage. However the second season's data indicated that the rate of water loss is the same. There were significant effects of the treatment on the levels of molybdenum in wine, but no differences were detected in sensory attributes.

Assess the influence of genotype on the variation in weight loss of ripening Shiraz berries:

Rather than investigate different clones of Shiraz we have looked more broadly at different varieties of Vitis vinifera. We have shown that Chardonnay and Shiraz have contrasting berry hydraulics. Chardonnay berries were found to hydraulically isolate more effectively from the vine than Shiraz. Also in 2005 a four fold larger hydraulic conductance out of post veraison Shiraz berries was measured compared to Chardonnay. These measurements strongly suggest that a degree of xylem connection is maintained during the ripening of Shiraz berries. It also leaves open the possibility of backlow of water from Shiraz berries via the xylem to the vine during the period of berry weight loss. We have also contrasted Saltana berries and found very different behaviour with a much higher conductance and xylem pressure.

In collaboration with industry assess the impact of berry shrivel on wine quality/style:

From the 2005-2006 molybdenum field trial we have made replicated batches of wine that have been analysed. The harvest was made to counteract the delaying effect, ie the delayed vines were harvested after the control vines so that similar relative amounts of weight loss, brix and TA were obtained. No difference was detected in wine attributes and sensory attributes. Anatomical studies on berries taken from Hardy's premium range were compared with Shiraz vines from the Coombe vineyard. The premium berries showed a more even development of loss of cell membrane permeability at advanced stages of ripening. This technique may be developed as a berry quality measure.

8. Recommendations

Future Research

Several lines of investigation have been suggested by this research. These are:

- Understanding the physiological mechanism for the rectification of water flow in Chardonnay berries, which is absent in Shiraz berries.
- Determining what signal results in the sudden onset of loss of pericarp cell viability at advanced stages of ripening.
- Determine what impact the loss of pericarp cell viability has on the biochemistry of flavour and aroma molecules and precursors.
- Determine if the technique of assessing berry cell viability can be used as a measure of berry quality.

The significance of the research for the industry relates to possible ways to delay or slow the loss of water by Shiraz berries. The cell death event that occurs at about the same time that loss of berry weight occurs may have a direct bearing on berry quality attributes.

9. Appendix 1: Communication

Posters at Conferences

Tilbrook J.1, 2, Pardo C.3, Kotula L.4 and Tyerman S.D. (2005) Differences in hydraulic properties of Vitis vinifera l. cv shiraz and chardonnay during berry development. ComBio Adelaide Sept 2005

Tilbrook J. Tyerman S.D. (2006) Hydraulics and cellular viability of grape berries during development. ComBio 2006 Brisbane Sept 2006

Seminars

S. Tyerman : Novel techniques for investigating the plumbing of plants: from the genes for the pores to the physiology of the pipes. CSIRO Hort Talk May 2004

S. Tyerman Xylem water transport into berries of grapevine during fruit development. 7th International Symposium on Grapevine Physiology and Biotechnology June 21-25, 2004 Davis California.

J. Tilbrook Differences in hydraulic properties of Vitis vinifera l. cv shiraz and chardonnay during berry development.CRCV Symposium June 2005

Reports

Tyerman S.D., Kaiser, B.N., Coleman, R., Shelden, M.C. and Tilbrook J. (2005) Investigating the hydraulics of grape vines: from the genes for the pores to the physiology of the pipes. Proceedings of the Twelfth Australian Wine Industry Technical Conference (eds R. Blair, P. Williams, S. Pretorius) pp 179-181.

Published papers

Tyerman, S.D.; Tilbrook, J., Pardo, C., Kotula, L.; Sullivan, W., Steudle, E. (2004) Direct measurement of hydraulic properties in developing berries of *Vitis vinifera* L. cv Shiraz and Chardonnay. **Aust. J. Grape and Wine Research** 10: 170-181.

Vandeleur, R., Niemietz, C.M. Tilbrook, J., and Tyerman, S.D. (2005) Roles of aquaporins in root responses to irrigation. **Plant and Soil** 274: 141-161.

Tilbrook J. and Tyerman SD (2006) Water, sugar and acid: how and where they come and go during berry ripening. *In* Finishing the job : optimal ripening of Cabernet Sauvignon and Shiraz : Proceedings, ASVO Seminar, Mildura Arts Centre, Mildura, Vic, Friday, 21 July 2006 / edited by David Oag, Kerry De Garis, Steve Partridge, Christopher Dundon, Melissa Francis, Russell Johnstone & Richard Hamilton. pp 1-7

10. Appendix 2: Intellectual Property

NIL

11. Appendix 3: References

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Tilbrook J. and Tyerman SD (2006) Water, sugar and acid: how and where they come and go during berry ripening. *In* Finishing the job : optimal ripening of Cabernet Sauvignon and Shiraz : Proceedings, ASVO Seminar, Mildura Arts Centre, Mildura, Vic, Friday, 21 July 2006 / edited by David Oag, Kerry De Garis, Steve Partridge, Christopher Dundon, Melissa Francis, Russell Johnstone & Richard Hamilton. pp 1-7

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12. Appendix 4 : Staff

Joanne Tilbrook (PhD student)

Professor Steve Tyerman

Dr Michael McCarthy

13. Appendix 5 Copies of published work

Direct measurement of hydraulic properties in developing berries of *Vitis vinifera* L. cv Shiraz and Chardonnay

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Abstract

Berries of *Vitis vinifera* L. cv Shiraz can undergo weight loss during later stages of ripening. Existing published views on how weight loss occurs are based on changes in capacity of the vascular system to import water during development (McCarthy and Coombe, *Australian Journal of Grape and Wine Research*, 5, 17–21, 1999). One important element of these views is the proposed cessation of water flow through the xylem after veraison. We have now measured the water flow into berries of Shiraz and Chardonnay as they develop using the pressure probe and the high pressure flow meter (HPFM). The pressure probe connected to the pedicel of individual berries provided measurements of single berry hydraulic conductance. By systematic excision of tissue segments of the berry and pedicel we determined where in the pathway hydraulic conductance changed during development. The HPFM was used on whole bunches showing that berries (including pedicels) represent parallel high hydraulic resistances and that the hydraulic resistance of the bunch axis was rather small. The hydraulic conductance per berry could be determined from excision experiments. There was close agreement between the pressure probe and HPFM measurements. Both showed a ten-fold reduction in hydraulic conductance of whole berries from veraison to full ripeness. Shiraz had hydraulic conductances that were 2- to 5-fold higher than those for Chardonnay. Shiraz maintained a higher hydraulic conductance past 90 days after flowering than Chardonnay. The decrease in hydraulic conductance occurred in both the distal and proximal parts of the berry for both varieties. The pressure probe also provided measurements of the xylem pressure that non-transpiring berries could develop. These pressures were –0.2 to –0.1 MPa until veraison and increased to zero when the juice osmotic potential reached about –3 MPa in Chardonnay and –4 MPa in Shiraz. The results suggest values of the reflection coefficient of the osmotic barrier around the xylem vessels of about 0.1–0.2 at veraison decreasing to 0 at harvest. It is suggested that in addition to changes in xylem anatomy, aquaporins in berry membranes may play a role in regulating hydraulic conductance. Water movement from the berry back to the parent vine via the xylem (backflow) may be an important component of berry weight loss in Shiraz, particularly if the phloem ceases functioning at high osmotic potentials near maximum weight. Backflow could account for a weight loss of 43 mg per day in Shiraz berries for a relatively small gradient of 0.1 MPa.

Abbreviations

$\mathbf{L_o}$ hydraulic conductance; $\mathbf{L_p}$ hydraulic conductivity; $\mathbf{R_o}$ hydraulic resistance; **DAF** days after flowering, **HPFM** high pressure flow meter; **TSS** total soluble solids

Keywords: Vitis vinifera, *berry ripening, berry shrivel, xylem function, xylem pressure, hydraulic conductance, hydraulic conductivity, pressure probe, HPFM, aquaporins, berry shrivel*

Introduction

Weight loss of *Vitis vinifera* L. berries during advanced stages of ripening may have impacts on sugar metabolism and flavour development (Coombe and McCarthy 2000), mineral nutrient concentrations of berries (Rogiers et al. 2000), and the practical estimation of final yields by viticulturists (McCarthy 1999). The most studied variety in this context has been Shiraz, which can loose over 20% of the maximum berry weight depending on maximum weight achieved (McCarthy 1997, Rogiers et al. 2004a,b). Weight loss in Shiraz berries has been observed in a range of climates (Smart et al. 1974, Davies and Robinson 1996, McCarthy 1997, Rogiers et al. 2000) and under different irrigation regimes (McCarthy 1997). McCarthy observed that the onset of weight loss in field grown vines occurred at about 90 days after flowering (DAF) irrespective of seasonal differences or irrigation regime. For potted Shiraz vines, deficit irrigation, nitrogen nutrition and rootstock

seemed to have an effect on the timing of the onset of weight loss with maximum weight being recorded between 74 and 85 DAF (Rogiers et al. 2004a). The extent of weight loss appeared to be related to the size of berries at their maximum weight that varied according to treatments. Larger berries at maximum volume (2 cm^3) showed larger percentage loss than smaller berries (1 cm^3) (Rogiers et al. 2004a).

To better define the cause of berry weight loss in Shiraz, McCarthy and Coombe (1999) calculated the components of berry weight during weight loss to show that it was loss of water that accounted for most of the loss of weight. The rate of accumulation of solutes per berry slowed after about 90 DAF and reached a plateau at 105 to 115 DAF. The changes in weight during different periods of berry development were explained in terms of variable contributions from xylem and phloem to berry water inflow. They proposed that xylem flow accounted for most of the water inflow up to veraison, during and immediately after which the xylem became non-functional. Water flow into the berry after veraison was proposed to occur predominantly via the phloem. Just before maximum weight was achieved it was proposed that the phloem became progressively non-functional resulting in water loss via transpiration exceeding water inflow. This model provides a good working hypothesis of berry weight loss where both xylem and phloem play key roles.

Historically, the change in xylem functionality during ripening has been investigated in three ways. Firstly the uptake of water soluble dyes has been used to examine the xylem transport in both excised (Findlay et al. 1987, Düring et al. 1987, Creasy et al. 1993) and attached berries (Rogiers et al. 2001). These have all demonstrated that an apparent blockage occurred in the peripheral and axial xylem vessels of post-veraison berries. The general pattern from studies on Muscat Gordo Blanco (Findlay et al. 1987), Pinot Noir and Merlot (Creasy et al. 1993), Riesling (Düring et al. 1987) and Shiraz (Rogiers et al. 2001) is that during veraison, dye accumulation virtually ceased in the peripheral xylem and became much reduced or ceased beyond the brush region of the axial xylem. Breaks in tracheids mainly in the peripheral xylem have been linked to cessation of dye transfer (Findlay et al. 1987, Creasy et al. 1993). These studies imply that there is a marked change in xylem function that militates against the use of certain dyes used as tracers. Studies based on dye molecules that are charged and of large molecular weight do not exclude the possibility that water molecules may still enter or leave the berry via the xylem and connections to the berry apoplast (Rogiers et al. 2001).

A second method to assess xylem function has been to measure the changes in K and Ca content of berries as they mature. The rationale is that Ca is xylem mobile and phloem immobile, while K is both xylem and phloem mobile. Lang and Thorpe (1989) recalculated data on de Chaunac grapes from Hrazdina et al. (1984) to show that Ca accumulation ceased at veraison. Creasy et al. (1993) showed for Pinot Noir that K uptake increased rapidly after veraison but Ca did not. Contrasting with these results, Rogiers et al. (2000) showed that Ca content continued to increase in post-veraison berries of Shiraz, although the K content increased at a greater rate so that the K/Ca ratio increased in post-veraison berries. This suggests that Shiraz berries may have a greater degree of xylem function after veraison compared to other varieties.

The third method of assessing the role of xylem and phloem during development has been to measure berry volume, weight or diameter, after treatments that impede the function of the phloem. These treatments, which have included girdling or heating of the pedicel, are compared to untreated attached berries, and berries that have been completely detached from the bunch but left in place to allow normal transpiration. With such experimentation Lang and Thorpe (1989) observed flow through the xylem from the berry to the parent plant (coined 'backflow'). For Italia grapes that had ceased volume expansion at full ripeness, 36% of the net water loss could be accounted for by xylem backflow.

In a highly original experiment, Greenspan et al. (1994) examined the diurnal swelling and shrinking patterns of treated berries of potted Cabernet Sauvignon and Zinfandel. They concluded that the role of the xylem in berry volume changes was much reduced after veraison while the contribution by phloem increased substantially. A subsequent study on field vines of Cabernet Sauvignon under different irrigation regimes confirmed the potted vine study, but also showed that some backflow through the xylem occurred (Greenspan et al. 1996). However, most water lost by the berry could still be accounted for by transpiration. From measurements of bunch and stem water potentials, Greenspan et al. (1996) observed that the water potential gradients were predominately directed into the berry. More recently, Rogiers et al. (2001) have used girdling experiments on Shiraz berries. Girdling at 67 DAF reduced the volume of the berries as expected, but detached berries reduced volume to a much greater extent. This indicated that water inflow still occurred through the xylem despite the apparent blockage reported by tracer dyes. If the xylem in the brush region remains connected to the berry apoplast in Shiraz berries after veraison, it would also be possible for backflow to occur if the pressure gradient is in the appropriate direction. Thus xylem backflow could account for some berry weight loss in Shiraz, which accompanies the cessation of phloem function proposed by McCarthy and Coombe (1999).

The pressure gradients that generate flow via the xylem/apoplast of the berry will depend on the degree to which the stem water potential of the parent plant is transmitted to the pedicel xylem and apoplast of the berries, and on the counter tension that the berry can develop within the apoplast. Water potentials of the xylem of the bunch stem have been observed to be similar or slightly more negative than the main stem xylem (Greenspan et al. 1996), indicating that stem xylem tension from the vine can be transmitted through to at least the xylem in the bunch rachis. Although berries develop strongly negative osmotic potentials (up to –5 MPa), these may not counter the apoplast tensions developed by the parent vine. It has been indicated that membrane

selectivity of pericarp cells is lost during ripening, i.e. the reflection coefficient is reduced (Lang and Thorpe 1989, Lang and Düring 1991). The reflection coefficient of a membrane is by definition the extent to which an osmotic gradient is translated into a pressure gradient across a membrane. It is likely that the barrier between the berry lumen and xylem lumen will have a low reflection coefficient even when membranes of pericarp cells have intact selective membranes (high reflection coefficient). Any reduction in reflection coefficient of berry membranes could render the berry's negative osmotic potential ineffective in preventing water loss to the parent vine.

From the discussion above, a key factor for understanding the role of the xylem in berry weight loss in ripening fruit is the way in which the hydraulic conductance and selectivity of the xylem-berry interface changes during development. This interface is likely to be analogous to a composite osmotic barrier consisting of pathways with different hydraulic conductances and reflection coefficients analogous to the composite transport model of membranes or roots (Steudle and Peterson 1998, Steudle and Henzler 1995). According to McCarthy and Coombe (1999), we would expect to find a sudden reduction in hydraulic conductance during veraison. Rogiers et al. (2004b) also hypothesise that a reduction in net vascular flow into the berry, concomitant with continued transpiration, leads to berry weight loss. Despite the importance of this reduction there appear to be no quantitative measurements of overall xylem hydraulic conductance, nor any elucidation of where changes in hydraulic conductance might occur within either the berry or the pedicel-berry system.

Here we report for the first time quantitative measurements of hydraulic conductance of pedicel-attached berries and parts thereof by using the root pressure probe (Steudle et al. 1993) and high pressure flow meter (HPFM, Tyree et al. 1995). Comparisons are made between Shiraz and Chardonnay berries. Chardonnay was chosen because it is not known to undergo substantial weight loss during later stages of ripening. We expected to observe that the hydraulic conductance of Shiraz was higher than that of Chardonnay and that the main region within the berry where hydraulic conductance changed during development would be in the brush region. Using the pressure probe we were also able to measure the xylem tensions that could be developed by non-transpiring detached berries. We expected to find a reduction in xylem tensions if the membranes in the osmotic barrier separating the xylem from the berry interior were to lose semipermeability during ripening or if the berries would tend to become hydraulically isolated due to a high hydraulic resistance in the xylem.

Material and methods

Fruit material

Hydraulic measurements were conducted on pre-veraison and post-veraison berries collected from *Vitis vinifera* cvs Shiraz (BVCR12) and Chardonnay (I10V1) growing on own roots in the Coombe Vineyard, Waite Campus of the University of Adelaide during the 2002–2003 and 2003–2004 seasons. In 2002–2003 Shiraz berries were selected from twelve vines that formed part of a randomised block rootstock trial. The Chardonnay berries were from twelve adjacent vines. Whole shoots were cut from the vine in the morning, the cut stem placed into water and transported to the laboratory (500 m). Berries were cut from the bunch with the pedicel underwater immediately before experiments. For 2003–2004, six replicate Shiraz and Chardonnay vines were selected from across the block and used for all measurements. Whole shoots collected with two bunches attached were transported to the laboratory with the cut stem in water. Samples were collected at least three times a day, because when the shoots were more than 3 hours in water the conductance appeared to fall sharply, presumably because of xylem occlusion caused by bacterial growth. Once in the laboratory the bunches were detached immediately prior to experiments at the proximal end of the peduncle by cutting with a sharp razor blade under water. The bunch closest to the proximal part of the shoot was used for HPFM measurements.

The dates of flowering (time for 50% capfall across the entire vineyard) were recorded for both seasons so that changes in berry physiology could be related to days after flowering (McCarthy 1999).

Pressure probe measurements

The root pressure probe (Figure 1a) was used to measure the water relations of whole berries and berries cut back at different positions. The root pressure probe is normally used to measure equilibrium root pressure and root hydraulic conductance (e.g. Steudle et al. 1993). Analogous to this we have connected the probe to the pedicel of single berries and assumed that the xylem provides a hydraulic connection between the probe and berry (analogous to the use of the instrument with roots). Dye injection experiments confirmed that the xylem was connected hydraulically to the pressure probe. To seal the pedicel with the instrument a piece of tubing 15–20 mm long (Tefzel-Schlauch tubes of ID 1.0 and 1.6 mm (OD 1.6 and 3.2 mm) depending on pedicel diameter) was flared at one end in order to provide a snug fit to the pedicel. The pedicel was gently pushed into the flared end and sealed with a cyanoacrylate glue (Super Glue or Loctite 401 or 406 adhesive with tubing treated with Loctite 770 primer). This provided a pressure-tight seal which was tested as part of the measuring procedure (see below). The berry and pedicel connected to the tube were sealed into the end of the pressure probe taking care to eliminate any air from the system. The water used to fill the tubing and probe was Millipore (0.2 μM) purified and de-aerated by vacuum treatment. The berry was covered with wet tissue paper to prevent transpiration. As the seal was tightened around the tubing the pressure was elevated in the system (Figure 1b). Measurements were made in the laboratory at temperatures between 20–22°C.

Steady state pressure

The pressure of the internal hydraulic system was allowed to relax to a steady state level after sealing (Figure 1b). In

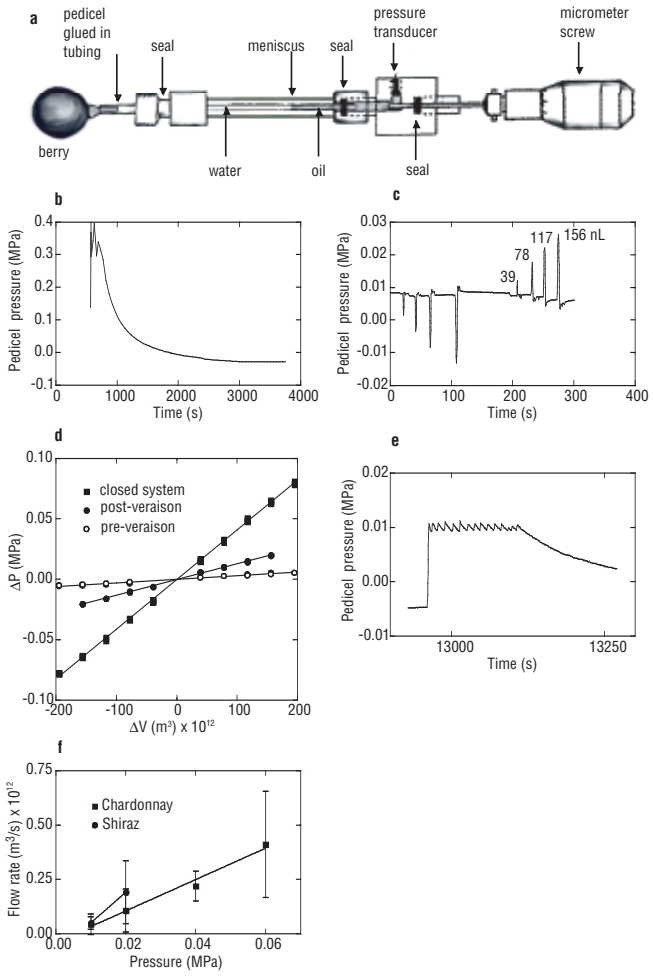


Figure 1. The pressure probe technique for measurement of berry hydraulic conductance and xylem tensions in the pedicel. (**a**) Diagram of the 'berry' pressure probe illustrating the method of attaching the berry pedicel to a piece of plastic tubing that is sealed into the probe. The oil/water meniscus can be used to measure volume flow into or out of the berry. The pressure transducer is connected to a computer and the micrometer screw is used to inject or withdraw water from the pedicel-attached berry system. (**b**) Initial equilibration stage after sealing the berry to the probe. The pressure slowly relaxes to a steady level that was always negative in berries pre-veraison and became positive during veraison and after veraison. (**c**) Pulses of pressure recorded when different volumes were injected or withdrawn from the berry. These are used to calculate elasticity of the system. (**d**) Pressure change as a function of volume change used to calculate elasticity. A closed (blocked) system was measured in order to check the hydraulic connection with the berry. (**e**) A pressure clamp experiment. The pressure is held at a constant level in this case by continued injection of volume into the berry. (**f**) The rate of volume injected is recorded and plotted as a function of the pressure. The slope of the relationship in (f) enables calculation of the hydraulic conductance of the berry. Berries were cut back at different positions during an experiment (see Figure 7a) in order to determine respective positions of major hydraulic resistances.

pre-veraison berries the pressure often became quite negative and in some instances caused cavitation in the probe. When this occurred it was not possible to carry out further measurements unless a constant water flow into the berry was allowed at higher pressures.

Elasticity

Subsequent to a steady state pressure being achieved, elasticity of the 'system' (measured as $\beta_s = \Delta P/\Delta V$) was determined by changing volume (using the micrometer screw and oil-water interface position in the measuring capillary) and recording the change in pressure (Figure 1c). 'System' in this case refers to a pedicel-attached berry plus probe and seals. The elasticity of the probe (β_p) with a blocked piece of tubing inserted in place of the pedicel was also measured. Figure 1d shows the change in volume with change in pressure for connected berries and blocked tubing. If β_s was equal to or higher than the value for blocked tubing (β_p), then it is possible that hydraulic continuity through the pedicel had not been obtained. Other criteria were used to test for hydraulic continuity: (1) glue was used to block the cut pedicel which should then show a large reduction of hydraulic conductance; (2) at the end of an experiment the cut surface of the pedicel was allowed to dry. If this caused a negative pressure to develop in the xylem, which returned to zero when a drop of water was placed on the cut surface it was assumed that a hydraulic connection was present.

Hydraulic conductance

To determine the hydraulic conductance, a pressure clamp procedure was used (Figure 1e). Initial experiments demonstrated that pressure relaxation curves combined with measurements of β, as usually used with roots (Steudle 1994), overestimated the hydraulic conductance through cut pedicels. Pressure clamp (volume relaxation) experiments entailed elevating the pressure by a certain recorded amount (between 0.01 and 0.05 MPa) and maintaining this increment in pressure while recording the rate of volume flow into the berry, when this was steady or nearly so (as during the HPFM technique). This could be done either using the movement of the oil-water interface or by following the movement required on the micrometer screw (both gave the same volume flow). Figure 1f shows steady flow rate as a function of pressure into whole berries from which hydraulic conductance was calculated. To determine limiting hydraulic conductances in parts of the berry and pedicel, distal portions were excised progressively. These positions coincided with: the base of the seeds, base of the berry; and base of the receptacle (torus) (Figure 7a). Hydraulic conductance (L_o) was divided by the berry surface area to give a value for 'hydraulic conductivity' (L_p) for comparison with other measurements on fruit in the published literature (Fishman and Génard 1998). The assumption behind using the fruit surface area is that the actual composite membrane surface area is proportional to berry size.

Hydraulic conductance of whole bunches using the High Pressure Flow Meter (HPFM).

An hydraulic conductance flow meter (HPFM-XP Dynamax, Houston Texas, USA) was used to measure hydraulic conductance of whole bunches and cut-back bunches. Use of the HPFM has been described for measuring root and shoot systems hydraulic conductance by Tyree and colleagues (Tyree et al. 1995, Bogeat-Triboulot et al. 2002). The HPFM was used as per manufacturer's instructions in either the transient or steady state mode of operation. The temperatures at which the measurements were made

were close to that used for the calibration of the instrument (between 20–22°C). After connecting the bunch via the cut peduncle and ensuring that no air had entered while sealing, transient measurements were begun. The pressure was slowly elevated at a constant rate while flow was recorded with the instrument. Flow was measured as the drop in pressure across a manifold connected with tubing of different and known hydraulic conductances. It was sometimes necessary to undertake a few trial measurements in order to use the appropriate flow range on the instrument and to test for the presence of air in the system after sealing. The hydraulic conductance was determined from the slope of the linear part of the volume flow versus pressure relationship (i.e. for steady flows, when all relevant capacities for water were infiltrated within the pedicel or berry). The pressure range for transient measurements was normally between 0.2 and 0.45 MPa. Measurements using the quasi steady state flow meter mode of the instrument where a constant pressure for a period of time was applied and flow rate measured (analogous to the pressure-clamp measurements with the pressure probe), gave hydraulic conductances not significantly different from those measured in transient mode on the same bunches ($P = 0.27$, two ANOVA with repeated measures on six replicated bunches, cut to the four segments, see below). When using either the HPFM or pressure clamp, it has to be ensured that infiltration of tissue by relatively large amounts of water does not change the hydraulic conductance being measured. No evidence of this was seen, and this is consistent with the tiny volumes injected relative to the volume of berries.

After at least four replicate measurements of hydraulic conductance, the measured bunch had the distal-most portion (about 25% of berries) severed with a sharp razor and the cut rachis (main bunch stem) was sealed with Super Glue. Another set of transient measurements was taken before the next most distal 25% (approx.) of berries was removed and the rachis again sealed. This process was continued until at least three segments of the bunch were removed. This provided hydraulic conductances for: (1) the whole bunch, (2) bunch minus first distal segment, (3) bunch minus first and second distal segments, (4) bunch minus first, second and third distal segments; called proximal segment. Berries were counted and weighed for each segment. Surface areas could be calculated from the known relationship between surface area and berry weight (see below).

Plots of hydraulic conductance versus the number of berries yielded linear to slightly concave-up curves (Figure 2a). Although in most cases a linear equation was statistically a better fit, we used an exponential equation to fit the relationship between hydraulic conductance and number of berries. The hydraulic conductance per berry was estimated from these fits assuming that the bunch stem and branches had a much higher hydraulic conductance than the berries themselves. This was checked by examining the hydraulic resistance (1/hydraulic conductance) as a function of berry number (Figure 2b). That relationship could be best fitted by an equation of the form: *Resistance* = *A* + *B*/*number of berries*, where *A* was

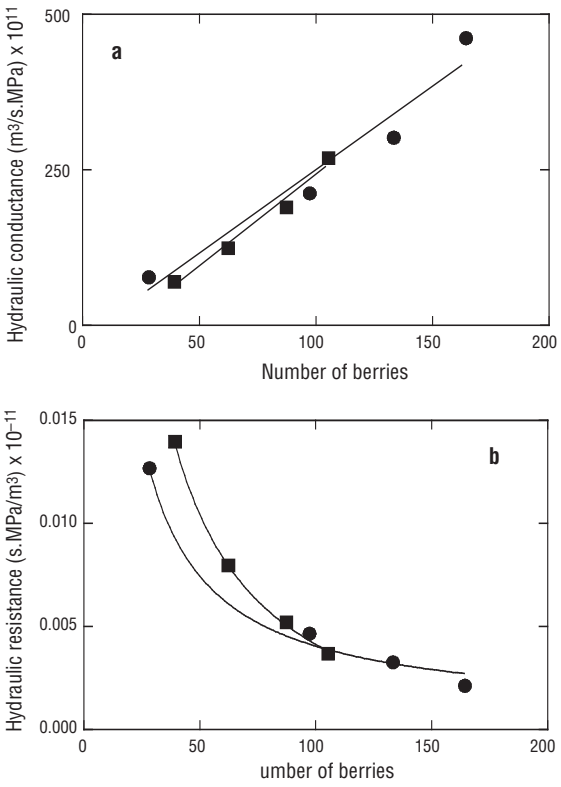


Figure 2. High pressure flow meter experiments performed on a whole bunch while excising successive segments of the bunch (distal to proximal) gave a close to linear relationship between hydraulic conductance and the number of berries (**a**) where two separate experiments are shown. (**b**) Resistance as a function of the number of berries could be fitted to an equation of the form $R_0 = A + B/(\text{berry number})$. A was small indicating that the rachis hydraulic conductance was much larger than that of the berries.

close to zero, to a good approximation. A value of A close to zero indicates a high conductance of bunch stem and branches. Hence, the whole bunch acted as a simple system of similar parallel hydraulic resistors, where component conductances were additive.

Berry weight

During the 2003–2004 season mean berry weight was recorded from 50–100 berries as 5-berry samples from proximal mid and distal parts of bunches on the six replicate vines. Berries were collected by the same method during 2002–2003 for Brix, pH and osmolality measurements.

Brix, pH and osmolality

Approximately 50 mL of juice was collected from berries selected for weight measurements. Brix was measured using a digital refractometer (temperature compensated, ATAGO Model PR101). pH was measured using a Cyberscan pH 310 Series (Eutech Instruments). Osmolality was measured on juice using a Wescor (Model 5500) water vapour pressure osmometer and converted to osmotic potential ($\Psi_\pi = -RTC$, where $R = 8.3143$ J/mol.K and T = absolute temperature in K).

Stem water potential

One leaf from each replicate vine was sealed in foil-covered zip-lock bags for at least one hour before water potential

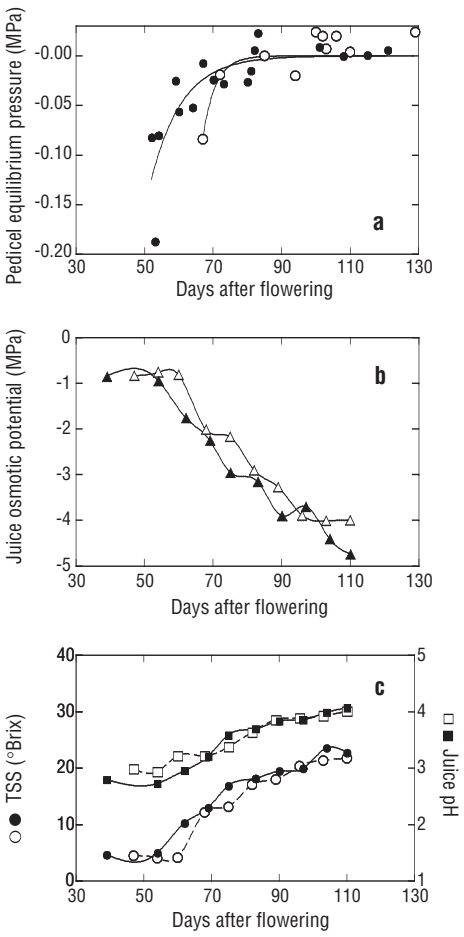


Figure 3. Pedicel equilibrium pressure (**a**) and ripening responses (juice osmotic potential, (**b**); TSS and pH (**c**)) of berries of Shiraz (closed symbols) and Chardonnay (open symbols) as a function of days after flowering collected during the 2002–2003 season. Comparison of pedicel equilibrium pressure with juice osmotic pressure at 50 DAF resulted in an estimate of the reflection coefficient of the osmotic barrier within the berry of 0.15.

was recorded using a pump-up portable pressure chamber (PMS Instrument Co.). Measurements were taken between 12 noon and 2 pm local time.

Berry deformability

Berry deformability was measured across the mid section of the berry at the widest point using a set of Harpenden Skinfold calipers that placed a constant force generated by a spring equivalent to 2.06 N. The deformation was measured as the millimeter reduction in diameter in response to the force. Five berries from each of four bunches of the six replicate vines were randomly selected to measure deformation.

Berry surface area

Berry surface area was calculated from measurements of length and diameter using the formula for the surface area of a prolate spheroid. For surface areas of berries on whole bunches used for the HPFM, a calibration was first obtained between berry surface area and berry weight on separately harvested berries. The relationship was best fitted by a quadratic equation of the form; surface area (mm^2) = A + B weight (g) + C weight $(g)^2$, where for Shiraz A = 544.6 mm^2, B = 1305 mm^2/g, C = –90.68 mm^2/g; and Chardonnay A = 486.7 mm^2 B = 1455 mm^2/g, C = –132.8 mm^2/g. Surface areas for HPFM experimental berries were then determined from the weights of the berries on the segments of the bunches.

Results

Pedicel equilibrium pressure related to post-veraison berry development

Pedicel equilibrium pressures were initially negative (subatmospheric) at veraison, which for the 2002–2003 growing season occurred at approximately 50 days after flowering (DAF) in both Shiraz and Chardonnay (Figure 3a). Pre-veraison berries could be measured, but often the negative pressures of less than –0.1 MPa generated would cause cavitation in the pressure probe. Through veraison and afterwards, the pedicel equilibrium pressures gradually increased and approached an asymptote at about zero, or at slightly positive pressures of no more than about 0.02 MPa. For both Chardonnay and Shiraz the pedicel equilibrium pressures reached zero MPa at about 80–90 DAF (see fitted curves in Figure 3a). The timing of this corresponded to juice osmotic potentials of about –3 and –4 MPa for Chardonnay and Shiraz, respectively (Figure 3b). TSS and pH increased to reach full ripeness values at about 110 DAF (about 22°Brix and pH 4 respectively) (Figure 3c).

In the 2003–2004 growing season more data were collected on berry hydraulic parameters over the time between veraison and about 100 days DAF. The pedicel equilibrium pressures behaved essentially as was observed in 2002–03, with a gradual increase from subatmospheric values of between –0.025 MPa and –0.05 MPa to slightly positive values of up to 0.025 MPa (Figure 4a). Similar to the behaviour recorded in 2002–03, observations in 2003–04 showed that Chardonnay reached pressures near zero near 80 DAF while Shiraz reached zero pressure at 90 DAF. This again corresponded to osmotic potentials of the juice of –3 MPa in Chardonnay and –4 MPa in Shiraz (Figure 4b). Osmotic potential of the juice declined more rapidly over time than for 2002–03, but reached similar peak values. Subsequent to these peak values of about –4.5 MPa in Chardonnay and –4.8 MPa in Shiraz, the osmotic potential increased again to values above –4 MPa (Figure 4b). For both Chardonnay and Shiraz there was a significant loss of berry weight after day 79 in Chardonnay and after day 75 in Shiraz (Figure 4c). This corresponded to a slight reduction in stem water potential measured at midday on the vines bearing the fruit (Figure 4d). However, subsequent irrigations that increased water potential to above –1 MPa for Shiraz (Figure 4d) did not correspond to a gain in weight of Shiraz berries (Figure 4c). Berry deformability increased as expected during ripening, reaching a maximum for Shiraz at 96 DAF (Figure 4e). A full series of measurements was not possible for Chardonnay due to the selected vines being harvested. Despite veraison occurring later in 2003–04 than in 2002–03, the berries reached maturity quicker with full ripeness (about 22°Brix) being reached at day 89 in Chardonnay and after day 96 in Shiraz (Figure 4f).

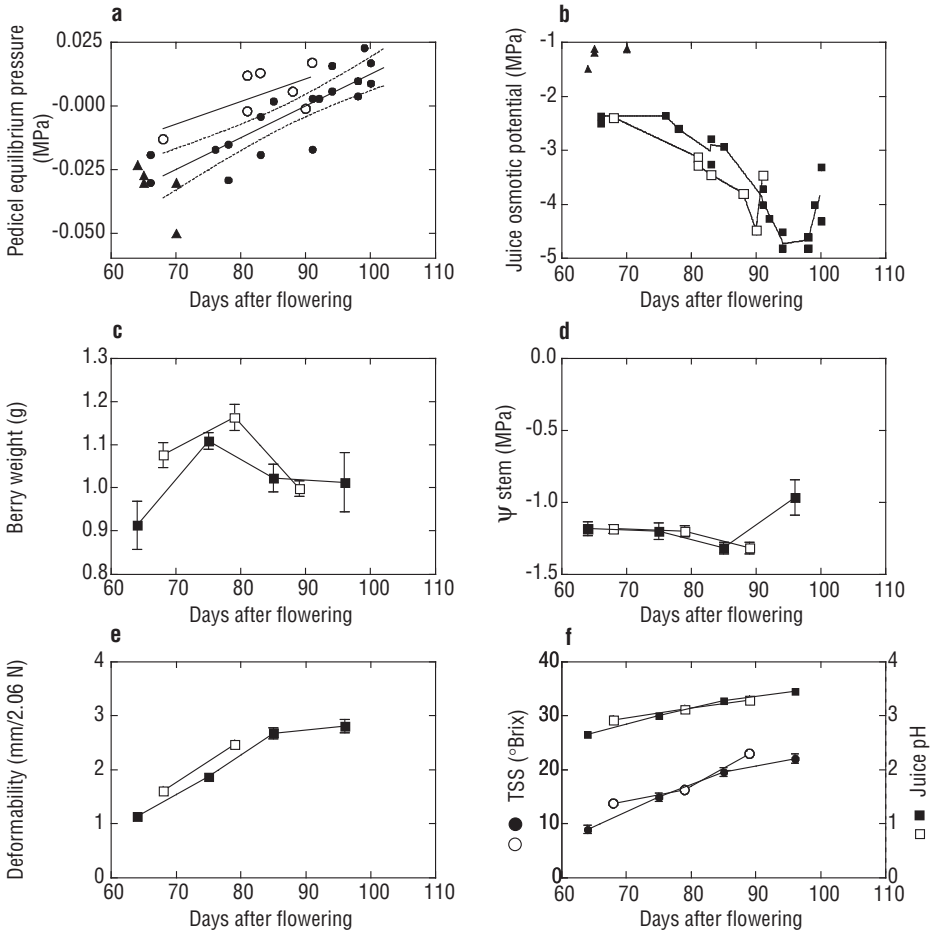


Figure 4. Pedicel equilibrium pressure (**a**) and berry physiological parameters of Shiraz (closed symbols) and Chardonnay (open symbols) as a function of days after flowering collected during the 2003–2004 season. Shown are: juice osmotic potential for pre-veraison berries (solid triangle), and post-veraison berries (solid square) (**b**); berry weight (**c**); stem water potential (**d**); deformability (**e**); and juice TSS and pH (**f**). With respect to pedicel equilibrium pressure, the same berries that were used in Figure 5 are shown here and were taken from the same shoots used for the HPFM experiments (Figure 6). All vales in figures c, d, e, and f are means (±SEM) from six replicate vines.

Hydraulic conductance of single berries measured with the pressure probe

Figure 5 shows the hydraulic conductances of Shiraz (a, c, e, g, left side) and Chardonnay (b, d, f, h, right side) berries during ripening in the 2002–03 (open symbols) and 2003–04 (closed symbols) and for the berries cut back at different positions (see Figure 7a). Different operators of the pressure probes collected data in each season yet there was good agreement in the magnitude of hydraulic conductances measured.

There was a trend of declining hydraulic conductance during ripening for both seasons and this was significantly so for whole berries of Shiraz (Figure 5a). Shiraz appeared to have higher hydraulic conductances than Chardonnay for whole berries and all cut positions (compare Figure a with b, c with d, e with f, g with h).

Hydraulic conductance of berries determined using the HPFM

Using the method of cutting back whole bunches while measuring the change in hydraulic conductance (see methods) it was possible to measure hydraulic conductance per berry during ripening in the 2003–04 season (Figure 6). These measurements correspond to the closed symbols in Figure 5a and 5b using the pressure probe on single whole berries. Since averages are taken over many more berries with the HPFM measurement there was inherently less scatter in the measurements of berry hydraulic conductance. There was remarkable agreement between the HPFM and pressure probe measurements of berry hydraulic conductance (compare Figure 6 with Figures 5a, 5b). Regression against DAF (fitted lines) showed a significant decline in berry hydraulic conductance for both varieties confirming the trends observed with the pressure probe measurements. Furthermore, Shiraz had significantly higher berry hydraulic conductance than Chardonnay, also confirming the validity of pressure probe measurements.

Hydraulic conductances have been converted to hydraulic conductivities (on a berry surface area basis) for the HPFM measurements (Table 1). A two-way repeated measures analysis of variance on the corresponding measurement dates showed that the berry hydraulic conductivity was significantly larger for Shiraz than the Chardonnay at the first and last sampling date corresponding to 68 DAF (Chardonnay) and 64 DAF (Shiraz), and 89 DAF (Chardonnay) and 85 DAF (Shiraz).

Discussion

Studies reported in this present paper on two grapevine varieties have provided the first published data on hydraulic properties of the pedicel and components of berries during development. Since the phloem would become blocked when the pedicel is excised, we assume that the measurements reflect water transport through the xylem and a composite osmotic barrier within the grape berry that are in series to the xylem. This has been confirmed by dye injection experiments (J. Tilbrook, pers. comm.) and is also the underlying assumption in previous

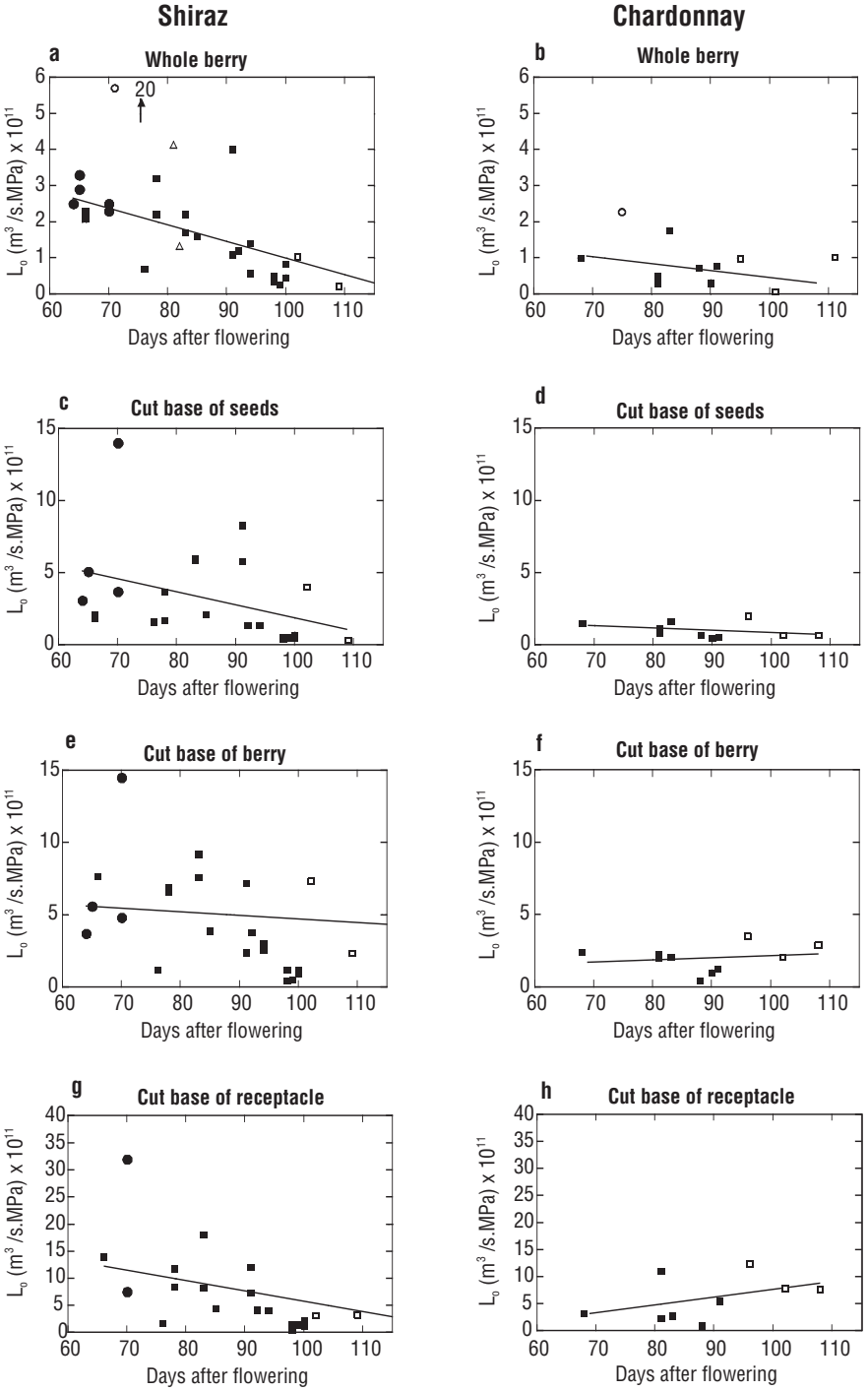


Figure 5. Berry hydraulic conductances *versus* days after flowering for Shiraz (**a, c, e, g,** left side) and Chardonnay (**b, d, f, h,** right side) measured with the pressure probe in two seasons (2002–2003 open symbols; 2003–2004 closed symbols). Berry tissue was excised at successive locations as indicated in Figure 7a. Regressions were fitted to the combined 2002–2003 and 2003–2004 data.

pedicel feeding experiments (Findlay et al. 1987) and in root pressure probe measurements (Steudle et al. 1993). When used in the pressure-clamp mode, the pressure probe and HPFM techniques gave values of berry hydraulic conductance that were in good agreement. The HPFM may be more generally suitable for measuring hydraulic conductances for other fruits during development.

The magnitudes of the 'hydraulic conductivity' (conductance divided by berry surface area, Table 1) are similar to the estimates made from modeling the dynamics of peach fruit expansion (2–3 × 10^{-4} g/cm.h, Fishman and Génard 1998). The actual composite membrane hydraulic conductivity in the berry will be determined by the relationship between berry surface area and the effective surface area of a 'composite membrane' (apoplast and symplast) within the berry. However, it is not clear what constitutes the composite membrane within a berry since there is no clear endodermis with a casparian band analogous to roots in berries, and the reflection coefficient of the osmotic barrier is close to zero in ripening fruit.

Our direct measurements show a substantial decrease in hydraulic conductance during and after veraison, as was previously implied by indirect observations. Our data do not indicate that the hydraulic conductance goes to near zero right after veraison. Rather, there is a continuous decline during ripening. This was particularly the case for Chardonnay. For Shiraz, higher hydraulic conductances were measured during all stages of development, which might correlate with this variety's propensity to exhibit berry weight loss. We must, however, acknowl-

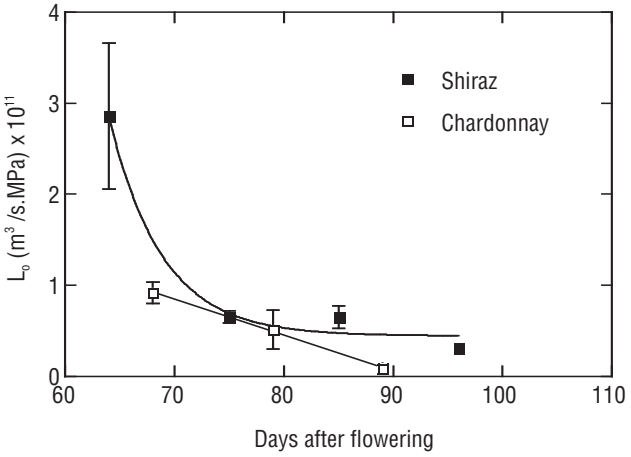


Figure 6. Berry hydraulic conductances *versus* days after flowering from HPFM experiments conducted in 2003–2004. Values are the mean of six replicate vines (one bunch per vine). Linear regression equations are significantly different between varieties ($P < 0.05$) and are: Shiraz (closed symbols); slope = -0.0755×10^{-11} m^3/s.MPa.day, intercept = 7.3×10^{-11} m^3/s.MPa. For Chardonnay (open symbols); slope = -0.040×10^{-11} m^3/s.MPa.day, intercept = 3.6×10^{-11} m^3/s^1,MPa. For Shiraz an exponential equation was statistically a better fit to the data (solid line). For the exponential equation: span = 1.4×10^{-5} m^3/s.MPa, plateau = 0.44×10^{-11} m^3/s.MPa, rate constant = 0.21/day.

edge that during our measurements on Chardonnay, there was also berry weight loss. Interestingly the onset of berry weight loss seemed to occur at similar hydraulic conductances for the two varieties (about 5×10^{-12} m^3/s.MPa from HPFM measurements).

Using the regression equations of hydraulic conductance *versus* DAF for different positions in the berry (Figure 5), we can calculate the hydraulic resistances ($1/L_o$) at two time points (70 and 100 DAF). These are plotted against cut positions (Figure 7a) for Shiraz and Chardonnay in Figure 7b and 7c respectively. Assuming that the resistances are in series, we can calculate by subtractions the intermediate series hydraulic resistances through each position in the berry. For example the hydraulic resistance between the base of the receptacle and the base of the berry (between position 3 and 2 respectively) is given by the hydraulic resistance measured for cut position 2 minus that measured for cut position 3. These component hydraulic resistances are plotted in Figure 7d and 7e for Shiraz and Chardonnay respectively.

Comparison of Figures 7d and 7e shows that the two varieties show similar behaviour in the way in which berry hydraulic resistances increase during development, but with large differences in the magnitude of the changes. In Chardonnay there is a much larger increase in hydraulic resistance through the proximal (brush) region and distal part of the berry. For Shiraz, the increase in resistance is about half of that observed in Chardonnay. We found no evidence for changes in resistance in the pedicel or receptacle region of the berry.

The large differences in the changes in hydraulic resistance, and hence hydraulic conductance, should have large effects on the hydraulic behaviour of the berries during ripening. For Shiraz, the berry does not become as hydraulically isolated in the brush region as was anticipated from dye uptake experiments (Rogiers et al. 2001). This may account for the continued Ca uptake after veraison (Rogiers et al. 2000). For Chardonnay the hydraulic isolation is more complete due to larger increases in hydraulic resistances in the proximal and distal parts of the berry. The behaviour of Chardonnay is in accord with experiments on Muscat Gordo Blanco showing dye travelling only to the brush region of the berry vasculature (Findlay et al. 1987).

It is interesting to speculate on the nature of the reduction in hydraulic conductance through berry development. Generally anatomical changes to the xylem vessels have been implicated in the interpretation of the dye movement studies. However one cannot exclude the possibility that xylem parenchyma cells that surround the berry xylem vessels become less water permeable during development. Aquaporins are largely responsible for high water permeabilities of plant cell membranes (Tyerman et

Table 1. Hydraulic conductivities of berries determined with the HPFM during the 2003–04 season on Shiraz and Chardonnay. Each measurement value is the mean of six replicates on bunches from different vines. A two way repeated analysis of variance was conducted on the data for the first three sample dates. The sources of variation and their significance are shown at the bottom of the table. A post-test (Bonferroni) was conducted to determine when differences occurred between varieties.

Date	Shiraz			Chardonnay			
	DAF	Berry L_p ($m^3/s.m^2.MPa$) × 10^9	SEM ($m^3/s.m^2.MPa$) × 10^9	DAF	Berry L_p ($m^3/s.m^2.MPa$) × 10^9	SEM ($m^3/s.m^2.MPa$) × 10^9	*P* value (Bonferroni post-tests)
21.01.04	64	22.5	6.18	68	5.71	0.67	$P < 0.05$
01.02.04	75	4.16	0.35	79	3.26	1.49	$P > 0.05$
11.02.04	85	3.78	0.79	89	0.52	0.14	$P < 0.001$
21.02.04	96	1.88	0.19				

Source of variation	% of total variation	*P* value
Interaction	6	0.0275
Variety	24	$P < 0.0001$
Date	46	$P < 0.0001$

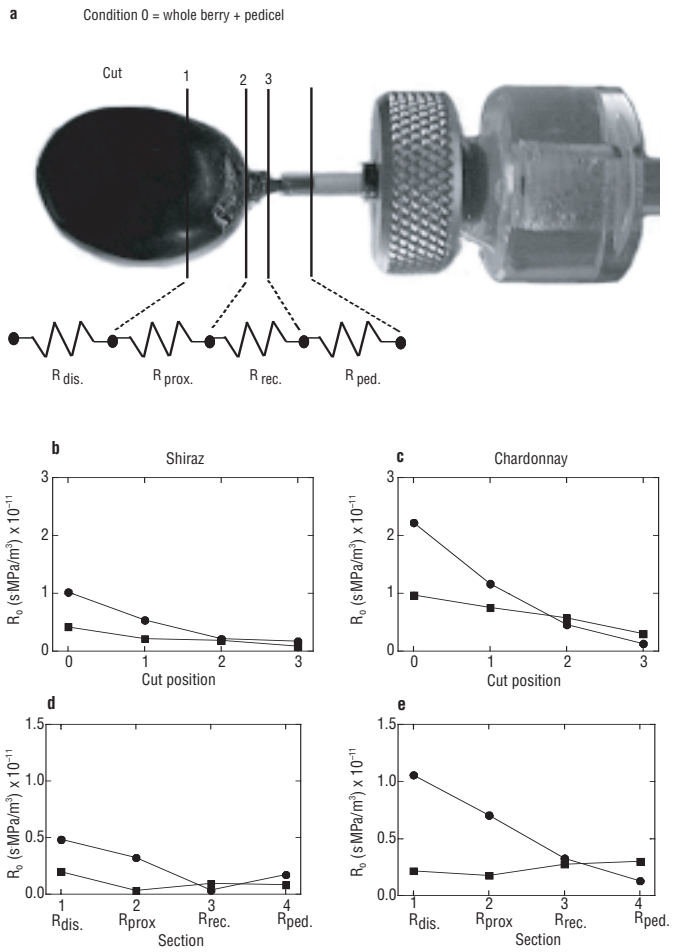


Figure 7. Calculating the positions in berries where hydraulic resistance (R_0) changed between 70 DAF (solid squares) and 100 DAF (solid circles). (**a**) Diagram of the cut positions along the berry, where condition 0 corresponds to the intact whole berry. (**b, c**) Hydraulic resistance as a function of cut position for Shiraz and Chardonnay respectively. (**d, e**) Hydraulic resistance for each section (see **a**) for Shiraz and Chardonnay respectively. This is calculated as R_0 at cut position 2 minus R_0 at cut position 3, R_0 at cut position 1 minus R_0 at cut position 2, and R_0 in condition 0 minus R_0 at cut position 1.

al. 2002) and have been implicated in berry development (Delrot et al. 2001). Also, composite transport in roots can have a large contribution from aquaporins (Tyerman et al. 2002), thus it would be surprising that this did not occur within berries given the similar hydraulic conductivities that we observe. Picaud et al. (2003) observed an increase in expression after veraison and at harvest, of one group of aquaporins associated with the plasma membrane (PIP1). However, this group of aquaporins generally shows low water permeability (Tyerman et al. 2002) that was also observed for the grapevine PIP1 by Picaud et al. (2004), and they may be more involved in neutral solute transport (Picaud et al. 2003). It remains to be seen what changes occur in the PIP2 group that generally shows very high water permeabilities. It would be worthwhile to test the water permeability of berry plasma membranes during development.

The degree of hydraulic connection between the berry and the parent vine may influence the propensity for berry weight loss at late stages of ripening in two ways. First, if transpiration continues from the berries and is able to generate sufficient xylem tension by cohesion tension from capillarity forces in berry cell walls, then higher berry hydraulic conductance (lower resistance) might limit berry weight loss by allowing greater water flow into the berry. On the other hand if the berry cannot generate sufficient tension in the xylem to counter the tension in the parent vine, as suggested by our measurements, then hydraulic isolation (low hydraulic conductance) would predispose towards prevention of weight loss.

If it is the effectiveness of hydraulic isolation from the parent vine at a particular stage of development that reduces the likelihood of berry weight loss, we can develop a hypothesis to explain why continued hydraulic connection via the xylem and berry composite membranes can lead to weight loss. Xylem backflow should be larger for an equivalent gradient directing water flow into the vine when the hydraulic conductance between the berry and the xylem of the pedicel is larger. Our measurements only provide hydraulic conductances for flow into the berry so it must be confirmed that reverse flow (corresponding to backflow) yields similar hydraulic conductances. Lang and Thorpe (1989) have shown for cv. Italia that backflow can account for 36% of the total water loss from the berry. Given that Greenspan et al. (1996) considered backflow to be a minor component in Cabernet Sauvignon, these contrasting results suggest that there are probably large varietal differences, as indicated in the present study.

Xylem backflow can only occur if there is an appropriate gradient to drive water flow through the composite membrane system in the berry to the adjacent stem xylem from the berry mesocarp cells. What is clear from the pressure probe measurements of equilibrium pressures of pedicel xylem in non-transpiring berries is that the very low osmotic potentials in the berry (down to –5 MPa) are not translated to negative pressures in the xylem, at least not for post-veraison berries. Rather there is a gradual increase in xylem pressures toward zero, which follows the pattern of declining hydraulic conductances. The berry pericarp cells have recently been reported to have much reduced turgor (almost zero) after veraison (Thomas et al. 2004), and unpublished results of ours using the cell pressure probe also indicate very low or zero turgor pressures in post-veraison pericarp cells. This might suggest that the water potential of these cells could be as negative as –5 MPa (the measured osmotic potentials). However, this is not transmitted through to the xylem conduits of the pedicel or adjacent vine stem. An apparent breakdown in compartmentation has been proposed for grape berries by Lang and Düring (1991). It would appear that the berry could only develop xylem tension through berry transpiration, and this is likely to decline during berry ripening (Rogiers et al. 2004b). Tensions developed by the parent vine would then prevail, and berries would shrink.

Our working hypothesis of how berry shrinkage might occur is illustrated in Figure 8. Due to solute accumulating in the apoplast or loss of semipermeability in the cell membranes of the xylem parenchyma cells, the berry is

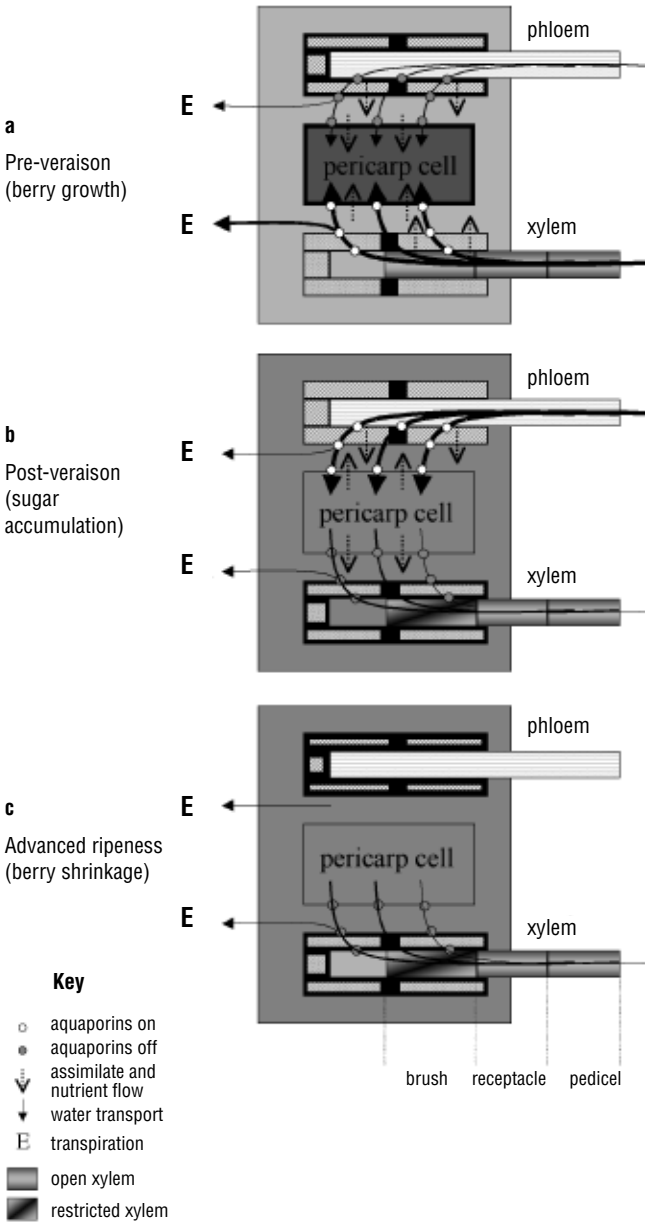


Figure 8. Cartoon summary of the hydraulics in grape berries incorporating the McCarthy and Coombe (1999) hypothesis and observations made in this report. It is hypothesised that aquaporins may be involved in changing flow during development in addition to xylem restrictions. **(a)** Pre-veraison berry has osmotically competent cells (reflection coefficient = 1) and the apoplast has low solute concentrations. Transpiration and cell expansion drive water inflow predominantly via the xylem. Aquaporins are activated in xylem parenchyma cells. **(b)** Berry after veraison where pericarp cell membranes lose osmotic selectivity (reflection coefficient goes to zero) and/or berry apoplast solute concentrations increase. Apoplast water potentials rise and cannot counter the tensions that could develop in the parent plant. Xylem/composite membrane hydraulic conductance has now become substantially reduced through a combination of xylem restriction and possibly closure of aquaporins in xylem parenchyma cells reducing backflow. Phloem translocation continues more rapidly and aquaporins are activated in the phloem. **(c)** Berry in the shrinkage phase where phloem translocation has ceased and where the xylem/composite membrane hydraulic conductance has not reduced sufficiently (e.g. Shiraz) to achieve hydraulic isolation from the parent vine, so that through a combination of transpiration and backflow the berry loses weight.

not able to develop xylem tensions despite the large osmotic potentials in the pericarp cells or berry apoplast (Figure 8b). Corresponding to this loss of *effective* osmotic gradients within the berry there would be a developmentally programmed decline in hydraulic conductance within the brush region and in the general distal region of the berry, as we have recorded. This reduction in hydraulic conductance could be due to a combination of reduced aquaporin activity in the xylem parenchyma or restrictions in the xylem vessels (Figure 8b,c). This hydraulic isolation would be required for two reasons: (1) to reduce xylem backflow; (2) to prevent an excessive water uptake from the vine that could result in the berry reaching dangerously high pressure (but note loss of selectivity in pericarp cells). Even with incomplete hydraulic isolation, provided phloem translocation continued, there would be ample flow into the berry to prevent weight loss. If on the other hand the hydraulic conductance does not decline sufficiently, then backflow will be larger, particularly during the day. If the phloem ceased to function, as proposed by McCarthy and Coombe (1999), this would exacerbate the situation by cutting off any supply of water to the berry (Figure 8c). Thus through a combination of reduced phloem function and relatively high hydraulic conductances within the xylem/composite membranes of the berry, Shiraz berries are more likely to display weight loss. The recent observation that berry size matters, that is, that larger berries are more likely to display weight loss (Rogiers et al. 2004a), may not be due to higher transpiration from larger berries as was proposed, but rather a higher hydraulic conductance of the internal composite membrane and xylem for larger berries which would increase the rate of backflow from the berry.

We can calculate the efflux of water due to backflow for a given pressure gradient using the hydraulic conductances that were measured for berries just before berry weight loss was observed (5×10^{-12} m^3/s.MPa), and which were sustained over time in Shiraz berries (Figure 7). For a relatively small average gradient of 0.1 MPa, Shiraz berries could loose 43 mg per day, which after one week would amount to about 30% of the weight of an average sized berry. Taking transpiration into account, some 15 mg per day estimated by Rogiers et al. (2004b), there is the potential for substantial weight loss through a combination of cessation of phloem loading, transpiration and backflow.

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Water, sugar and acid: how and where they come and go during berry ripening

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Introduction

The largest components of the juice from ripe berries are water, sugar (glucose and fructose) and the organic acids, mostly malate and tartrate. Altogether these make up 99.5% of the mass of the juice (Figure 1A). Water, sugar and acid influence the flavour components in the berry, either: through being required for the synthesis of these components; affecting their concentration through volume of the berry; or, by indirectly affecting their biochemical pathways. This trio will also affect the sensory properties of the finished wine by enhancing or diminishing the features carried by the flavour and colour components. The concentration of the solutes (sugars and organic acids) also account for most of the relatively large osmotic potential of the sap of berry cells. The relative proportion of the osmotic potential accounted for by sugars and acids switches over at veraison from acid dominant to sugar dominant (Figure 1B). The osmotic potential of the sap will influence the water balance of the berry depending on where the sap is distributed in the berry and how the cell membranes behave.

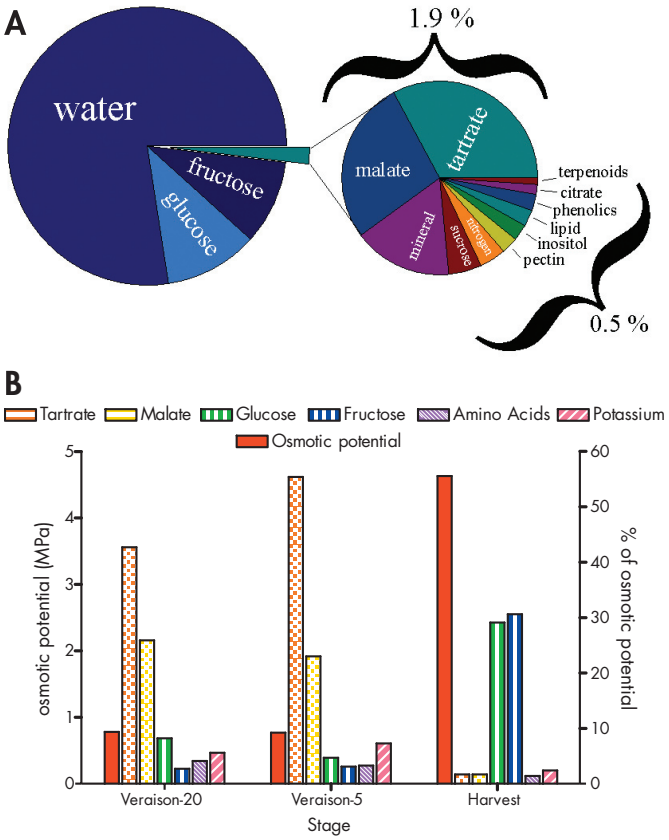


Figure 1. (A) Relative concentrations (g/L) of components of free run juice at harvest. Water glucose and fructose make up 98.1% of the total. Of the remaining components (1.9%) malate and tartrate make up 1.1% and 'minor' components including flavour components make up 0.5%. Data calculated from Combe and Iland (2004).
(B) Osmotic potential of juice and proportion of osmotic potential (as %) contributed by organic acids, sugars, and amino acids and potassium for Cabernet Sauvignon berries at three stages of development. Plotted from Diakou et al. (1997).

If there should be a problem with water balance of the berry, or sugar concentration, or acid concentration, the outcome can seriously compromise grape and wine quality or reduce the efficiency of wine making. A study of Cabernet Sauvignon and Merlot related to climate in Bordeaux over the last 50 years revealed that the quality of the vintage was highly correlated with sugar to acid ratio of the juice (Jones and Davis 2000). Therefore to allow us to *finish the job* in an informed way it is imperative that the mechanisms of accumulation and loss of water, sugar, and acid during the ripening phase of the berry are fully understood. In this paper a basic summary of the understanding of the physiology of ripening will be provided and there will also be a focus on some recent developments in understanding in this area. We will not examine recent developments in understanding of seed ripening since this will be examined separately in this volume.

Berry ripening dynamics

The dynamics of berry ripening, as they would pertain to Cabernet Sauvignon or Shiraz grapes are shown in Figure 2. This figure illustrates the double sigmoid increase in berry weight with time after flowering, upon which are superimposed several key events including: the accumulation of solutes; changes in conductance of the vascular system; transpiration; and, berry water relations (pericarp cell turgor and apoplast pressure). This is modified from the diagram provided by Coombe and Iland (2004) to provide an update with new information about xylem conductance, transpiration, and berry water relations, to be discussed further below. Also superimposed is a preliminary generalisation of the change in pericarp cell integrity; an important indication of how the pericarp cells can sustain osmotic gradients and support compartmentalised biochemical reactions.

Formation stage

In the formation stage (often referred to as Stage I), the berry increases in size through a combination of cell division and cell expansion (Ojeda et al. 1999). Water influx to the berry occurs via the xylem and phloem. At this stage the berry is hydraulically well connected to the parent vine (Greenspan et al. 1994) and direct measurements of xylem hydraulic conductance show the highest values (Tyerman et al. 2004, Figure 3). The berry transpires and the few stomata that are present, of the order of a few dozen, appear to be functional (Rogiers et al. 2004a). This corresponds to a negative apoplast pressure measured indirectly with a pressure probe (Tyerman et al. 2004), as would be expected for an organ that is transpiring and drawing water through the xylem from the vine. The pericarp cells have a normal turgor pressure, 0.2 to 0.27 MPa, depending on depth into the pericarp (Thomas et al. 2006), and the berries have low deformability (e.g. Huang and Huang, 2001). In respect of its water relations, the berry is behaving like a young leaf, albeit one with low photosynthetic activity, and being reliant on phloem import of sugars for growth and respiration.

Accumulation of organic acids occurs in the formation stage. The organic acids are ultimately derived from sucrose metabolism (Ruffner et al. 1983). Tartrate, the deprotonated anionic form of tartaric acid, is synthesised early and peaks in concentration before veraison. For an example of these processes in Cabernet Sauvignon see Diakou et al. 1997. Tartrate is synthesised from ascorbate (vitamin C), as is oxalate that is also present in the berry (DeBolt et al. 2004). Oxalate, a minor acid component, is precipitated as calcium oxalate crystals in specialised cells called idioblasts (DeBolt et al. 2004). Tartrate is soluble, and probably stored in the vacuole (Terrier and Romieu 2001). It does not appear to be metabolised, and though its concentration decreases by dilution, the content per berry remains stable. Recently a key enzymatic step in the formation of tartrate from ascorbate has been characterised (DeBolt et al. 2006). This was done by combining an understanding of genomics with profiling of organic acids in members of the Vitaceae. One species of Vitaceae was discovered not to synthesise tartrate and there was a higher than normal concentration of ascorbate present in the berries. By examining libraries of expressed genes from the *Vitis vinifera* berry at stages corresponding to the period of tartrate synthesis, several candidate genes for this process were identified. One step in the biosynthesis of tartrate that is proposed to be 'rate limiting' is the conversion of L-idonate to 5-keto D-gluconate with the enzyme L-idonate dehydrogenase. The gene for this enzyme was identified by virtue of its conspicuous absence in the species of Vitaceae that does not accumulate tartrate. This work is highly significant because it now allows the determination of how the enzyme is controlled and what factors might determine high or low tartrate content of berries.

Malate concentration continues to increase in the berry juice until veraison. The key enzyme in malate synthesis is phosphoenol pyruvate carboxylase (PEPC) that produces oxaloacetate that is subsequently reduced to malate. Malate production is subject to negative feedback through suppression of PEPC activity by malate. This does not occur as malate is compartmentalised in the vacuoles (Terrier and Romieu 2001). After veraison, there is a decline in the concentration and per berry amount of malate. This decline is attributed to malate being a respiratory substrate, where the degree of malate loss is positively correlated with temperature since the rate of respiration is temperature dependent. It is likely that the vascular tissues, which have a high respiratory demand during sugar import, act as a sink for malate in the berry (Coombe 1987).

Both malate and tartrate are accumulated in the vacuole and a transport system for the acids resides on the vacuolar membrane (Terrier et al. 1998). This is likely to be an ion channel based on results obtained from other plants. The inward gradient for organic anion accumulation is generated by hydrogen ion pumps that create an inside positive voltage and a low pH in the vacuole (Terrier et al. 1998).

Calcium and potassium accumulate in the berry during the formation stage, calcium being delivered via the xylem since it is non-mobile in the phloem, and potassium is largely delivered via the

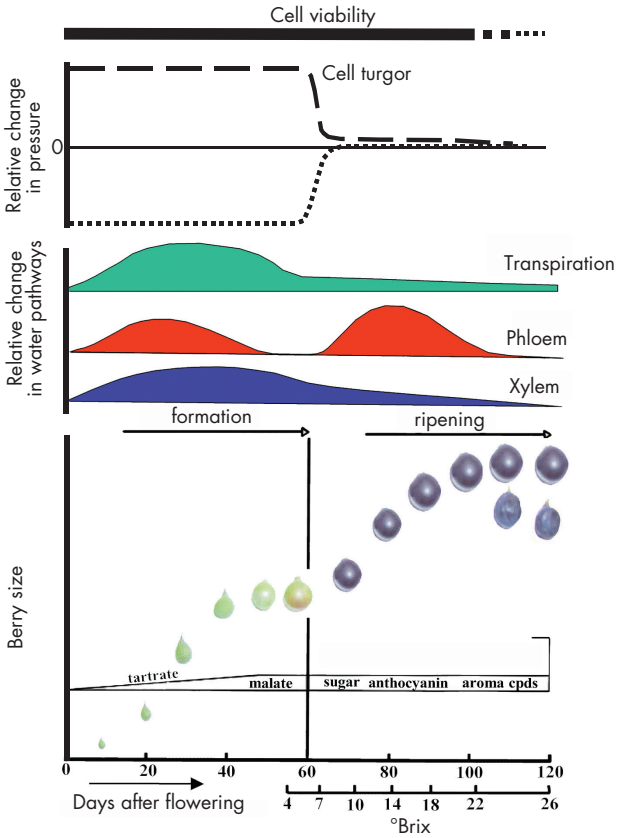


Figure 2. Change in berry size through development illustrating the double sigmoid characteristic of growth, and corresponding relative changes in the activity of xylem, phloem and transpiration (see references in text). Veraison is indicated by the vertical line at 60 days after flowering. Also shown are the approximate times when various compounds start to accumulate in the berry or where they peak (malate and tartrate). In the case of potassium there are two periods of accumulation corresponding to the double peaks in phloem translocation into the berry (Mpelasoka et al. 2003). Berry shrinkage is indicated where in Shiraz the time of onset occurs at approximately 90 days after flowering (McCarthy 1999). Change in pericarp cell turgor (Thomas et al. 2006) and apoplast tension (negative pressure, Tyerman et al. 2004) are indicated, along with a preliminary indication of the change in pericarp cell viability late in ripening. Diagram redrawn from Coombe and Iland (2004).

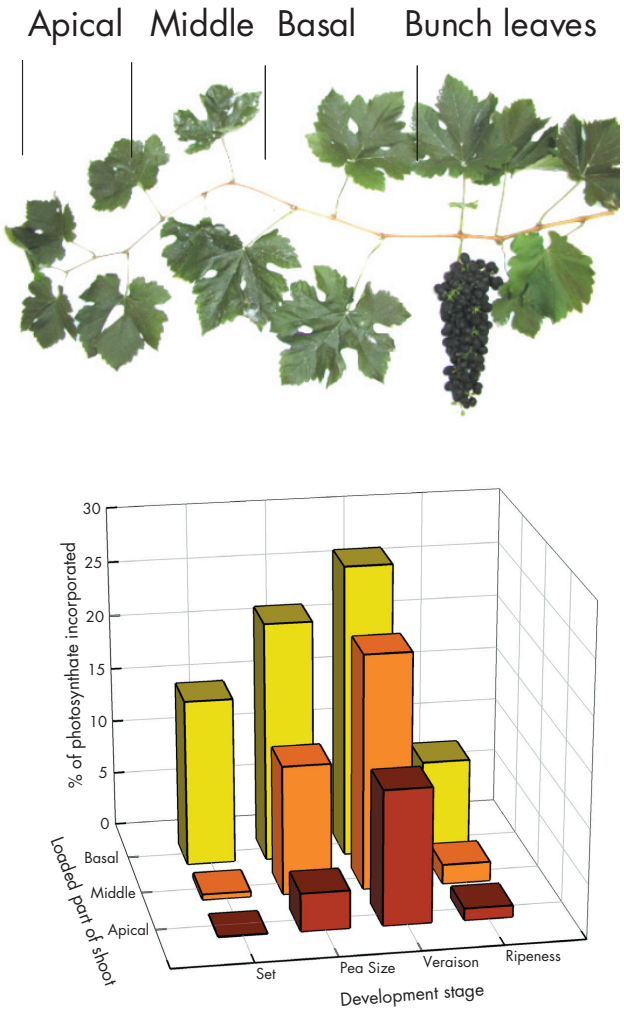


Figure 3. Percentage of ^{14}C labelled photosynthate incorporated into Cabernet Sauvignon berries from leaves at different regions of the shoot at four stages of development. Except for bunch leaves, leaves contributed progressively more photosynthate to the bunch the closer they were to the bunch. The bunches showed the strongest sink strength at veraison. Data plotted from Hunter and Visser (1988).

phloem. The concentration of these two ions present in the berry can be used to indicate the relative changes in phloem and xylem derived fluxes throughout development (Rogiers et al. 2006a). Other important nutrients also show particular patterns of accumulation. Phloem mobile elements, including potassium, phosphorus, sulphur, magnesium, boron, iron and copper accumulate throughout berry development (formation and ripening stages), while the xylem-mobile elements including calcium, manganese and zinc accumulate mostly before veraison (Rogiers et al. 2006b).

Veraison and subsequent ripening

The onset of berry ripening, termed inception by Coombe (1992), is signalled by berry softening, accumulation of sugars and anthocyanin accumulation in the skin. Berry volume rapidly increases about five days after the beginning of sugar accumulation and softening (Coombe 1992; Huang and Huang 2001). Berry softening may be the consequence of a much reduced turgor pressure of berry pericarp cells. The subsequent berry enlargement is proposed to be due to a two step process of cell wall loosening, first in the pericarp cells then subsequently in the skin tissues (Huang and Huang 2001). The turgor pressure of pericarp cells was recently measured with the cell pressure probe before, during, and after veraison (Thomas et al. 2006). The turgor pressure declined to an extremely low value of about 0.05 MPa in post-veraison berries, but importantly, not to zero. The pericarp cells also behaved as competent osmometers, indicating that the cell membrane was still intact and remained selectively permeable. This is an important observation allowing the mechanism of increased sugar loading in to the berry to be better understood. The observation is also corroborated by the measures of cell viability described below.

Translocation of sugars occurs via mass flow in the phloem cells from sources to sinks. Sucrose is translocated and there is a measurable concentration gradient in sucrose from leaves to bunches along the stem (Hunter and Ruffner 2001). The sources in a grape vine can be the stored carbohydrate in the wood and roots, or the photosynthesising leaves; once the leaves have reached a certain stage of development approximated by half their final area (Hale and Weaver 1962). At early stages, the bunch is a relatively weak sink and the growing shoot tip will compete for translocated sugars. The bunch becomes a progressively stronger sink for sugars as veraison is approached, and the relative contribution of different leaves on the shoot depends on their maturity and distance from the bunch (Figure 3). The leaves surrounding the bunches contribute quantitatively little to the carbohydrate in the berries (Hunter and Visser 1988).

The ratio of source (supply) to sink (demand) can have an impact on the way the source responds. Leaf trimming experiments have revealed a large capacity for photosynthesis to be up-regulated in the remaining leaves that compensates for the loss of source leaves (Poni and Giachino 2000). Even relatively large defoliations can have no significant effect on yield per vine in Cabernet Sauvignon, although a reduced accumulation of sugars in the berries is evident (Poni and Giachino 2000). A similar effect is seen for Chardonnay where 75% defoliation from as early as four weeks after flowering had no effect on berry diameter, cluster weight or clusters per vine, although total soluble solids was reduced. This was clearly at the expense of stored carbohydrate in the vine roots and wood that had following on effects in the next season's growth and flowering (Bennett et al. 2005). Only when all leaves were removed on Pinot Noir vines was there a substantial and significant reduction in berry volume (Petrie et al. 2000). There was a clear reduction in berry sugar content and berry dry weight, and at only moderate loss of leaf area was there a reduction in percentage soluble solids. There was no difference in the linear relationship between berry water content and seed dry weight with leaf removal, though there was a substantial decrease in the slope of the relationship between berry dry weight and seed dry weight. This experiment has revealed a decoupling between sugar inflow and water inflow to the berry. Petrie et al. (2000) suggest that the rate of sugar accumulation is directly related to the photosynthetic capacity, while berry growth is determined by other factors.

Trunk or cordon girdling, which severs the phloem cells, has been known to increase yield. This is due to cutting off the sinks in the wood and roots so that more sugars are translocated to the bunches. Although this undoubtedly happens, it has been shown by Williams et al. (2000), that such treatment also leads to stomatal closure to about half the initial stomatal conductance. This corresponded to an increase in leaf water potential and led the authors to suggest that the increase in berry size could also be in response to a more favourable water status particularly at the formation stage. Girdling *above* the bunch on the shoot was investigated by Hunter and Ruffner (2001). Despite cutting off the normal source of sugar from the shoot above the bunch, it was shown that there was no effect on the concentrations of malate, tartrate glucose and sucrose at ripeness. There was actually an increase in dry mass per berry and sucrose per berry (though not significant) in the girdling treatment that was attributed to a change in water balance of the berry. This illustrates that the berries are a strong sink and that sources can be 'reallocated' when the normal sources for sugar loading are removed.

The rapid accumulation of sugars that begins at veraison must result in a substantial alteration of membrane transport of sugars and redistribution of concentration gradients within the cell walls and cells of the berry. Zhang et al. (2006) have recently shown that the pathway of phloem unloading abruptly changes at veraison from symplasmic (transport from phloem cells to parenchyma cells via cytoplasmic connections through plasmodesmata) to apoplasmic (transport from the phloem cells across the cell membrane into the cell wall space). If the phloem cells were to maintain a functional symplasmic (direct) connection to the parenchyma cells, the turgor pressure of the parenchyma cells would be higher and this would tend to oppose the bulk flow through the phloem (Patrick 1997). The shift to unloading in to the cell wall space, combined with an increase in the activity of cell wall invertase, results in a decrease in the osmotic potential (rise in fructose and glucose concentration) in the cell wall space surrounding the phloem cells. This leads to a greater driving force for water outflow from the phloem cells. The increased outflow of water from the phloem cells necessarily drives greater sugar flow to the berry via mass flow. As expected for bulk flow there is a coupling between sugar transport and water transport, and there is a correlation between sugar increase per berry and berry transpiration (Rebucci et al. 1997). However, as the Petrie et al. (2000) experiment showed the ratio between the two may not be constant under extreme situations.

The reduction in turgor that is measured over veraison is likely to be due to *controlled* accumulation of solutes in the apoplast rather than an increase in cell membrane leakiness of the pericarp cells as has been proposed by Lange and Düring (1991). It is likely that the majority of apoplast solutes are the monosaccharides, fructose and glucose, given that these accumulate in the berry at this stage. Invertases, that hydrolyse sucrose to glucose and fructose, must be active at the onset of sugar accumulation. Invertase activity measured on a per gram basis, peaks at veraison (Dreier et al. 1998) and at this point the glucose to fructose ratio approaches one, as would be expected. Dreier et al. (1998) have shown that a soluble

acid invertase can be released from the berry from the onset of veraison, indicating that there is increased invertase activity in the cell wall space in the berry. However, it was proposed that this invertase is released from the vacuole into the wall space due to a proposed loss of membrane integrity. This proposal is not supported by subsequent measurements of cell viability (see below) or cell water relations (Thomas et al. 2006). Therefore either a cell wall located invertase is the key, or sucrose is hydrolysed in the vacuole by the vacuolar invertase and the hexoses are subsequently released selectively to the outside of the cells. A cell wall invertase has been identified in ripe berries (Sarry et al. 2004) and Hayes (2006) has identified a transcript of a cell wall invertase gene that increases in expression up to veraison in Cabernet Sauvignon. Recently Zhang et al. (2006) have shown that the expression and activities of cell wall invertase increases in grape berries at the onset of ripening.

Coincident with the increased import of sugar, it is not surprising that the genes for some monosaccharide transporters and sucrose transporters exhibit increased expression in the ripening berry. There are three hexose transporters that are highly expressed *VvHT1*, *VvHT2* and *VvHT3* (Hayes 2006). *VvHT1* selects for glucose over fructose (Vignault et al. 2005) and is expressed during the formation stage in the phloem-associated cells. *VvHT2* and *VvHT3* are more highly expressed during ripening but their location is unknown (Hayes 2006). The expression of the sucrose transporters *VvSUC11* and *VvSUC12* also increased when ripening commenced, but again, until the location of the transport and its mode of operation are known it is difficult to speculate on the significance of these changes (Davies et al. 1999).

Later stages of ripening

In order to achieve the flavour ripeness and seed maturity that is commercially desirable, berries often need to be left unharvested after they have achieved maximum weight. In Shiraz and probably other varieties as well, including Cabernet Sauvignon (McCarthy 1999), there is a reduction in berry volume, which in extreme cases appears as berry shrivel. For Shiraz, greater than 20% of the maximum berry weight can be lost depending on maximum weight achieved (McCarthy 1997; Rogiers et al. 2004b). Shiraz berries have been observed to loose weight in a range of climates (Smart et al. 1974; Davies and Robinson 1996; McCarthy 1997; Rogiers et al. 2000) and under different regimes of irrigation (McCarthy 1997). McCarthy (1999) showed that maximum berry weight was unrelated to the degree of ripeness, berry size or a particular date, rather the lowest coefficient of variation between treatments and years was achieved when weight maximum was measured from days after flowering. The onset of weight loss in field grown vines occurred at about 90 days after flowering (McCarthy 1999). Rogiers et al. (2004b) found that the berries on potted Shiraz vines started to loose weight after 74 to 85 days after flowering depending on irrigation, nitrogen nutrition and rootstock. It is not clear why the timing after flowering of maximum berry weight, or indeed 10° and 15°Brix (McCarthy 1999), should be so uniform. McCarthy (1999) explains this as in terms of an integrative effect of sugar supply from the whole vine. It is also interesting that the peak of seed dry weight (91-92 days after flowering) closely corresponds with the peak in berry fresh weight in Shiraz (Ristic and Iland, 2005).

McCarthy and Coombe (1999) showed that it was a reduction in water content that resulted in most of the loss of weight in Shiraz berries. The changes in weight during different periods of berry development were explained in terms of variable contributions from xylem and phloem to berry water inflow. McCarthy and Coombe (1999) proposed that the xylem accounted for most of the water inflow until veraison, after which the xylem became non-functional. Water flow in to the berry during ripening was proposed to occur predominantly via the phloem. At near maximum weight it was proposed that the phloem become non-functional resulting in water loss via transpiration exceeding water inflow.

Recent measurements of xylem functionality have demonstrated that the xylem do not become completely blocked after veraison (Tyerman et al. 2004, Bondada et al. 2005; Keller et al. 2006), as proposed by McCarthy and Coombe (1999) and that there are differences in behaviour between varieties. Shiraz only looses about 60% of xylem conductance (Figure 4) while in Chardonnay over 90% of the conductance is lost after veraison. Also the gradient for water flow into the berry via the xylem is much reduced as indicated by an increase in apoplastic pressure and a reduction in berry transpiration. Xylem were demonstrated to be functional when an appropriate gradient was artificially imposed on berries (Bondada et al. 2005). This opens the possibility that flow back from the berry into the vine could account for some of the berry weight loss. Such backflow has recently been demonstrated directly using dye tracers where it was suggested that this pathway allowed recycling of excess phloem-derived water back to the vine (Keller et al. 2006). The degree to which this happens under different circumstances and in different varieties is yet to be ascertained, and will require a quantitative analysis of the water budget of a berry. Using data published on berry transpiration by Rogiers et al. (2004a) and calculating the rate of water inflow via the phloem using the formulae presented by Dreier et al. (2000) these rates have been compared as shown in Figure 5. Also included in the figure is the flow that could occur via the xylem under a relatively small (0.1 MPa) gradient, using measurements of hydraulic conductance. As can be seen in Figure 5, all of the flows (transpiration, phloem inflow and xylem backflow) are quite similar, and this indicates that there is a fine balance between phloem inflow, transpiration, and xylem flow in the ripening berry.

An important issue that has been discussed in relation to initiation of sugar accumulation is the integrity of the cell membranes in the berry pericarp cells. It has been indicated that membrane integrity of pericarp cells is lost during ripening, (Lange and Thorpe 1989; Lang and Düring 1991) and this has also been supported by measurements of invertase leakage (Dreier et al. 1998). However, there appear to have been no attempts to measure cell membrane integrity at different stages of development. A combination of dyes has been used to examine the changes in the membrane integrity

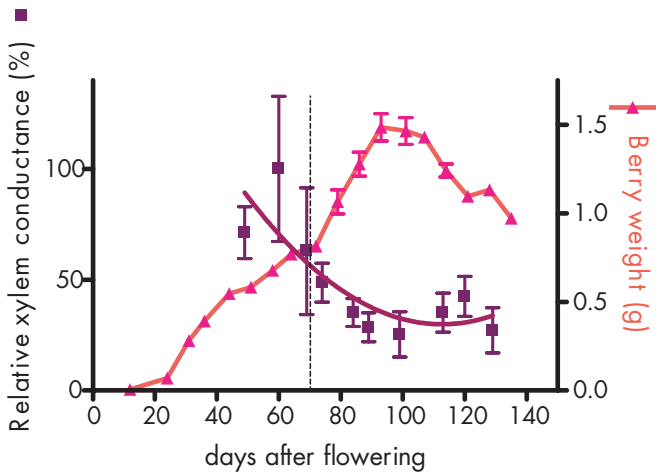


Figure 4. Relative change in xylem-berry hydraulic conductance (maximum = 100%) measured during berry development and corresponding changes in berry weight for Shiraz berries grown in the Coombe vineyard at the Waite Campus University of Adelaide during the 2004-2005 season.

of berry cells during development. One dye indicates living cytoplasm as green fluorescence and the other registers DNA as orange fluorescence only when the membrane becomes permeable to the dye. Slicing a berry in half along the longitudinal axis allows the dyes to be presented to the cells in the appropriate buffer after removing contents of cut cells. Figure 6 shows an example of the image that can be obtained under fluorescence that reveals a surprising amount of detail, including the peripheral cytoplasm in pericarp cells. Dark areas or orange-yellow areas are indicative of non-viable cells. For post-veraison berries of Chardonnay and Shiraz, up to about 95-100 days after flowering, there was no evidence of membrane breakdown. Near maximum weight there was a relatively sudden breakdown in the pericarp cells towards the centre of the berry (orange fluorescence indicating access to DNA), which subsequently diminished at later stages to reveal a dark non-staining area corresponding to non-viable cells. The periphery of the berry appeared to remain intact and the vascular cells also remained viable. This was observed in both Shiraz and Chardonnay although Shiraz showed a more uneven pattern. It is believed that this simple method could also be used to reveal patterns of cell viability that may relate to the quality of the ripening berries. Loss of cell membrane integrity would signal relatively large changes in metabolism in the berry and it is possible that this may impact on the development of flavour compounds.

A model of sugar and water flow in the ripening berry

Figure 7 illustrates the changes that occur in the ripening berry based on the evidence that is available to date. In pre-veraison berries transpiration dominates. The apoplast solution has a high osmotic potential (low solute concentration), but is under tension, reflecting the hydraulic connection with the parent vine through fully conducting xylem. The pericarp cells have high turgor and their osmotic pressure is largely accounted for by malate, tartrate and potassium. Phloem unloading occurs via direct cytoplasmic connections to the parenchyma cells and pericarp cells.

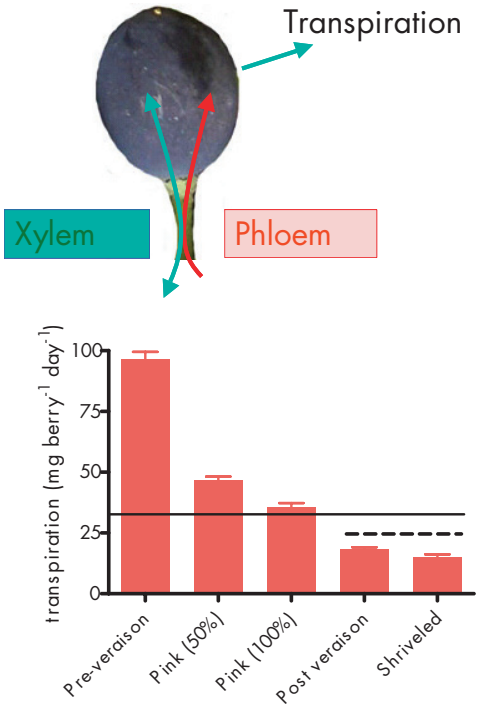


Figure 5. Flows in and out of Shiraz berries. Transpiration rates (vertical bars) were obtained from Rogiers et al. (2004a). The solid horizontal line is an estimate of the average inflow of water calculated using the formula of Dreier et al. (2000) based on the rate of rise in TSS during ripening. The dashed line indicates the flow that would occur in a Shiraz berry via the xylem for a 0.1 MPa gradient.

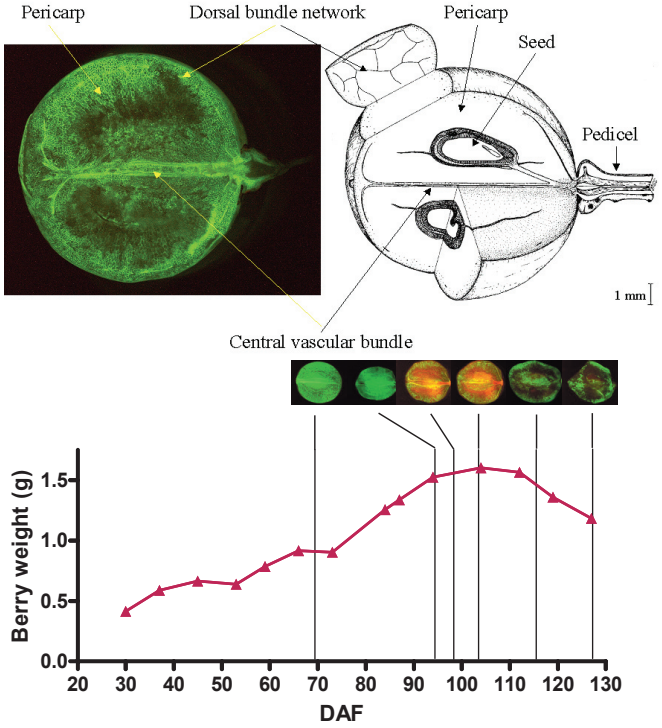


Figure 6. Measures of cell viability in Shiraz berries at different stages of ripening during the 2005-2006 season. The top image shows the degree of detail that can be detected via the simple halving of the berry down the longitudinal axis, taking care to slice between the seeds. The position of the seeds can be generally observed using a bright background illumination. There are two dyes that are used in these images, one that fluoresces green when taken up into intact cell cytoplasm (fluorescene diacetate), and one that fluoresces orange when bound to DNA that is only accessible to the interior of the cell when the cell membrane becomes leaky (propidium iodide).

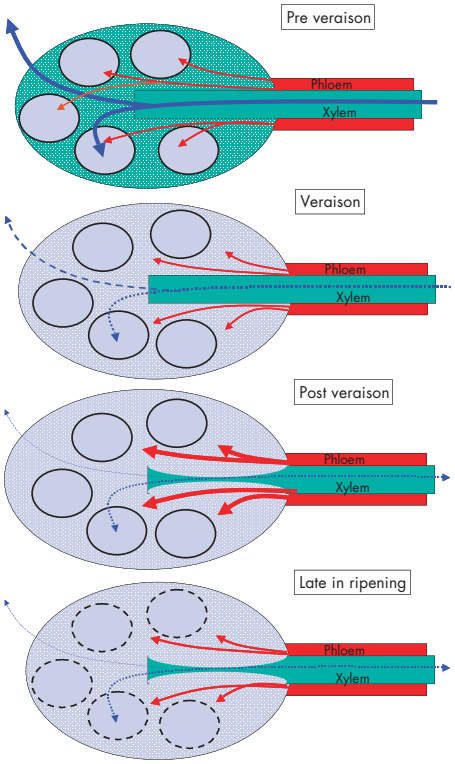


Figure 7. Changes in xylem, phloem and transpiration components of berry water balance during four stages in ripening. *Preveraison*: Transpiration dominates; the apoplasm solution has a low solute concentration (high osmotic potential), but is under tension. The pericarp cells have high turgor. Phloem unloading occurs via the symplasm. *Veraison*: There is a change to phloem unloading into the apoplasm and with an increase in apoplasm invertase activity there is an increase in apoplasm solute concentration (decreased osmotic potential). Turgor of pericarp cells drops. Sieve tube pressure gradient increases. Transpiration drops and apoplasm pressure increases. *Postveraison*: Xylem hydraulic conductance is reduced, but this is variety dependent. Transpiration is reduced. Hexose transport into pericarp cells is increased to match increased phloem transport. *Late ripening*: Xylem hydraulic conductance is normally reduced. Pericarp cells become leaky near maximum berry volume, but vascular cells retain integrity. Phloem inflow is drastically reduced. Control on xylem hydraulic conductance may be lost in some varieties (e.g. Shiraz).

At veraison there is a loss of connectivity via the plasmodesmata so that unloading of sugar has to occur across the phloem cell membranes in to the cell wall space. Combined with increased activity of cell wall invertase, the apoplast osmotic potential decreases (increased solute concentration). This accounts for the observed reduction in turgor of pericarp cells. The pericarp cells remain viable and show membrane selectivity. Their osmotic potential becomes dominated by glucose and fructose, although in the data shown from Diakou et al. (1997) in Figure 1, only 60% of the osmotic potential is accounted for by these sugars. Due to the change in apoplast osmotic potential the sieve tube pressure gradient increases allowing greater mass flow to the berry. Membrane transport of hexose into pericarp cells increases to match increased phloem transport. Transpiration from the berry is reduced and the apoplast tension decreases.

During and after veraison the hydraulic conductance of the berry xylem system declines. This tends to hydraulically isolate the berry from the parent vine (Greenspan et al. 1994; Keller et al. 2006) though there may be a difference in the hydraulic conductance depending on the direction of flow. Late in ripening the hydraulic conductance of the xylem is much reduced. Pericarp cells loose membrane integrity near maximum berry volume, but living vascular cells (vascular parenchyma and phloem cells) retain integrity. Phloem inflow is reduced and will eventually stop, resulting in reduced water supply and increased likelihood of berry weight loss. Control of the hydraulic conductance of the xylem system may be lost in some varieties; for example in Shiraz there is less shut down in the hydraulic connection between the xylem and the berry apoplast, suggesting that this control is variety dependent and perhaps related to problems such as berry shrivel (Tyerman et al. 2004).

Overview and future research

Although there have been some interesting developments recently that are highlighted in this paper, there is still much to learn about how the major metabolites and water in the berry are accumulated and how these can impact on the development of the flavour and aroma compounds and their precursors. We are closer to understanding the accumulation of tartrate with the identification of the gene and enzyme that may control the rate of accumulation (DeBolt et al. 2006). Compartmentation within the cell and between different cell types will no-doubt depend on specific transporters for organic anions that may be expressed at different times during development. Examination of the expression of key genes that code for invertases and sugar transporters and their location in time and space will also provide a better understanding of the control of sugar accumulation. Aquaporins (water channels) are likely to be involved in regulating water flow given their dominance in regulating flows within leaf tissue and root tissues.

The master controllers of these processes are yet to be revealed. Many changes in the levels of gene transcripts occur with softening and shortly thereafter before changes in sugar and acids are detected (Terrier et al. 2005). There is evidence that auxin, ethylene and abscisic acid may be involved in regulating the ripening process (Davies et al. 1997; Chervin et al. 2004). Recently it was shown that a brassinosteroid (castasterone), and its precursor dramatically increase in concentration in the berry at veraison. The brassinosteroids are essential for normal plant development. Application of castasterone stimulates ripening while an inhibitor delays ripening (Symons et al. 2006). Knowledge of the growth regulators involved could lead to practical applications in altering the timing of the commencement of ripening and perhaps even later events such as berry weight loss, where the effects of growth regulators have yet to be closely examined.

Some consideration should be given to the way cells in the berry senesce since a preliminary examination of this has indicated some interesting contrasts between berries sourced from different vineyards (Tilbrook et al. paper in preparation). The death of cells in the berry would signal some substantial changes in metabolism that would surely impact on the flavour and aroma compounds.

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14. Appendix 6 Budget reconciliation

15. CRCV Attachment 1: Project Summary and Impact Form

Attachment 1 – Project Summary & Impact Form

(To Be attached to all Final Reports.)

Project Leaders are required to submit reports to Program Managers for compilation for the information of the Executive Management Group, the CRCV Board and the Australian Government's CRC Program

The format and reporting dates have been developed to satisfy the requirements of the CRC Program and the CRCV. This form seeks to collect information from Project Leaders about how the project has performed against the CRCV Commonwealth Agreement.

Project Title	Shiraz berry weight loss at sub-optimal maturity – defining the problem
Period Report Covers	January 02 – Sept 06
CRCV Project Code Number	CRCV 2.4.1 CRV 01/02a
Organisation	Cooperative Research Centre for Viticulture

Objectives

- To gain an understanding of the extent, degree and possible causes of the phenomenon known as Shiraz berry weight loss
- Investigate the physiological & anatomical reasons for shrivel
- Investigate the synchrony of the annual onset of berry weight loss.
- Determine the influence of some vineyard management practices and climatic influence on berry weight loss
- Assess the influence of genotype on the variation in weight loss of ripening Shiraz berries

In collaboration with industry assess the impact of berry shrivel on wine quality/style

For each objective summarise in a minimum of two sentences progress over the life of the project.

To gain an understanding of the extent, degree and possible causes of the phenomenon known as Shiraz berry weight loss:

We have applied a new technique using a pressure probe to determine the water relations parameters of whole berries. There is compelling evidence that Shiraz berries have quite different hydraulic properties compared to varieties that normally do not show berry weight loss. The main feature of Shiraz berries is an inability of the xylem in the berry to seal off completely after veraison and in particular the display of a high outward directed (to the vine) hydraulic conductance.

Investigate the physiological & anatomical reasons for shrivel:

There is quantitative evidence for berry shrinkage to be partially accounted for by backflow into the vine, due to continued hydraulic connection in Shiraz berries. Dye tracer studies have shown the presence of backflow in Shiraz berries after veraison.

Investigate the synchrony of the annual onset of berry weight loss:

This component of the project was not continued due to higher priorities for time in the berry physiology components of the project.

Determine the influence of some vineyard management practices and climatic influence on berry weight loss:

We have examined the effect of molybdenum spraying which is being used to treat poor fruit set in some varieties. There were significant effects of the treatment on the timing of maximum weight but no difference in the subsequent rate of berry weight loss. Levels of molybdenum in wine, but no differences were detected in sensory attributes.

Assess the influence of genotype on the variation in weight loss of ripening Shiraz berries:

Rather than investigate different clones of Shiraz we have looked more broadly at different varieties of Vitis vinifera. We have shown that Chardonnay and Shiraz have contrasting berry hydraulics. Chardonnay berries were found to hydraulically isolate more effectively from the vine than Shiraz. We have also contrasted Saltana berries and found very different behaviour with a much higher conductance and xylem pressure.

In collaboration with industry assess the impact of berry shrivel on wine quality/style:

From the 2005-2006 molybdenum field trial we have made replicated batches of wine that have been analysed. The harvest was made to counteract the delaying effect, ie the delayed vines were harvested after the control vines

so that similar relative amounts of weight loss, brix and TA were obtained. No difference was detected in wine attributes and sensory attributes. Anatomical studies on berries taken from Hardy's premium range were compared with Shiraz vines from the Coombe vineyard. The premium berries showed a more even development of loss of cell membrane permeability at advanced stages of ripening. This technique may be developed as a berry quality measure.

Progress Against Milestones

(Update progress against CRCV milestones as documented in the Commonwealth Agreement and CRCV Strategic Plan)

Milestone	**Year**							**Progress**	**Revised Date for Achievement of Milestone**
	1	**2**	**3**	**4**	**5**	**6**	**7**		
Appoint PhD Student	---							PhD student appointed	
Develop PhD Research Proposal	---							Completed	
Initiate glasshouse & field experiments on synchrony	---------							Completed	
Develop experimental techniques	---------							Completed	
Sampling from existing CRCV sites	--------							Completed	
Commence genotype studies	----------							Completed	
Annual reporting to funding bodies	----------							As required	
Prepare articles & reports for industry	----------							Partly completed	
Apply for funding extension for PhD student	-							Not relevant	

Amendments to Milestones

Amendments to Milestones and Timelines require approval by Executive Management Group. Significant amendments will require approval in writing by the Commonwealth CRC Program.

Show Progress as Achieved, On Schedule, Delayed, or No-Longer Relevant. If Delayed or No Longer Relevant provide further detail.

Progress since the beginning of the project

The Report length is 500 word limit. Information provided in this Report will be used to compile CRCV Reports as public documents. The report must not contain information of a confidential nature concerning the Centre or its intellectual property.

In contrast to other grape varieties, berries of *Vitis vinfera* L. cv Shiraz reach a maximum weight around 90 days after flowering then lose up to 25% of that weight prior to harvest. It has generally been accepted that water flow into and out of berries via the xylem ceases at veraison and water continues to enter only via the phloem. The first direct measurements of pedicel xylem water flow by us suggest otherwise.

Using a modified pressure probe for single berries and a flow meter for bunch measurements, water flow into berries via the xylem was found to gradually decrease after veraison. In 2005 Shiraz showed an eight fold and Chardonnay a four fold reduction in hydraulic conductance into berries from veraison to harvest. Around day 90, water flow into Shiraz berries plateaued rather than continue to decrease, coinciding with the commencement of weight loss. Chardonnay berries were found to hydraulically isolate more effectively from the vine than Shiraz. Systematic excision of the berries located the regions where hydraulic conductance changed from veraison to harvest. Distinct differences were found between Shiraz and Chardonnay. We also determined the hydraulic conductance for flow out of the berries for Shiraz and Chardonnay. Here we observed a ten fold higher hydraulic conductance for Shiraz that was similar to hydraulic conductance for flow in to the berry at the equivalent stage of development. This indicates that Shiraz and Chardonnay have contrasting hydraulics. Chardonnay is able to rectify flow in some way so that the system acts like a valve preventing flow out of the berry. For Sultana this comparison could not be done because very negative pressures had to be applied which caused cavitation. These measurements strongly suggest that a degree of xylem connection is maintained during the ripening of Shiraz berries. It also leaves open the possibility of backlow of water from Shiraz berries via the xylem to the vine during the period of berry weight loss. Dye tracer studies with Lucifer yellow were performed on intact bunches on potted vines. These clearly showed backflow from Shiraz berries back to the vine but not from Chardonnay berries at similar development stages.

The cell membranes of the berry are likely to play a role in these phenomena so we examined the changes in cell leakiness using vital stains at key stages in development. Two stains were used, fluorescein diacetate (FDA) and propidium iodide (PI). Both stains showed loss of cell membrane integrity during later stages, but PI showed a sudden accessibility of DNA that then subsided just after the peak in berry weight. This technique shows promise in berry quality assessment.

During the 2004-05 season we also conducted a preliminary trial with a standard molybdenum foliar spray on Shiraz. This was based on a casual observation by Mardi Longbottom that berry weight loss was not observed in Merlot that had been treated with molybdenum. The 2004-05 season preliminary experiment on Shiraz vines in the Coombe vineyard showed that peak berry weight was delayed with molybdenum treatment, so a full factorial treatment was carried out on SARDI vines at Nuriootpa during 2005-06 season. ABA was measured in berries during ripening in this experiment because ABA synthesis involves an enzyme step which requires a molybdenum cofactor. Wines were also made from this trial and sensory analysis performed. Although peak berry weight was also delayed there was no significant difference observed between molybdenum treated and control vines in berry weight loss or in wine properties and sensory scores. There was however a large increase in the molybdenum content of the wines.

Likely use of research outcomes by Industry and Others
(We are looking for your opinions and observations)

How successful was the project in meeting its scientific objectives (please underline)

SOMEWHAT MOSTLY COMPLETELY

Who is currently using the outcomes of the project? (please underline)

NO-ONE RESEARCHERS AUSTRALIAN GRAPEGROWERS

AUSTRALIAN WINERIES OTHER(please specify)

Who will be using the outcomes of the project within 5 years? (please underline)

NO-ONE RESEARCHERS AUSTRALIAN GRAPEGROWERS

AUSTRALIAN WINERIES OTHER(please specify)

Have their been any unexpected benefits of the research?

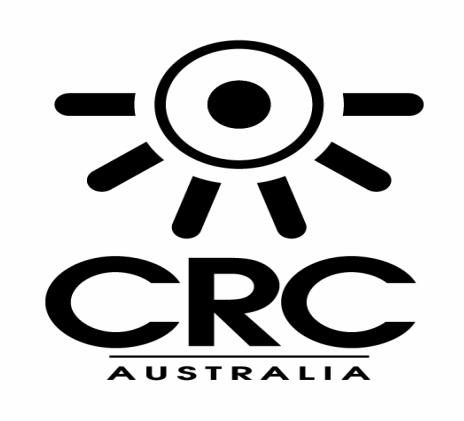
Yes or No

Please detail: The research has demonstrated that shoot manipulation and shoot to root signalling may be important in regulating root capacity for water uptake.

What is your best estimate of how long it will be before the Australian Industry is using the outcomes of this research project to best effect (maximum benefit)?

NOW	1-2 YEARS	3-5 YEARS	6-10 YEARS	OTHER (please specify
	XX Some components		XXX	

16. CRCV Attachment 2: CRCV Annual Report Requirements





Attachment 1 – Project Summary & Impact Form

(To Be attached to all Final Reports.)

Project Leaders are required to submit reports to Program Managers for compilation for the information of the Executive Management Group, the CRCV Board and the Australian Government's CRC Program

The format and reporting dates have been developed to satisfy the requirements of the CRC Program and the CRCV. This form seeks to collect information from Project Leaders about how the project has performed against the CRCV Commonwealth Agreement.

Project Title	Shiraz berry weight loss at sub-optimal maturity – defining the problem
Period Report Covers	January 02 – Sept 06
CRCV Project Code Number	CRCV 2.4.1 CRV 01/02a
Organisation	Cooperative Research Centre for Viticulture

Objectives

- To gain an understanding of the extent, degree and possible causes of the phenomenon known as Shiraz berry weight loss
- Investigate the physiological & anatomical reasons for shrivel
- Investigate the synchrony of the annual onset of berry weight loss.
- Determine the influence of some vineyard management practices and climatic influence on berry weight loss
- Assess the influence of genotype on the variation in weight loss of ripening Shiraz berries
- In collaboration with industry assess the impact of berry shrivel on wine quality/style

For each objective summarise in a minimum of two sentences progress over the life of the project.

To gain an understanding of the extent, degree and possible causes of the phenomenon known as Shiraz berry weight loss:

We have applied a new technique using a pressure probe to determine the water relations parameters of whole berries. There is compelling evidence that Shiraz berries have quite different hydraulic properties compared to varieties that normally do not show berry weight loss. The main feature of Shiraz berries is an inability of the xylem in the berry to seal off completely after veraison and in particular the display of a high outward directed (to the vine) hydraulic conductance.

Investigate the physiological & anatomical reasons for shrivel:

There is quantitative evidence for berry shrinkage to be partially accounted for by backflow into the vine, due to continued hydraulic connection in Shiraz berries. Dye tracer studies have shown the presence of backflow in Shiraz berries after veraison.

Investigate the synchrony of the annual onset of berry weight loss:

This component of the project was not continued due to higher priorities for time in the berry physiology components of the project.

Determine the influence of some vineyard management practices and climatic influence on berry weight loss:

We have examined the effect of molybdenum spraying which is being used to treat poor fruit set in some varieties. There were significant effects of the treatment on the timing of maximum weight but no difference in the subsequent rate of berry weight loss. Levels of molybdenum in wine, but no differences were detected in sensory attributes.

Assess the influence of genotype on the variation in weight loss of ripening Shiraz berries:

Rather than investigate different clones of Shiraz we have looked more broadly at different varieties of Vitis vinifera. We have shown that Chardonnay and Shiraz have contrasting berry hydraulics. Chardonnay berries were found to hydraulically isolate more effectively from the vine than Shiraz. We have also contrasted Saltana berries and found very different behaviour with a much higher conductance and xylem pressure.

In collaboration with industry assess the impact of berry shrivel on wine quality/style:

From the 2005-2006 molybdenum field trial we have made replicated batches of wine that have been analysed. The harvest was made to counteract the delaying effect, ie the delayed vines were harvested after the control vines

so that similar relative amounts of weight loss, brix and TA were obtained. No difference was detected in wine attributes and sensory attributes. Anatomical studies on berries taken from Hardy's premium range were compared with Shiraz vines from the Coombe vineyard. The premium berries showed a more even development of loss of cell membrane permeability at advanced stages of ripening. This technique may be developed as a berry quality measure.

Progress Against Milestones

(Update progress against CRCV milestones as documented in the Commonwealth Agreement and CRCV Strategic Plan)

Milestone	**Year**							**Progress**	**Revised Date for Achievement of Milestone**
	1	**2**	**3**	**4**	**5**	**6**	**7**		
Appoint PhD Student	---							PhD student appointed	
Develop PhD Research Proposal	---							Completed	
Initiate glasshouse & field experiments on synchrony	---------							Completed	
Develop experimental techniques	---------							Completed	
Sampling from existing CRCV sites	--------							Completed	
Commence genotype studies	----------							Completed	
Annual reporting to funding bodies	----------							As required	
Prepare articles & reports for industry	----------							Partly completed	
Apply for funding extension for PhD student	-							Not relevant	

Amendments to Milestones

Amendments to Milestones and Timelines require approval by Executive Management Group. Significant amendments will require approval in writing by the Commonwealth CRC Program.

Show Progress as Achieved, On Schedule, Delayed, or No-Longer Relevant. If Delayed or No Longer Relevant provide further detail.

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