Water Transport in Grape Berry and Pre-Harvest Berry Dehydration

Johannes Daniel Scharwies

School of Agriculture, Food and Wine

The University of Adelaide

A thesis submitted to the University of Adelaide in fulfilment of the requirement for the degree

Master of Agricultural Science

February, 2013

Abstract

Pre-harvest berry dehydration is reported for different grape varieties in many countries and may be exacerbated by global warming. In Australia a net water loss of up to 30% from berries of the most commonly grown red grape variety *Vitis vinifera* L. cv. Shiraz at the end of ripening is a serious problem causing significant yield losses and higher sugar concentration. The current hypothesis is that this water loss occurs due to a diminishing water inflow into berries at the end of ripening while transpiration prevails. Water backflow from berries to the parent vine via the xylem may contribute to this loss. The approach in this study was to compare hydraulic properties and quantify water uptake and water loss in different varieties to identify the contributing factor(s) in berry water relations that may cause net water loss. Different patterns of hydraulic resistance for water inflow were detected in the rachis, pedicels, and berries in bunches of Chardonnay, Grenache, and Shiraz during berry development. An increase in whole bunch hydraulic conductance was correlated to berry growth in Grenache, but not in Chardonnay and Shiraz. At the end of ripening, berry hydraulic resistance decreased in Shiraz whilst it increased in Chardonnay. This may prevent water backflow from Chardonnay berries to the parent vine, but this difference appeared to occur after weight loss began in Shiraz, and in Grenache, which did not display weight loss, there was a low berry hydraulic resistance. A lower hydraulic resistance in bunches of Grenache corresponds to this variety having more isohydric regulation of plant water potential reported in the literature. Using an *in vitro* transpiration assay on excised berries where both water loss and water uptake were measured, berries of all varieties showed net water uptake in early stages of development switching to a net water loss after veraison. A twofold higher net water loss from mature berries of Shiraz compared to Chardonnay was caused by lower water uptake in Shiraz rather than a higher transpiration rate. I conclude that the difference may be caused by a lower water potential difference as driving force for water uptake into Shiraz berries, since an equal or higher hydraulic resistivity was measured in Chardonnay berries. Finally, a computer model was developed to simulate transpiration and water uptake in the field under changing evaporative conditions during the 2011-12 season. This model demonstrated that water uptake via the xylem and phloem was able to balance peaks in transpiration due to high VPD events just after veraison, but was not able to compensate these peaks at the end of ripening. Compared to Grenache and Chardonnay there was greater sensitivity of Shiraz berries to high VPD at the end of ripening due to insufficient compensation of transpirational water loss by xylem water uptake which may explain this variety's propensity to display significant weight loss.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Acknowledgements

I wish to thank my supervisors Prof. Steve Tyerman and Dr. Brent Kaiser for their guidance, encouragement, and support throughout my Master studies. In particular, thank you, Steve, for all the interesting discussions, excellent ideas, the support to attend meetings, workshops, and conferences, and your great trust to give me the freedom to try whatever experiment seemed to be interesting. I'm looking very much forward to have you as a supervisor for my proposed PhD. Moreover, I would also like to thank Dr. Joanne Tilbrook for her external supervision and for sharing her knowledge about her previous experiments on grape berry water relations with me.

Special thanks go to Wendy Sullivan for her great support in the glasshouse and laboratory. Wendy has a fantastic way to keep everything running and helped me a lot in setting up instruments, growing my plants, and obtaining materials for my research.

I also wish to thank all members of the Tyerman, Gilliham, and Kaiser Laboratory for the fabulous two years that I had in this group so far.

For their financial support, I would like to acknowledge the German Academic Exchange Service (DAAD) for providing me with a living allowance and for covering part of my tuition fees. Moreover, I would like to thank the Gerlacianum foundation for a top-up scholarship, Yalumba and the Australian Society of Viticulture and Oenology for scholarships to attend symposia in Australia and overseas, and The University of Sydney to support my attendance at a workshop.

My greatest gratitude goes to my family, my girlfriend, and friends. Thank you Mum, Dad, Christoph, Daniel, and Grandma for all your support and the wonderful Skype meetings which make me feel closer to you. You are the best family anyone could have. Thanks you, Lina, for your love, your patience, and support during my writing process. Home Is With You.

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Chapter 1 – Introduction

1.1 Preface

Australia ranks in the top 10 of wine producing countries worldwide, and even in the top five of the largest wine exporters (IOV, 2012).

A total amount of 1.56 million tonnes wine grapes have been grown in Australia for the production of 1,117.8 million litres of wine in the 2010-11 season (ABS, 2012). More than 47% of these wine grapes were produced in South Australia (740,475 tonnes in 2010-11).

To compete in the global wine market, high quality grape production is required. One of the most important factors in grapevine production is vine water status. Deficit irrigation strategies have been shown to have beneficial effects on berry flavour development (Chapman, Roby, Ebeler, Guinard, & Matthews, 2005). However, sufficient water supply is a prerequisite to obtain good berry yields (Freeman & Kliewer, 1983).

Australia's most popular grape variety Shiraz (*Vitis vinifera* L.) is very susceptible to the pre-harvest berry dehydration disorder (ABS, 2010; Krasnow et al., 2010; McCarthy, 1999; Sadras & McCarthy, 2007). Berries affected by this disorder lose weight just before harvest and reach higher sugar concentrations. Finally this leads to higher alcohol content in the wine which is produced from these grapes. Consumer preferences for low-alcohol wines and higher taxation on high alcohol wines thus make these grapes less suitable for wine production and force grape growers and wineries to look into options to reduce this disorder in the field (Kontoudakis, Esteruelas, Fort, Canals, & Zamora, 2011). Even though pre-harvest berry dehydration has been studied extensively, the pivotal cause of this phenomenon is still unknown.

1.2 Pre-harvest berry dehydration – one of multiple berry shrivel disorders

Many different disorders are reported that cause grape berries to shrivel on the vine (Krasnow et al., 2010). Besides pre-harvest berry dehydration that is most common in Australian Shiraz, sunburn, bunch-stem necrosis, and sugar accumulation disorder have been observed in grapevine. Even

though they all induce berry shrivel, these disorders have a different cause and different effects on grape berry development and composition.

Figure 1. Grape bunch of Vitis vinifera L. cultivar Shiraz affected by pre-harvest berry dehydration during the 2011-2012 season in the Coombe vineyard at the University of Adelaide Waite Campus in the Adelaide region (South Australia)

Pre-harvest berry dehydration (Figure 1) had not been properly addressed until McCarthy (1997, 1999) observed berry weight reduction prior to harvest in field-grown Shiraz over four consecutive seasons always about 90 days after flowering. The reported reductions of berry weight ranged from 20% (McCarthy, 1997; S. Rogiers, Keller, Holzapfel, & Virgona, 2000) up to 30% (S. Y. Rogiers, Greer, Hatfield, Orchard, & Keller, 2006; Tyerman et al., 2004). Although most significant effects of pre-harvest berry dehydration have been observed in Shiraz, the same phenomenon was also reported for Carignane, and Cabernet Sauvignon berries (Freeman & Kliewer, 1983; McCarthy, 1999). It has been shown that the observed reduction in berry weight is caused by a net water loss from the berries rather than by a loss of solutes or loss of dry matter (McCarthy & Coombe, 1999; S. Rogiers et al., 2000). Therefore, an imbalance between water uptake and water loss has to exist at the end of ripening in these varieties. This imbalance could be caused by a lower water uptake due to a change in driving force and/or hydraulic conductance of the grape bunches, or higher water loss due to an increase in transpiration or even water backflow from the berries to the parent vine (Greer & Rogiers, 2009; Tilbrook & Tyerman, 2009). So far, the actual contributions of these factors are still unknown.

A similar pre-harvest dehydration disorder has been observed in ripening kiwifruit berries (*Actinidia chinensis* var. chinensis 'Hort16A') in California (Thorp et al., 2007).Likewise, an imbalance between water uptake and water loss has been identified as the cause for a net water loss (Clearwater, Luo, Ong, Blattmann, & Thorp, 2012).

In comparison, grape bunches affected by sunburn show visible browning and cracking of the berries (Krasnow et al., 2010). Bunch-stem necrosis and sugar accumulation disorder can affect grape bunches anytime after veraison and cause a lower sugar content than in normal berries. Moreover, grape bunches affected by bunch-stem necrosis can be identified due to the necrotic rachis tissue. In cases of pre-harvest berry dehydration disorder usually most grape bunches of the canopy are affected, whereas grape bunches affected by bunch-stem necrosis and sugar accumulation disorder occur more randomly within the canopy and sometimes only parts of bunches are affected.

1.3 Grape berry development and water relations

Grape berry water relations are closely related to grape berry development and vice versa. Since pre-harvest berry dehydration always occurs 90-100 days after anthesis (McCarthy, 1999; S. Rogiers et al., 2000; Tilbrook & Tyerman, 2009) and affects berry water relations some connection to grape berry development seems likely.

Grape berry development comprises two rapid growth phases that are linked by a period of almost zero berry weight change (Coombe & McCarthy, 2000). During the first rapid growth phase, which is also called berry formation phase, berry growth is due to cell division and cell expansion (Coombe, 1960). Both seeds and pericarp exhibit the same growth rate and develop in a similar way. Cell division of the pericarp takes place only during the first two weeks after anthesis, whereas cell division of the skin can last up to four weeks after anthesis (Considine & Knox, 1981; Ojeda, Deloire, Carbonneau, Ageorges, & Romieu, 1999). Water uptake into berries occurs mainly via the xylem at this stage (Greenspan, Schultz, & Matthews, 1996; Greenspan, Shackel, & Matthews, 1994). At the end of the berry formation phase, berry growth slows down, berries soften, change colour, and sugar accumulation starts (Coombe, 1960, 1992). This cardinal point during berry development is called veraison. Before the inception of veraison, the seeds have fully developed.

After veraison, a second rapid growth phase starts that is only due to cell expansion (Coombe, 1960). During this phase, which is also called berry ripening phase, sugar accumulates in the berries and acidity declines (Coombe & McCarthy, 2000). To facilitate sugar transport to the berries, water

uptake into the berries is generally assumed to switch from xylem to phloem supply at veraison (Greenspan et al., 1996; Greenspan et al., 1994). Moreover, phloem unloading changes from a symplastic to an apoplastic pathway to allow bulk flow of assimilates (i.e. sugar) into the berries (Xia & Zhang, 2000; Zhang et al., 2006). At the end of the berry ripening phase, sugar and berry weight reach their maximum, and flavour development commences (Coombe & McCarthy, 1997). In some varieties, such as Shiraz, a decrease in berry weight can occur at the end of ripening while flavour development occurs, and lead to a concentration of sugar in the berries (McCarthy, 1999). This decrease in weight is due to a net water loss from the berries that might be caused by an imbalance between water uptake and transpiration or even water backflow via the xylem (Greer & Rogiers, 2009; Tilbrook & Tyerman, 2009).

1.3.1 Xylem and phloem water uptake into grape berries

Coombe and Bishop (1980) and Greenspan et al. (1996; 1994) discovered that pre-veraison berries exhibit diurnal phases of expansion and contraction due to transpiration and potentially water backflow to the parent vine during the day, and rehydration during the night. Moreover, they learned that pre-veraison berries are more sensitive to vine water deficits than post-veraison berries. After veraison, the amplitude of the diurnal cycles of expansion and contraction reduced significantly. They used experiments where shoot and berry transpiration were altered and measurements of changes in berry volume in comparison between intact berries, heat girdled berries (phloem blocked), and detached berries, to calculate rates of xylem inflow, phloem inflow and transpiration. From these experiments they deduced that water transport into pre-veraison berries is mostly via the xylem, whereas in post-veraison berries the phloem provides most of the water supply.

Rogiers et al. (2000; 2006) monitored calcium, and potassium levels in grape berries during the course of berry development to study the contribution of xylem and phloem to berry hydraulics. They found that calcium which is exclusively transported via the xylem was strongly accumulated in pre-veraison berries, but was highly variable in post-veraison berries. In contrast, accumulation of potassium which is mainly transported via the phloem occurred at a higher rate in post-veraison berries. Potassium and sugar accumulation showed also the same pattern during the course of berry development (S. Y. Rogiers et al., 2006). When girdling was used to block the phloem pathway into berries, an inhibition of potassium and sugar accumulation was observed, the berries remained smaller, but no change in calcium accumulation was detected. This supports the hypothesis that pre-veraison water transport into the berries is due to the xylem and post-veraison water transport is due to the phloem.

However, a study by Fishman, Genard, and Huguet (2001) advises caution on biased results (i.e. flow rates of xylem and phloem) from girdling experiments. The interdependence between phloem and xylem inflow could result in erroneous measurements when one pathway is blocked. Moreover, heat girdling could also affect the xylem conduits (Windt, Gerkema, & Van As, 2009). Using nuclear magnetic resonance (NMR) flow imaging techniques, Windt et al. (2009; 2006) showed that xylem water uptake into a tomato truss continued during development and that most water was imported via the xylem and not the phloem.

A study on water relations in ripening kiwifruit supports the observation that xylem water uptake continues in post-veraison fruits (Clearwater et al., 2012). Moreover, xylem and phloem contributed apparently equally to the fruit water supply late in development.

1.3.2 Integrity of the xylem in post-veraison berries

Dye uptake studies have been widely used to investigate the function of xylem for water uptake into grape berries (Creasy, Price, & Lombard, 1993; During, Lang, & Oggionni, 1987; Findlay, Oliver, Nii, & Coombe, 1987; S. Y. Rogiers et al., 2001).

It was observed that eosin which stains xylem vessel elements was taken up by pre-veraison berries showing a good distribution in the vascular system, whereas in post-veraison berries it showed a patchy distribution throughout the dorsal vascular network and was not taken up by ripe berries (During et al., 1987; Findlay et al., 1987). Therefore, it was assumed that xylem function was disrupted in post-veraison berries due to the stretching of xylem vessels during berry expansion.

In accordance, Creasy et al. (1993) found the same decline in eosin dye uptake by post-veraison berries, but noticed that the decline of dye uptake occurred before the onset of berry expansion. In their opinion, dye uptake was probably not only impaired due to a disruption of the vascular network, but also by a loss of fruit parenchyma compartmentation (Lang & During, 1991).

Rogiers et al. (2000) expressed their doubts regarding dye tracer experiments to investigate xylem function. Their experiments also showed that calcium uptake continued in post-veraison berries. Since calcium is exclusively transported by the xylem, there must be either a non-vascular pathway for calcium uptake to the berries or dye uptake experiments were interpreted incorrectly. They hypothesized that there might exist size exclusion filters in the xylem preventing dye uptake, the dye might bind irreversible in the pedicel, or the dye becomes invisible in the berry (S. Y. Rogiers et al., 2001).

Bondada et al. (2005) and Keller et al. (2006) showed that dye uptake in post-veraison berries is possible if the required uptake gradient is applied. Using a pressure membrane or a wicking method

applying a gradient in water potential at the stylar end of post-veraison berries, Bondada et al. (2005) showed uptake of basic fuchsine into the vascular system of the berries. The other way around, Keller et al. (2006) obtained reverse dye uptake into post-veraison berries by applying dye to the stylar end of berries. A loss of gradient for dye uptake into post-veraison berries might be due to high concentrations of solutes in the apoplast, an increased hydrostatic pressure, and/or compartmentation breakdown.

Chatelet et al. (2008a, 2008b) investigated the xylem integrity in post-veraison Chardonnay berries. They observed a few nicks in the xylem of post-veraison berries, but also observed intact vessels. Moreover, xylem vessels seem to be able to stretch and new vascular bundles were added in the course of berry development.

In summary, according to the literature the xylem remains functional in post-veraison berries, but dye studies indicate that there might be a reduced gradient for water uptake via the xylem.

1.3.3 Apoplastic phloem unloading in post-veraison berries

In most plant tissues nutrients are symplastically unloaded from the phloem to the corresponding sink tissue, since the unloading process via existing plasmodesmata between the post-phloem parenchyma and sink tissue is a low resistance pathway (Lalonde, Tegeder, Throne-Holst, Frommer, & Patrick, 2003; Patrick, 1997). However, Ruan and Patrick (1995) observed that phloem unloading in tomato fruit switched from a symplastic to an apoplastic pathway in older fruits, when accumulation changed from starch to hexoses. They assumed that this change is due to the osmotic character of the hexoses. A symplastic unloading would probably lead to a reversed osmotic gradient between the sink tissue and the phloem. To prevent this, the phloem must become symplastically isolated from the sink tissue and apoplastic unloading allows continued bulk flow of assimilates.

Indeed, Xia and Zhang (2000) observed that plasmodesmata between sieve-elements and their surrounding cells were blocked by electron-opaque globules in post-veraison grape berries. This observation indicates a symplastic isolation of the phloem. The switch from symplastic to apoplastic unloading was confirmed by movement of carboxyfluorescein and green fluorescent protein-tagged viral movement protein. These membrane impermeable tracers were confined to the phloem in post-veraison berries (Zhang et al., 2006). Moreover, the expression of cell wall acid invertases increased in sieve-elements of post-veraison berries which facilitate a faster unloading of sugars from sieve-elements.

A decrease of fruit parenchyma cell pressure and berry firmness was observed by Thomas et al.

(2006; 2008) shortly before the onset of sugar accumulation in Chardonnay, Pinot Noir, and Cabernet Sauvignon. Even after veraison there was a low, but still measureable cell pressure of fruit parenchyma cells indicating that parenchyma cell membranes should be still intact. Moreover, they observed that rapid growth and sugar accumulation did not commence before cell pressure dropped to 0.1 MPa, after a maximum of 0.3 MPa during stage I of berry development. Hence, they concluded that the decrease of cell pressure might be a mechanistically important component for apolastic phloem unloading and the activation of sugar accumulation. Matthews, Thomas, and Shackel (2009) showed that the decline of cell pressure and the onset of sugar accumulation and colour formation were delayed by 14 days when berries were enclosed in plastic boxes to suppress berry expansion. Therefore, they proposed that the drop in cell pressure of the fruit parenchyma might trigger gene expression specifically related to sugar accumulation.

More precise observations of solute accumulation were done by Wada, Matthews, and Shackel (2009). They found that an increase of apoplastic solutes and a decrease of fruit parenchyma cell pressure occurred more than 10 d before onset of berry colour change in field grown Cabernet Sauvignon. Moreover, both apoplast and fruit solute potential reached about -4 MPa late in ripening berries which suggests highly coordinated solute transport across fruit parenchyma cell membranes.

1.4 Net water loss from post-veraison berries

There are two potential pathways for water loss from grape berries: transpiration via the berry surface, and water backflow from the berries to the parent vine via the xylem. A net water loss from post-veraison grape berries can be due to an imbalance between water uptake and water loss via transpiration (Greer & Rogiers, 2009; S. Y. Rogiers et al., 2006; S. Y. Rogiers, Hatfield, Jaudzems, White, & Keller, 2004), water backflow from the berries to the parent vine via the xylem during weather conditions of high evaporative demand (Choat, Gambetta, Shackel, & Matthews, 2009; Tilbrook & Tyerman, 2009), or a combination of these effects. A hypothesis was proposed by Tilbrook and Tyerman (2009) to explain why some varieties are more affected by pre-harvest berry dehydration than others. This hypothesis relates to observations on varietal differences in hydraulic conductance for water inflow and backflow from grape berries via the xylem and varietal differences in the degree of pericarp cell death in post-veraison grape berries (Fuentes, Sullivan, Tilbrook, & Tyerman, 2010; Tilbrook & Tyerman, 2008, 2009; Tyerman et al., 2004).

1.4.1 Imbalance between water uptake and transpiration

Water loss via transpiration is inextricably linked with the plants need for carbon dioxide uptake from the atmosphere (Schulze, 1986). The regulation of this process is mainly facilitated by the opening and closure of stomata. Interestingly, regulation of whole plant water relations in grapevine seems to be variety dependent (Schultz, 2003). Some varieties like Grenache exhibit a better stomatal control causing smaller changes in plant water potentials during water deficit than other varieties like Shiraz which shows a large decrease in plant water potential during water deficit. These differences are referred to as isohydric and anisohydric behaviour, respectively. Moreover, R. K. Vandeleur et al. (2009) observed a positive relation between transpiration rate and root hydraulic conductance in grapevine. Diurnal changes of root hydraulic conductance did not change in Chardonnay (anisohydric) during water deficit, whereas a reduction of diurnal changes was observed in Grenache (isohydric). The regulation of this process was hypothesized to be due to changes in aquaporin expression and/or regulation. To date, differences in grape bunch water relations between isohydric and anisohydric varieties have not been investigated.

On a grape bunch and berry level, S. Y. Rogiers et al. (2004) and Greer and Rogiers (2009) conducted detailed studies to unveil characteristics of berry transpiration in Shiraz. They found that stomata of grape berries become progressively non-functional during the course of berry development due to obstruction by waxes (S. Y. Rogiers et al., 2004). Moreover, the amount of total wax per berry surface area decreased during veraison and then remained stable after veraison and did not change during the pre-harvest berry dehydration phase. Weight change measurements on detached berries (S. Y. Rogiers et al., 2004) and *in* vivo gas-exchange measurements on intact grape bunches (Greer & Rogiers, 2009) showed that berry transpiration was high in pre-veraison berries and declined during the course of fruit development. These contradictory results between decreasing or constant wax amount per surface area and decline in transpiration per surface area might be due to the fact that the conductance of the grape epidermis rather depends on structural or compositional features than actual wax layer thickness (S. Y. Rogiers et al., 2004). They concluded that the observed net water loss from grape berries of the variety Shiraz at the end of ripening was not due to an increase of transpiration rate, but might have been rather caused by a decline in water uptake rate whilst transpiration continued.

Since direct measurements of water uptake into grape bunches or berries are very difficult, flow rates have been estimated by studies of calcium and potassium accumulation in ripening berries of Shiraz (S. Rogiers et al., 2000; S. Y. Rogiers et al., 2006). Variable calcium content in post-veraison berries

made it difficult to estimate the contribution of the xylem water supply with this technique. However, strong potassium accumulation in post-veraison berries until harvest indicated a constant phloem water supply concurrently with pre-harvest berry dehydration in a first study (S. Rogiers et al., 2000). In a second study, lower potassium accumulation was detected when pre-harvest berry dehydration occurred (S. Y. Rogiers et al., 2006). Therefore, an imbalance between water supply and water loss could be due to a decreasing phloem water supply at the end of ripening (S. Y. Rogiers et al., 2006).

Additionally, daily rates of water loss and water uptake were assessed by in situ measurements of bunch weight (Greer & Rogiers, 2009). The results suggest that declining water uptake rates in post-veraison berries are exceeded by water loss due to transpiration at the onset of berry weight loss. However, evidence by direct measurement of water uptake rates is still lacking.

1.4.2 Xylem backflow and the hydraulic connection of grape bunches and berries

Xylem backflow was proposed by different groups (Choat et al., 2009; Greenspan et al., 1996; Greenspan et al., 1994; Keller et al., 2006; Lang & Thorpe, 1989; Tilbrook & Tyerman, 2009; Tyerman et al., 2004). Berry water loss due to xylem backflow is likely to occur during times of high evaporative demand when transpiration causes a decrease in leaf water potentials and the stem water potential drops below the bunch water potential.

Interestingly, Greenspan et al. (1996; 1994) observed a much smaller amplitude of daily contractions in post-veraison berries compared to pre-veraison berries. Hence, they concluded that backflow is likely to occur in pre-veraison berries, but may have no or only little effect in post-veraison berries. However, according to measurements on stem and bunch water potential there was a positive gradient for water flow from the bunches back to the parent vine after veraison (Greenspan et al., 1996). Moreover, they did not know that berry transpiration decreases during the course of development (S. Y. Rogiers et al., 2004). Thus, the decline in daily contraction rates might be due to lower berry transpiration and may not necessarily reflect backflow characteristics. To verify this assumption, the amount of water loss due to transpiration and backflow has to be quantified independently.

Direct evidence for the amount of xylem backflow is difficult to achieve, since independent measurements for xylem and phloem flows are hard to conduct. One way to achieve a better idea about the possible flow between the berries and the parent vine is to determine the characteristics of the connection between the berries and the shoot via the xylem by measuring the hydraulic conductance or hydraulic resistance for water flow along this pathway (Choat et al., 2009; Tilbrook &

Tyerman, 2009; Tyerman et al., 2004). A decline of hydraulic conductance for water flow into grape bunches and individual berries of the varieties Shiraz and Chardonnay during the course of fruit development was measured (Tilbrook & Tyerman, 2009; Tyerman et al., 2004). Even though grape bunches and berries of these varieties both showed a general decline in hydraulic conductance, the conductance for inflow remained higher in the variety Shiraz than in Chardonnay at the end of ripening. In the table-grape variety Sultana, the hydraulic conductance for inflow was even much higher than in Chardonnay and Shiraz (Tilbrook & Tyerman, 2009). Measurements on hydraulic conductance for water backflow from berries revealed a much higher conductance in Shiraz compared to Chardonnay at the stage of pre-harvest berry dehydration in Shiraz. From these results Tilbrook and Tyerman (2009) concluded, that the higher hydraulic conductance for backflow in Shiraz compared to Chardonnay, which is not susceptible to pre-harvest berry dehydration, could facilitate a net water loss due to backflow. In Sultana which never shows pre-harvest berry dehydration another mechanism must exist that prevent water backflow, since berries from this variety showed a high hydraulic conductance. Direct evidence for xylem backflow in post-veraison berries of potted Shiraz vines was obtained by using the apoplastic tracer Lucifer Yellow (Tilbrook & Tyerman, 2009). In contrast, backflow ceased in post-veraison Chardonnay berries.

More detailed studies on individual berries with pedicels attached revealed that the berries provided the main part of the hydraulic resistance which is the inverse of hydraulic conductance, whereas the pedicel and receptacle resistances remained unchanged (Tyerman et al., 2004). Comparable to this, Choat et al. (2009) showed a much higher hydraulic resistance of the berry compared to the pedicel in post-veraison Chardonnay with the difference that they also found a high resistance in the receptacle as well. The increase of hydraulic resistance of the berry and the receptacle was due to a decrease of functional vascular bundles indicated by using the dye tracer acid fuchsine. Furthermore, high amounts of sequestered materials were found in post-veraison receptacles of Chardonnay berries.

Lang and Thorpe (1989) and Keller et al. (2006) supposed that the increase of solutes in the berry apoplast of post-veraison berries should cause a positive osmotic pressure and thus xylem backflow. Choat et al. (2009) also pointed out that a comparably low concentration of solutes in the phloem during post-veraison solute accumulation (Zhang et al., 2006) would require a high amount of water recycling to concentrate solutes in berries. Therefore, excess water has to be recycled via the xylem.

1.4.3 Pericarp cell death and xylem pressure in relation to pre-harvest berry dehydration

The integrity and structure of cell membranes in grape berries is important during the process of sugar accumulation, to generate a negative water potential for water uptake, and to resist tension that might be generated from the parent vine during times of high evaporative demand (Lang & During, 1991; Tilbrook & Tyerman, 2008).

Post-veraison grape berries of the variety Sultana showed no significant mesocarp cell death compared to 25 – 40% cell death in post veraison grape berries of the varieties Chardonnay and Shiraz, which is also associated with much higher berry deformability in Chardonnay and Shiraz (Tilbrook & Tyerman, 2008). Moreover, slightly positive xylem pressure was measured for post-veraison grape berries of Chardonnay and Shiraz, whereas post-veraison grape berries of Sultana had negative xylem pressures. This corresponds with the idea that berries with intact cell membranes can generate negative xylem pressure (Tilbrook & Tyerman, 2008). At the same time, it would be difficult for berries of Shiraz to withstand a negative water potential gradient towards the parent vine since berries of Shiraz maintain a good hydraulic connection concurrently with low cell membrane integrity (Tilbrook & Tyerman, 2008; Tyerman et al., 2004).

A comprehensive study of mesocarp cell death in berries of 22 red and white grape varieties at harvest maturity showed the general trend that higher cell death was correlated to pre-harvest berry dehydration (Fuentes et al., 2010). With respect to the onset of cell death in grape berries it is interesting to note that ten genes associated with programmed cell death processes are induced after veraison in berries of the variety Pinot Noir (Pilati et al., 2007).

1.4.4 Hypotheses on pre-harvest berry dehydration

There are currently two different opinions regarding the cause of pre-harvest berry dehydration:

Greer and Rogiers (2009) postulate that pre-harvest berry dehydration is caused by a reduction of water inflow into grape berries while berry transpiration continues. The reductions of water inflow could be due to lower xylem and/or phloem inflow.

Tilbrook and Tyerman (2008, 2009) attribute pre-harvest berry dehydration to a combined effect of post-veraison hydraulic conductance, and post-veraison cell vitality.

Pre-harvest berry dehydration does not occur if: 1.) the hydraulic connection between the berries

and the vine is maintained as well as mesocarp cell integrity (e.g. Sultana); 2.) the berries become hydraulically isolated from the vine when mesocarp cell death rises (e.g. Chardonnay).
If berries remain hydraulically connected to the vine and compartmentation breakdown of mesocarp cells starts, a loss of xylem tension due to a decline in osmotic potential will cause a reduction in xylem inflow. Moreover, berries would be more susceptible to berry weight loss by xylem backflow due to the lack of osmotic barriers counteracting the negative water potential in the vine.

1.5 Aquaporins in plant water relations

Besides the presence of physical barriers like high amounts of sequestered materials (Choat et al., 2009), changes in expression and/or regulation of aquaporins are likely to be involved in the regulation of hydraulic conductivity of plant vascular systems (Javot & Maurel, 2002; Tyerman, Niemietz, & Bramley, 2002).

Aquaporins are water permeable pores which belong to the membrane intrinsic proteins (MIPs) (Johanson et al., 2001). The MIP superfamily comprises plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), nodulin-like intrinsic proteins (NIPs), small basic intrinsic proteins (SIPs), rarely found GlypF-like intrinsic proteins (GIPs) (Gustavsson, Lebrun, Norden, Chaumont, & Johanson, 2005), hybrid intrinsic proteins (HIPs), and x intrinsic proteins (XIPs) (Johanson & Danielson, 2008) which have all been found in grapevine. The characteristic topology of all these hydrophobic proteins are six membrane-spanning alpha-helixes (Reizer, Reizer, & Saier, 1993).
Aquaporins have been shown to have significant influence both on root (Alleva et al., 2006; Boursiac et al., 2008; R. K. Vandeleur et al., 2009) and shoot water transport of plants (Cochard et al., 2007; Postaire et al., 2010). Some aquaporins are not only highly permeable to water, but also to H_2O_2, glycerol, urea, formamide, boric acid, silicone, arsenite CO_2, NH_3, and NH_4^+ (Tyerman et al., 2002; Vera-Estrella & Bohnert, 2011).
Heterologous expression of plant aquaporins in systems like *Xenopus laevis* oocytes is commonly used to characterize protein functions (Bellati et al., 2010; Shelden, Howitt, Kaiser, & Tyerman, 2009). In the same way isolated plasma membrane vesicles have been used for functional characterization (Alleva et al., 2006).
For *in situ* observation, mercury chloride and other aquaporin inhibitors were used to study the influence of aquaporin activity on plant water relations (Niemietz & Tyerman, 2002). However, it is important to keep in mind that mercury chloride in particular does not only inhibit aquaporins, but also a large range of other proteins and thus has a significant influence on metabolic processes in

plants which may affect the results of experiments (Javot & Maurel, 2002; Lovisolo, Tramontini, Flexas, & Schubert, 2008).

1.5.1 Aquaporin expression in grape berries

Pilati et al. (2007) found a high expression of aquaporins in pre-veraison Pinot Noir berries which is consistent with a higher hydraulic conductance at that stage (Tyerman et al., 2004), but some aquaporins were also induced during ripening.

In 2008, Fouquet et al. identified 28 putative aquaporin genes in the grapevine genome (Jaillon et al., 2007) and identified nine aquaporin cDNAs in *Vitis vinifera* cv. Cabernet Sauvignon. A different nomenclature for several of these genes was suggested by Shelden et al. (2009) using bioinformatics and phylogenetic analysis. Due to high homologies at both the nucleotide and amino acid levels, differences between aquaporins are difficult to detect. In the same study, 13 aquaporin cDNAs were identified in *Vitis vinifera* cv. Cabernet Sauvignon.

Fouquet, Leon, Ollat, and Barrieu (2008) observed a global decrease in aquaporin expression in the course of development of Cabernet Sauvignon berries. However, most aquaporins seemed to be regulated in a pattern related to ripening (Table 1).

Table 1. Aquaporin genes expression in grape berries of the variety Vitis vinifera L. cv. Cabernet Sauvignon during grape berry development (redrawn from Fouquet et al., 2008).

Group	Expression during grape berry development	Gene name
1	No significant variation	*VvPIP1;1*
2	Expression decreases at veraison	*VvPIP1;2*, *VvPIP2;2*, *VvTIP1;2*, *VvTIP2;1*
3	Expression decreases after veraison	*VvPIP1;3*, *VvTIP1;1*
4	Expression increases at veraison	*VvPIP2;1*, *VvPIP2;3*

In contrast, Picaud, Becq, Dedaldechamp, Ageorges, and Delrot (2003) reported higher *VvPIP* expression in older stages of Chardonnay, Ugni, and Pinot meuniere berries. A global increase of aquaporin expression was also observed in post-veraison Chardonnay berries (Choat et al., 2009). Although expression of most aquaporins decreased prior to harvest, the general expression level remained high.

The results of these studies show that it is not straightforward to infer changes in hydraulic conductivity only from aquaporin expression patterns in grape berries. Instead post-translational

regulation and localization might have an important role in regulation of water transport in berries as it does for other plant organs.

1.5.2 Influence of aquaporins on root water transport in grapevine

R. K. Vandeleur et al. (2009) investigated the role of aquaporins in roots of the isohydric variety Grenache and the anisohydric variety Chardonnay in relation to diurnal and drought stress responses. They discovered that aquaporin *VvPIP2;2* was constitutively expressed without any influence of time during the day, or plant water status. In contrast, *VvPIP1;1* was highly regulated. The expression was correlated to the diurnal changes in hydraulic conductance. Expression was increased in water stressed Chardonnay, whereas no changes were detected in water stressed Grenache which correlated with the changes in root hydraulic conductivity. In turn, expression increased in Grenache when re-watered. Characterization in *Xenopus laevis* oocytes showed no difference in water transport from controls for *VvPIP1;1*, but increase of water permeability for *VvPIP2;2*. Interestingly, co-expression of both aquaporins showed a larger increase in water permeability. A similar phenomenon was shown for *Zea mays* aquaporins (Fetter, Van Wilder, Moshelion, & Chaumont, 2004). Co-expression in this case led to a re-localization of *PIP1* to the plasma membrane. Therefore one might conclude that not only expression of individual aquaporins is important for regulation of water transport, but also interaction between particular isoforms can occur which depends on co-localisation in particular cell types. This would need to be considered in any investigation of aquaporin control of berry hydraulic conductance.

1.5.3 Mechanisms for aquaporin regulation

Several different mechanisms for regulation of plant aquaporins have been shown including regulation by phosphorylation, cytosolic pH, calcium, abscisic acid, and salicylic acid. Moreover, changes due to light/dark treatments, differences in turgor, and high osmotic concentrations have been observed.

Regulation by phosphorylation has been demonstrated for the seed-specific aquaporin α-TIP (Maurel, Kado, Guern, & Chrispeels, 1995). Cytosolic pH sensing of aquaporins was observed in isolated root plasma membrane vesicles of *Beta vulgaris* (Alleva et al., 2006). Water permeability was reduced at pH-values lower than 7 and strong aquaporin inhibitory effects due to increased cytosolic calcium concentrations were also reported. Subsequently, pH-sensing due to interaction

between aquaporins was shown by co-expression of *BvPIP1;1* and *BvPIP2;2* (Bellati et al., 2010). Abscisic acid caused an increase of aquaporin expression in roots of *Nicotiana tabacum* (Mahdieh & Mostajeran, 2009). In contrast, H_2O_2 seems to decrease aquaporin activity due to the release of hydroxyl radicals and subsequent oxidative gating (Ye & Steudle, 2006). A similar pathway might be addressed by salicylic acid (Boursiac et al., 2008). Salicylic acid seems to induce the accumulation of reactive oxygen species (ROS) in cells which might regulate aquaporin activity by initiating intracellular aquaporin re-localisation via a signalling pathway. The effect of salicylic acid on root hydraulic conductance in *Arabidopsis thaliana* was reduced when accumulation of ROS was prevented.

In *Arabidopsis thaliana*, a dark treatment enhanced the expression of several PIP genes resulting in a higher hydraulic conductivity of excised rosettes (Postaire et al., 2010). In contrast, in walnut leaves a strong correlation was found between light induced increase of leaf specific hydraulic conductance and expression of *JrPIP2;1* and *JrPIP2;2* (Cochard et al., 2007). For *Arabidopsis thaliana* roots, expression of aquaporins haven been shown to be modulated by the circadian clock (Takase et al., 2011). Both light and turgor seem to affect the water permeability of midrib parenchyma cells in leaves of *Zea mays* (Kim & Steudle, 2007). Finally, it has been assumed that high concentration of solutes in cells affect the aperture of aquaporin pores by causing the channels to collapse (R. Vandeleur, Niemietz, Tilbrook, & Tyerman, 2005; Ye, Wiera, & Steudle, 2004). This might be of high importance in post-veraison grape berries as they contain very high concentrations of solutes at this stage.

1.6 Conclusion and aims of research

This literature review shows that much effort has been taken to understand pre-harvest berry dehydration and discover possible causes for a net water loss from post-veraison grape berries. An imbalance between water inflow into berries, berry transpiration and possible backflow of water from the berries to the parent vine under conditions of high plant transpiration was discovered to be the most likely cause for a net water loss from berries before harvest (Greer & Rogiers, 2009; Tilbrook & Tyerman, 2009). However, the exact contribution of each of these components is still unknown.

1.6.1 Xylem flow was found to remain possible in post-veraison berries

It has been shown that the xylem is still functional in post-veraison grape berries (Bondada et al., 2005; Chatelet et al., 2008a, 2008b; Keller et al., 2006). Therefore, xylem flow remains a possibility

in post-veraison berries.

Studies on cumulative flows of xylem and phloem into fruits, and water loss through transpiration and possible backflow have been conducted in grape berries (Greenspan et al., 1996; Greenspan et al., 1994; Lang & Thorpe, 1989; S. Rogiers et al., 2000) and also in kiwifruit (Clearwater et al., 2012). Evidence for backflow from post-veraison grape berries to the parent vine via the xylem was reported by Tilbrook and Tyerman (2009) using dye tracer studies.

1.6.2 A decline of berry surface conductance was observed during ripening, but the extent of varietal differences is unknown

Gas exchange (i.e. transpiration, respiration) of grape berries has been investigated for single excised berries (S. Y. Rogiers et al., 2004) and for intact grape bunches (Greer & Rogiers, 2009) in the variety Shiraz. A general decline of grape berry transpiration in the course of berry development was discovered by these studies. There are currently no studies that compare grape berry transpiration between different varieties.

1.6.3 Hydraulic conductivity was shown to reduce during ripening, but more detail is required on varietal differences and the sites of changes across the whole bunch and berry structures

A decline in hydraulic conductance for water inflow into grape bunches and single berries has been discovered during the course of berry development and varietal differences became evident (Tilbrook & Tyerman, 2009; Tyerman et al., 2004). There were also differences between varieties in hydraulic conductance for water backflow (Tilbrook & Tyerman, 2009). These changes in grape berry hydraulic conductance were attributed to physical changes in the conductive tissue as well as to changes in aquaporin expression and activity (Choat et al., 2009; Fouquet et al., 2008). Even on a whole plant level, differences in water management led to the distinction between isohydric and anisohydric varieties (Schultz, 2003). Investigating this phenomenon, R. K. Vandeleur et al. (2009) found different gene expression patterns of aquaporins in roots between isohydric and anisohydric cultivars. No comparisons of hydraulic properties of grape bunches and berries between isohydric and anisohydric varieties have been done yet.

Varietal differences are also reported for the amount and pattern of mesocarp cell death in post-veraison grape berries (Fuentes et al., 2010; Tilbrook & Tyerman, 2008), and a variety specific correlation between mesocarp cell death and pre-harvest berry dehydration was discovered

(Fuentes et al., 2010; Tilbrook & Tyerman, 2008).

1.6.4 Research aims

From the literature review varietal differences in berry and vine hydraulic properties were indicated. Therefore, the aim of this research will be to measure hydraulic properties of grape bunches from different varieties with more detail on the different structural elements of the bunches (i.e. rachis, pedicels, and berries) to give a better idea how grape bunches work hydraulically as a complex system. Secondly, a comparative study on water uptake into grape berries and berry transpiration across varieties in a controlled environment may show if pre-harvest berry dehydration which occurs only in some varieties like Shiraz is linked to varietal difference in these features. Finally, a model of berry hydraulics will be designed which takes berry surface conductance, xylem water uptake, berry growth, and sugar accumulation from the preceding experiments into account to estimate changes of phloem inflows into post-veraison berries over time under the assumption of constant phloem sap concentrations. This integrative approach aims to asses which component of differences in berry surface conductance, xylem, and phloem water uptake make grape berries from different varieties more sensitive to a net water loss under conditions of high evaporative demand at the end of berry ripening.

Chapter 2 – Hydraulic properties of grape bunches

2.1 Introduction

Pre-harvest berry dehydration which can cause a net water loss of up to 30% from grape berries of the *Vitis vinifera* L. cultivar Shiraz at the end of ripening (McCarthy, 1999; S. Y. Rogiers et al., 2006) has been studied in relation to changes in hydraulic properties of grape bunches. The rational was that a net water loss could be caused either by an imbalance between water uptake and transpiration, or by water backflow from the berries to the parent vine. Backflow would be more likely if the hydraulic conductivity for xylem flow to the berry was high.

In early studies, During et al. (1987) and Findlay et al. (1987) used the xylem staining dye eosin to investigate xylem integrity and xylem water uptake into grape berries. They found even distribution of the dye in the vascular system of pre-veraison berries, but only a very patchy distribution throughout the dorsal vascular network in post-veraison berries, and no uptake in ripe berries. Therefore, the xylem in grape berries was regarded as being non-functional during ripening. However, there was always doubt if the decline in dye uptake explained a change in xylem integrity (S. Rogiers et al., 2000). Studies on the accumulation of calcium in grape berries which is not mobile in the phloem showed a continuous uptake even at the end of ripening (S. Rogiers et al., 2000). Using a pressure membrane technique, Bondada et al. (2005) showed that uptake of the dye basic fuchsine through the xylem into berries is even possible at the end of ripening if an appropriate gradient in water potential was applied. For berries still attached to the vine, Keller et al. (2006) observed that the dye basic fuchsine moves through the vascular system back to the bunch stem when applied at the stylar end of berries. Detailed studies of xylem anatomy and development in grape berries demonstrated that despite some minor nicks in the xylem of post-veraison berries, xylem vessels remained intact and even new formation of xylem vessels occurred during grape berry expansion (Chatelet et al., 2008a, 2008b). In conclusion, these studies show that there are still intact xylem vessels in post-veraison grape berries, but a water potential gradient is required for dye uptake through these vessels into berries.

Another approach to study xylem properties in grape berries was taken by Tyerman et al. (2004) who

measured the hydraulic conductance of grape berries and grape bunches by using the pressure probe, and high pressure flow meter. By using these techniques they avoided relying on a driving force for dye uptake and rather measured the physical properties for water flow through the xylem. They found a decline of hydraulic conductance in grape berries and grape bunches during the course of berry ripening. When comparing the varieties Chardonnay which is not affected by pre-harvest berry dehydration and Shiraz, which is severely affected by pre-harvest berry dehydration, they found a higher hydraulic conductance in grape berries of Shiraz at the end of ripening. The same difference between Chardonnay and Shiraz was also observed at a bunch level (Tilbrook & Tyerman, 2009). Berries of Shiraz had also a higher hydraulic conductivity for backflow than berries of Chardonnay at the end of ripening. Therefore, they concluded that berries of Shiraz would be much more susceptible to a negative water potential generated by the plant causing water backflow from the berries to the parent vine under conditions of high transpiration. Interestingly, berries of *Vitis vinifera* L. cultivar Sultana, a table grape variety that is not affected by pre-harvest berry dehydration, had a significantly larger hydraulic conductance compared to both Chardonnay and Shiraz. When measuring the hydraulic conductance of grape berries with pedicels attached in more detail, Tyerman et al. (2004) found that the berries provided the main resistance, whereas the resistance of the pedicel and receptacle were smaller. The resistance of the bunch stem (i.e. rachis) was regarded as rather small or even negligible. Choat et al. (2009) measured similar resistances for berries, but found also a higher resistance in the receptacle.

Studies from the literature show that there are varietal differences in hydraulic properties for grape berries and grape bunches. So far, the varieties Chardonnay, Shiraz, and Sultana have been compared. Therefore, the hypothesis that post-veraison hydraulic isolation of berries is pivotal to protect them from a net water loss in the presence of mesocarp cell death will be tested on the grape variety Grenache together with Chardonnay and Shiraz which have been used in previous studies (Tilbrook & Tyerman, 2009). From the literature, large differences in whole plant hydraulics have been found between Grenache and Shiraz which was the motivation to choose this variety to compare to (Schultz, 2003). Concurrently, a more detailed study on the contribution of the rachis, the pedicels, and the berries on the total bunch hydraulic resistance will be conducted since the rachis hydraulic properties have not been investigated before. The hypothesis is that an increase in hydraulic resistance in berries of Grenache at the end of ripening should protect them against negative water potentials in the parent vine since mesocarp cell death causes a loss an osmotic barriers which could otherwise maintain a negative osmotic potential. A low hydraulic resistance is expected to be measured in the rachis of grape bunches, whereas the highest resistance should be

found in the pedicel-berry junction or the berry itself which would bring about the proposed hydraulic isolation.

2.2 Material and methods

2.2.1 Plant material

Vitis vinifera L. cultivars Chardonnay clone I10V1, Grenache clone 137, and Shiraz clone BVRC12 were grown in the Coombe vineyard, 34° 58' S 138° 37' E, at the University of Adelaide Waite Campus in the Adelaide region (South Australia) on own roots. Chardonnay was planted in 2006 with a row spacing of 3 m, a vine spacing of 1.8 m, and flowering which was determined as 50% cap-fall across all vines in the row, occurred on the 24th of October in year 2011. Grenache was planted in 2002 with a row spacing of 3 m, a vine spacing of 1.8 m, and flowering occurred on the 28th of October in year 2011. Shiraz was planted in 1993 with a row spacing of 3 m, a vine spacing of 2.7 m, and flowering occurred on the 29th of October in year 2011.

2.2.2 Sampling regime

Shoots with bunches of grapes were collected from the varieties Chardonnay, Grenache, and Shiraz at the Coombe vineyard seven times during the 2011-12 season to gather information on berry development and to measure the hydraulic properties of the grape bunches. The sampling started from a pre-veraison, pea-sized berry stage and was carried out until late harvest. For each sampling date 15 shoots with bunches were collected per variety during a period of three days. Each day a mix of shoots with bunches from all three varieties were sampled between 7am and 8am in the morning and stored with their cut ends in buckets filled with water at room temperature, without direct sunlight until the experiment.

2.2.3 Measurement of hydraulic conductance

A sensitive 5 g/h LIQUI-FLOW flow meter (Bronkhorst High-Tech B.V., Ruurlo, Netherlands) connected to the XYL'EM apparatus (Instrutec, Montigny les Cormeilles, France) were used to measure the hydraulic properties of the bunches of grapes and their three major structural elements (berries, pedicels, rachis) in detail. The instrument was filled with purified (Milli-Q Plus; Merck Millipore, Billerica, MA, USA) and degassed (1.0 x 5.5 Mini Module(TM); Membrana GmbH,

Wuppertal, Germany) water to avoid blockages and embolisms. For the measurement one of the collected shoots with a bunch of grapes was immersed in a container filled with degassed water and the bunch was cut adjacent to the main stem. After recutting the peduncle with a sharp, fresh razor blade, the tubing of the flow sensor was immediately connected to the bunch of grapes and the connection was carefully sealed with a wire "hose clamp" to avoid leakages. To pressurize the system, the sample was perfused at a pressure of 200 kPa for two minutes. Subsequently the pressure-flow response was measured at 0.15 MPa, 0.10 MPa, 0.05 MPa and 0.00 MPa each for five minutes (Figure 2). Figure 2 shows a typical sequence of measurements and demonstrates the protocol for all measurements reported here.

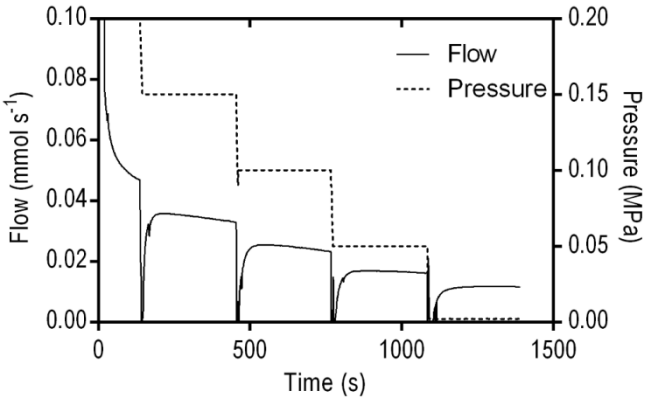

Figure 2. XYL'EM apparatus measurement on a bunch of grapes. The flow rate of purified, degassed water into the bunch (solid line) is shown in relation to pressure changes of the fluid (dashed line) applied using the machine.

The same approach was used to measure the samples of rachis with pedicels attached and the rachis proper. For these measurements the berries were removed by hand from the submerged bunches and pedicels were removed by using dissection scissors. Measurements on different varieties and parts of bunches were spread equally throughout the day to avoid detecting time dependent effects on grape hydraulics.

2.2.4 Grape bunch developmental parameters

For all bunches used in the hydraulics experiment the amount of berries was quantified and dimensions (length, width) of the main bunch stem were measured. Fresh weight of all berries, pedicels, and rachis were measured on a fine balance (OHAUS Corporation, Parsippany, NJ, USA). Five berries were chosen randomly from each bunch and berry fresh weight, berry diameter, and height (Digitronic Caliper MW110-DBL, Moore & Wright, Bowers Metrology Ltd., Bradford, West Yorkshire, UK) were measured individually. Berry sugar concentration was determined from the

mixed sample of these five berries by using a digital refractometer (PR-101, ATAGO CO., LTD, Tokyo, Japan).

2.2.5 Data analysis and statistics

Output data on pressure and flow rate from the XYL'EM apparatus were analysed for the local maximum flow rate at each pressure step by using the MATLAB software (MathWorks, Natick, MA, USA). The statistical software R (R Core Team, 2012) was used to determine the hydraulic conductance of each specimen as the slope from a linear regression between pressure and flow rate subsequently (Figure 3). The intercept of this regression gave information about a possible negative driving force for water flow into the specimen.

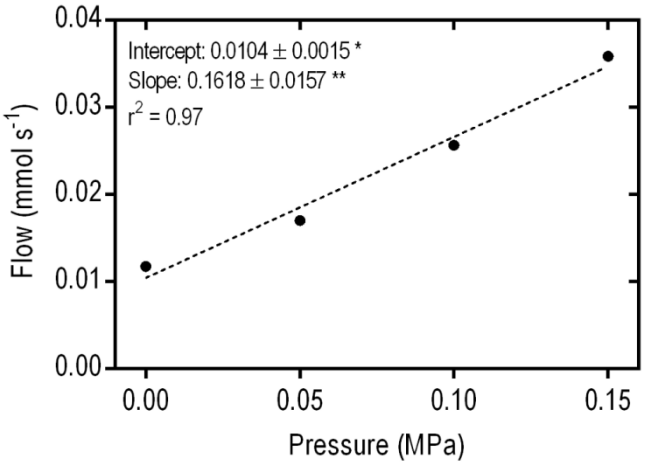

Figure 3. Calculation of hydraulic conductance. Linear regression (dashed line) between the pressure and flow rate from the four-pressure-step measurements by the XYL'EM apparatus on a bunch of grapes shown in Figure 2. Asterisks indicate significance (* $p \leq .05$, ** $p \leq .01$).

Furthermore, the MATLAB software was used for batch processing of the raw data. Berry volumes (Eq.1) and berry surface (Eq.2-6) areas from the single sampled berries were calculated based on the berry diameter (d) and berry height (h) measurements.

$$V = \frac{4}{3} \times \pi \times \left(\frac{d}{2}\right)^2 \times \left(\frac{h}{2}\right) \quad 1)$$

Since most berries were not perfectly spherical, berry surface areas were calculated for perfectly spherical berries ($S_{spherical}$) (Eq. 2), for oblate spheroidal berries (S_{oblate}) (Eq. 3,4) with a larger

diameter, and for prolate spheroidal berries ($S_{prolate}$) (Eq. 5,6) with a larger height.

$$S_{spherical} = 4 \times \pi \times \left(\frac{d}{2}\right)^2 \quad 2)$$

For oblate spheroids the ellipticity (e_{oblate}) is defined by

$$e_{oblate} = \sqrt{1 - \frac{\left(\frac{h}{2}\right)^2}{\left(\frac{d}{2}\right)^2}} \quad 3)$$

and was used in the general formula for oblate spheroids to calculate the berry surface area:

$$S_{oblate} = 2 \times \pi \times \left(\frac{d}{2}\right)^2 + \pi \times \left[\frac{\left(\frac{h}{2}\right)^2}{e}\right] \times \ln\left(\frac{1+e}{1-e}\right) \quad 4)$$

For prolate spheroids the ellipticity ($e_{prolate}$) is defined by

$$e_{prolate} = \sqrt{1 - \frac{\left(\frac{d}{2}\right)^2}{\left(\frac{h}{2}\right)^2}} \quad 5)$$

and was used in the general formula for prolate spheroids to calculate the berry surface area:

$$S_{prolate} = 2 \times \pi \times \left(\frac{d}{2}\right)^2 + 2 \times \pi \times \left[\frac{\left(\frac{d \times h}{4}\right)}{e}\right] \times \sin^{-1} e \quad 6)$$

Finally, all data were collected in an Excel sheet (Microsoft Office Excel 2007, Microsoft Corporation, Redmond, WA, USA).
Berry densities were calculated as the quotient between berry weight and berry volume, and pedicel and rachis weights were calculated in relation to the quantities of berries on a bunch and the total berry surface area. The total berry surface area was computed as the product of the average surface area of a berry on a bunch which was derived from the single sampled berries and the total quantity of berries on a bunch.

The specific hydraulic conductivity of bunches, rachis with pedicels attached and the rachis proper was calculated from the hydraulic conductances in relation to the total berry surface area since a normalisation is necessary to compensate for different sizes of bunches. Normalizing hydraulic conductance against surface area is a common method used for roots and leaves (Sack & Holbrook, 2006) and a linear relationship has been found between hydraulic conductance of bunches and the numbers of berries (Tilbrook & Tyerman, 2009; Tyerman et al., 2004).

The hydraulic resistivity of bunches, rachis with pedicels attached and the rachis proper was computed as the inverse of the hydraulic conductivity of these parts. Since resistances in a system such as a bunch add up when they are in series (rachis + pedicels + berries) the hydraulic resistivity of the berries was calculated as the difference between the bunch resistivity and the resistivity of the rachis with pedicels attached (Choat et al., 2009). In the same way, the hydraulic resistivity of the pedicels was calculated as the difference between the resistivity of the rachis with pedicels attached and the resistivity of the rachis proper.

GraphPad Prism 5 for Windows (GraphPad Software, San Diego, California, USA) was used for the statistical tests (linear regression, correlation, one-way ANOVA) and all figures have been created in this programme.

2.3 Results

2.3.1 Grape bunch development

Berries of all three grape varieties showed the typical two phase pattern of grape berry development. After an initial rapid growth phase berry growth came to a halt at the end of the berry formation phase (Figure 4). About 60 days after anthesis (DAA) berries of Grenache and Shiraz started to get soft and changed colour which is called veraison. In Chardonnay berry formation took about 5 days longer and veraison occurred at about 65 DAA. Before veraison berry density (Figure 4 B) and sugar concentration (Figure 4 C) did not change much in all three varieties. Compared to almost equal sugar concentrations berry density differed between the three varieties with berries of Chardonnay having the highest density, Shiraz an intermediate density, and Grenache the lowest density during this first phase of grape berry development.

After veraison the berry ripening phase started with a second rapid growth phase, an increase of berry density, and an increase in berry sugar concentration at a similar rate in all three varieties. Maximum berry weight was measured at about 95 DAA in Grenache and Shiraz (Figure 4 A). In Chardonnay the second rapid growth phase was about 15 days longer and maximum berry weight was measured at 115 DAA. Berries of Grenache had on average an almost 50% higher berry weight than berries of Shiraz at that stage whereas berries of Chardonnay had on average a lower berry weight than Shiraz. When maximum berry weight was measured berry density was almost equal in all three varieties and did not change for Chardonnay and Grenache thereafter (Figure 4 B). Berry sugar concentration plateaued at 24°Brix in Grenache and did not change between the last two sampling dates of the experiment (Figure 4 C). In Shiraz berries lost about 21% of berry fresh weight from 95 till 123 DAA (Figure 4 A). During that time berry density and sugar concentration increased significantly and was higher than berries of Chardonnay and Grenache (Figure 4 B, C). Finally berries of Shiraz had a sugar concentration of 30°Brix. Similar but later, berries of Chardonnay lost about 10% of berry weight from 115 till 128 DAA with a final sugar concentration of 26°Brix (Figure 4 A, C).

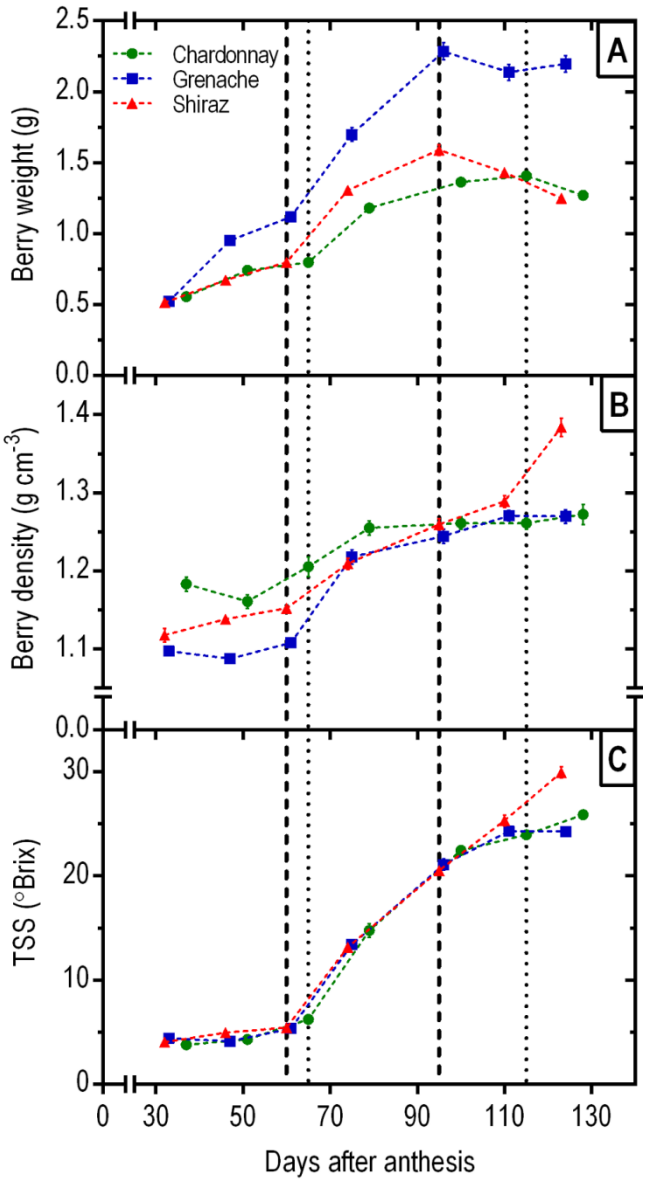

Figure 4. Developmental parameters of ripening grape berries. Berry weight (A), berry density (B), and berry total soluble solids (C) of ripening grape berries of the Vitis vinifera L. cultivars Chardonnay (green solid circles), Grenache (blue solid squares), and Shiraz (red solid triangles) during the 2011-12 season. Veraison occurred at 60 days after anthesis (DAA) (left dashed line) for Grenache and Shiraz, and at 65 DAA (left dotted line) for Chardonnay. Maximum berry fresh weight was measured at 95 DAA (right dashed line) for Grenache and Shiraz, and at 115 DAA (right dotted line) for Chardonnay. Means ± SEM, n = 75 (5 per 15 bunches of grapes) at each time point.

The main stem structure of bunches divided into pedicels (Figure 5 A) and rachis (Figure 5 B) showed a significant weight increase during the course of berry development in all three varieties. Whereas there were no significant differences between the three varieties in pedicel weight per berry and the increase in pedicel weight, significant differences were observed in rachis weight per berry

for all three varieties. Grenache had the highest rachis weight per berry, Shiraz had an intermediate rachis weight per berry, and Chardonnay had the lowest rachis weight per berry (Figure 5 B). However, no significant differences between rachis weight increases were detected between the varieties. Considering that pedicels and rachis are supportive structural elements important for berry growth, pedicel weight and rachis weight were also related to the total berry surface area per bunch (Figure 5 C, D). This revealed a general weight decrease of pedicel and rachis tissue that was available to support the berries during the course of grape berry development in all three varieties.

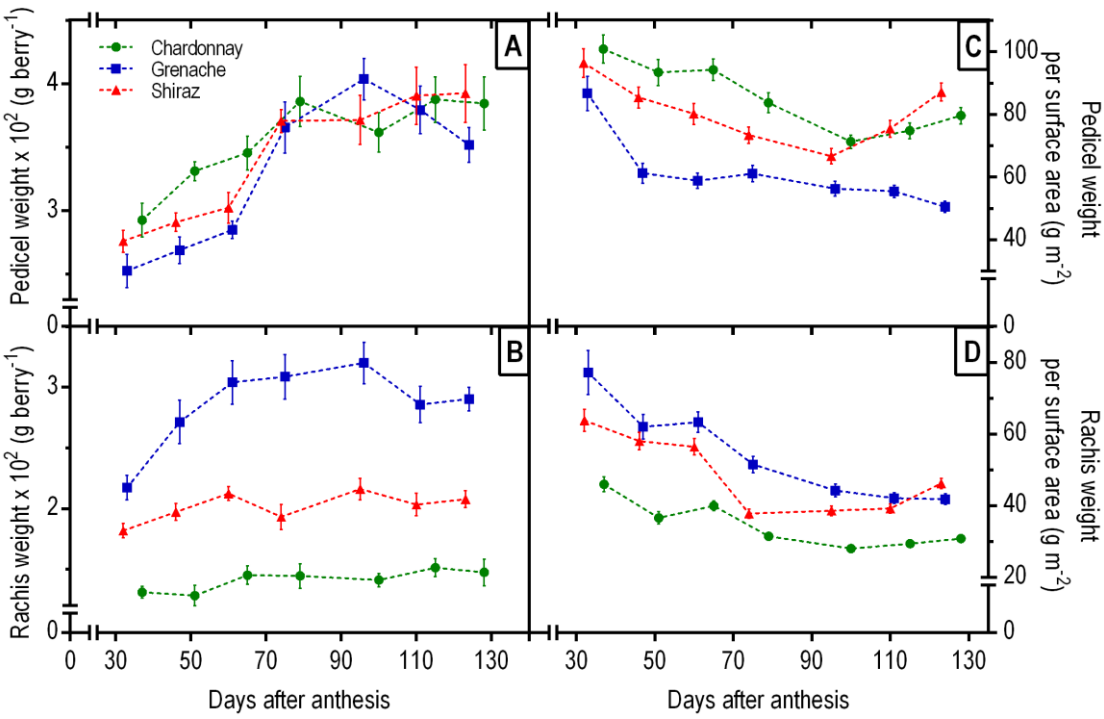

Figure 5. Development of pedicels and rachis. Pedicel weight per berry (A), rachis weight per berry (B), pedicel weight per total berry surface area (C), and rachis weight per total berry surface area (D) of ripening grape bunches of the Vitis vinifera L. cultivars Chardonnay (green solid circles), Grenache (blue solid squares), and Shiraz (red solid triangles) during the 2011-12 season. Means ± SEM, n = 15 at each time point.

These measurements of grape bunch developmental parameters were used to relate changes in hydraulic properties of these grape bunches to developmental changes in the course of grape berry development.

2.3.2 Grape bunch hydraulic properties

A very highly significant positive correlation ($p < .001$) was found between the increase in total berry surface area and bunch hydraulic conductance for grape bunches of the variety Grenache during the course of berry development (Figure 6 A). On the contrary, a significant negative correlation ($p = .023$) for this relation was found in Shiraz. In Chardonnay, the hydraulic conductance showed no significant ($p = .951$) change as the berry surface area increased. Pre-veraison bunches with a small total surface area had a similar hydraulic conductance in all three varieties. While the total berry surface area increased during development, the bunch hydraulic conductance increased in Grenache as well, but remained almost constant in Chardonnay and Shiraz.

The main stem structure (rachis + pedicels) of bunches from the variety Grenache had a higher hydraulic conductance compared to Chardonnay and Shiraz, but no significant correlation was found between the hydraulic conductances of rachis + pedicels in all three varieties and the increase in total berry surface area which goes along with bunch maturation (Figure 6 B).

For the rachis proper, only in the variety Grenache was there a very highly significant positive correlation ($p < .001$) between the increase in total berry surface area and rachis hydraulic conductance (Figure 6 C). Likewise, rachis of the variety Grenache had a higher hydraulic conductance compared to Chardonnay and Shiraz.

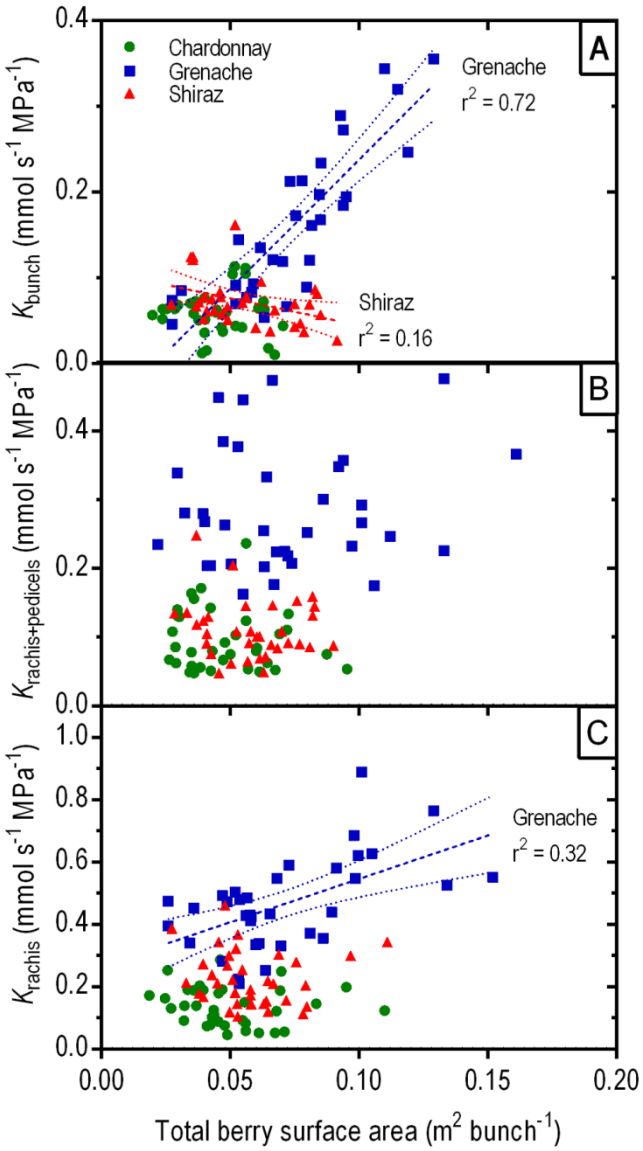

Figure 6. Hydraulic conductance – total berry surface area correlation analysis. Correlation of whole grape bunch hydraulic conductance (A), hydraulic conductance of rachis with pedicels attached (B), and hydraulic conductance of the rachis proper (C) to the total berry surface area of all pooled samples from ripening grape bunches of the Vitis vinifera L. cultivars Chardonnay (green solid circles) from 37 to 128 days after anthesis (DAA), Grenache (blue solid squares) from 33 to 124 DAA, and Shiraz (red solid triangles) from 32 to 123 DAA during the 2011-12 season. Square Pearson correlation (r2), and linear correlations (dashed lines) with 95% confidence intervals (dotted lines) are shown. Where no linear correlation is shown, no significant ($p \leq .05$) correlation was found.

To be able to work out the hydraulic contribution for all components of bunches (e.g. berries, pedicels, and rachis) hydraulic conductivities per total berry surface area were calculated and converted into hydraulic resistivities. The values for hydraulic resistivities of bunches, rachis + pedicels, and the rachis proper were plotted in a stacked diagram (Figure 7). Therefore, the areas in

between the dashed lines represent the shares of the berries, pedicels, and rachis in the total bunch hydraulic resistivity.

Simple linear regressions showed no significant ($p = .200$) change of total bunch hydraulic resistivity over time in Grenache (Figure 7 B inset), whereas significant increases of total bunch hydraulic resistivity were detected in Chardonnay ($p = .010$) (Figure 7 A inset) and Shiraz ($p = .002$) (Figure 7 C inset).

The contribution of each component in bunches (e.g. berries, pedicels, and rachis) to total bunch hydraulic resistivity was assessed. In Chardonnay, the rachis hydraulic resistivity was significantly ($p = .010$) larger than the berry hydraulic resistivity at 79 DAA (Figure 7 A). Three weeks later at 100 DAA this difference was still almost significant ($p = .081$). At the last measurement date around 128 DAA there was an almost significant ($p = .066$) higher hydraulic resistivity of the berries compared to the pedicels. In general, the rachis contributed most of the whole bunch hydraulic resistivity until 100 DAA. Afterwards, the berry hydraulic resistivity increased.

In Shiraz, a similar trend was discovered where berry hydraulic resistivity increased during later developmental stages (Figure 7 C). Berry resistivity peaked at 110 DAA, but dropped again afterwards. Also pedicel and rachis hydraulic resistivities increase during development. Nonetheless, no significant differences were detected in hydraulic resistivities between the berries, pedicels, and rachis part at any stage of development.

In contrast, the berry hydraulic resistance accounted for the major component of bunch resistance in early stages of development in Grenache and decreased afterwards (Figure 7 B). At 33 DAA, the berry hydraulic resistivity was significantly ($p = .013$) higher than the pedicel hydraulic resistivity. Two weeks later at 47 DAA, the berry hydraulic resistivity had increased and was very highly significant higher ($p < .001$) than the hydraulic resistivities of pedicels and rachis. After another increase two weeks later at 61 DAA, the berry hydraulic resistivity reached a maximum resistivity at which point it was highly significantly different to the pedicel ($p < .001$) and rachis ($p = .003$) resistivities. At 96 DAA, the rachis hydraulic resistivity was almost significantly ($p = .096$) higher than the berry resistivity. In general, the pedicel hydraulic resistivity increased during development, while the rachis hydraulic resistivity did not change much.

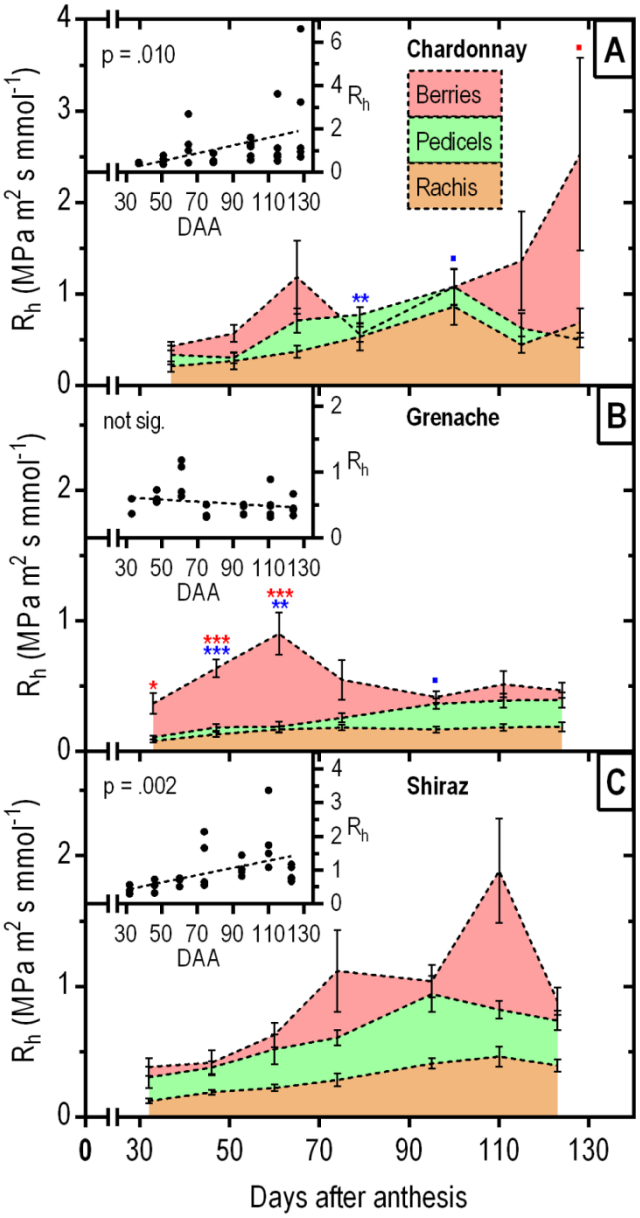

Figure 7. Profile of grape bunch hydraulic resistivities during bunch development. Hydraulic resistivity (Rh) of the berries (red area), the pedicels (green area), and the rachis (brown area) of ripening grape bunches (combined area) of the Vitis vinifera L. cultivars Chardonnay (A), Grenache (B), and Shiraz (C) during the 2011-12 season. The hydraulic resistivities calculated from the measured hydraulic conductivity of the total grape bunches (upper dashed line), the rachis with pedicels attached (middle dashed line), and the rachis proper (lower dashed line) are shown as means (centre of the error bar) ± SEM (n = 4-5 measurements at each time point) which define the hydraulic resistivity of the berries, pedicels, and rachis. Asterisks denote significant differences between the berries and pedicels (asterisks in red), and between the berries and rachis (asterisks in blue) according to one-way ANOVAs for each group; a dot denotes almost significant differences (˙ $p \leq .100$; * $p \leq .050$, ** $p \leq .010$, *** $p \leq .001$). There were never differences between the pedicels and rachis. Insets: All individual measurements of hydraulic resistivity of total bunches from each variety were plotted against time in days after anthesis (DAA). Linear regressions (dashed lines) are shown and text indicates whether the slope of these regression were non-zero.

Since flowering dates differed not more than five days between Chardonnay, Grenache, and Shiraz, total bunch hydraulic resistivities were compared between all three varieties for each time point of the measurements (Figure 8 A). For the first four time points, no significant differences were detected between the three varieties. From about 100 DAA, Grenache had a significant lower bunch hydraulic resistivity compared to Chardonnay ($p = .012$) and Shiraz ($p = 0.17$).Two weeks later, bunches of Grenache still had an almost significant ($p = .070$) lower hydraulic resistivity than bunches of Shiraz. For the last measurement point, an almost significant ($p = .082$) lower hydraulic resistivity was found for bunches of Grenache compared to Chardonnay. There was also a very large difference between the average hydraulic resistivities for bunches of Chardonnay and Shiraz, but it was not significant.

The same comparison was carried out for berry hydraulic resistivities and pedicel hydraulic resistivities of all three varieties, but no significant differences were found (data not shown). The most significant differences were found when comparing the rachis hydraulic resistivities between the three varieties (Figure 8 B). Except from the second time point of the measurements, Grenache had always an almost significant ($p = .057$) lower to a highly significant ($p = .003$) lower rachis hydraulic resistivity compared to Chardonnay. For the third time point of the measurements an almost significant ($p = .083$) lower rachis hydraulic resistivity was detected in Shiraz compared to Chardonnay. This difference between Shiraz and Chardonnay became significant ($p = .045$) at the fifth time point of the measurements. At the penultimate time point of the measurements, the rachis hydraulic resistivity was significant higher in both Chardonnay ($p = .050$) and Shiraz ($p = .043$) compared to Grenache.

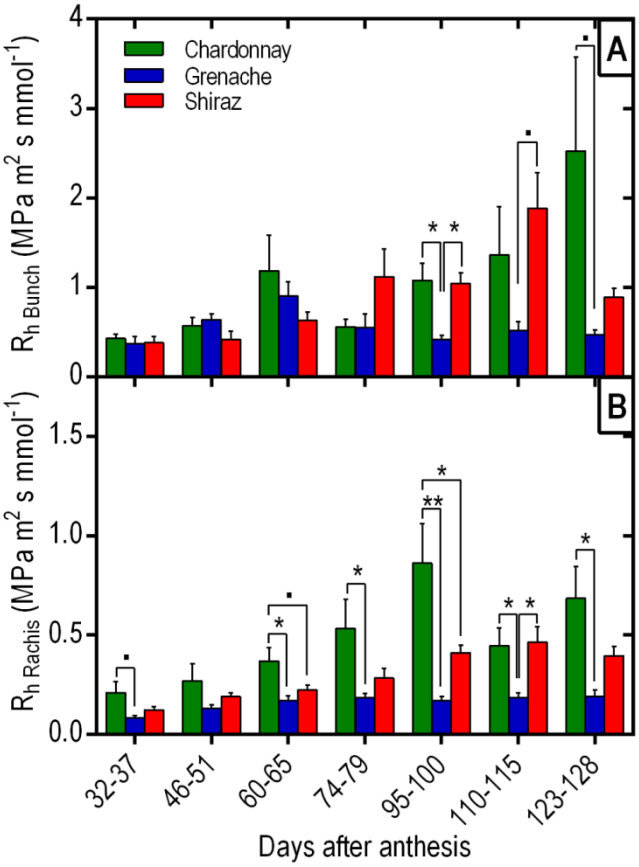

Figure 8. Varietal comparison for whole grape bunch and rachis hydraulic resistivities. Comparison of the whole grape bunch hydraulic resistivity (A), and the hydraulic resistivity of the rachis proper (B) between ripening grape bunches of the *Vitis vinifera* L. cultivars Chardonnay (green), Grenache (blue), and Shiraz (red) at the seven measurement time points during the 2011-12 season. Means ± SEM, n = 4-5 measurements per variety at each time point. Asterisks indicate statistical significant differences within a group according to one-way ANOVAs; a dot denotes almost significant differences (˙ $p \leq .100$; * $p \leq .050$, ** $p \leq .010$, *** $p \leq .001$).

When doing the flow measurements on whole bunches at zero applied pressure, a significant small inward flow rate was still detected depending on the variety and time in development (Figure 2). A high flow rate was measured for pre-veraison bunches of Shiraz compared to Chardonnay and Grenache. Closer to harvest the flow rate at zero pressure declined in Chardonnay and Shiraz, but increased significantly ($p < .001$) in Grenache which was shown by a simple linear regression. No significant change was measured for bunches of Chardonnay and Shiraz at the last three time points of the measurements.

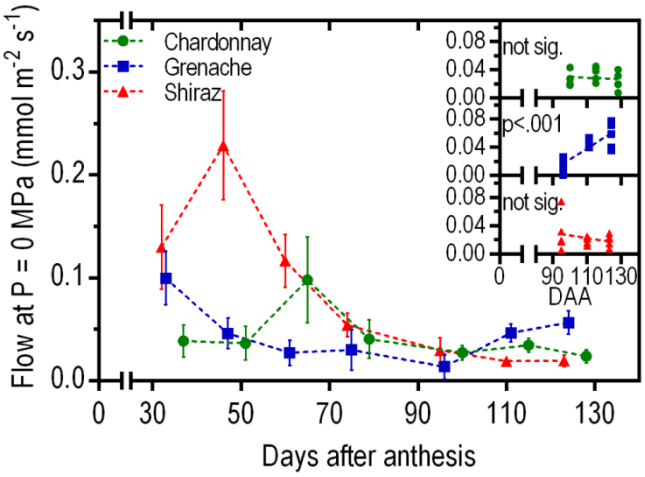

Figure 9. Flow rate at zero pressure into grape bunches of the *Vitis vinifera* L. cultivars Chardonnay (green solid circles), Grenache (blue solid squares), and Shiraz (red solid triangles) during the 2011-12 season. Means ± SEM, n = 4-5 measurements at each time point. Insets: Linear regressions (dashed lines) for the individual flow rates at zero pressure measured during the last three measurement time points for the three varieties. The text indicates whether the slopes of these regressions were non-zero.

2.4 Discussion

The highly significant positive correlation between whole bunch hydraulic conductance and total berry surface area in Grenache shows that this variety adjusts its hydraulic conductance according to berry growth during the course of berry development (Figure 6). This also implies that the bunch hydraulic conductivity which is the hydraulic conductance normalized to total berry surface area does not change over time which is shown in the inset of Figure 7 B. Therefore, Grenache as an isohydric variety behaves differently to the anisohydric varieties Chardonnay and Shiraz which show a decrease of whole bunch hydraulic conductivity (i.e. an increase in hydraulic resistivity) during the course of berry development (Figure 7 A inset, C inset). In Shiraz there was a negative correlation between the increase in total berry surface area and whole bunch hydraulic conductance (Figure 6 A). A decrease of whole bunch hydraulic conductivity in Chardonnay and Shiraz has been reported by Tilbrook and Tyerman (2009) before, but no studies have been conducted on isohydric varieties like Grenache. Hence, this is the first time that an adjustment of whole bunch hydraulic conductance to berry growth is reported. This adjustment could be due to growth of existing xylem vessel or even the formation of new xylem vessels as it is reported by Chatelet et al. (2008b). It is possible that different patterns of aquaporins expression in Grenache compared to Chardonnay and Shiraz could contribute to this behaviour as it was reported for roots of Chardonnay and Grenache (R. K. Vandeleur et al., 2009). Interestingly, all three varieties had the same whole bunch hydraulic

conductance at a pre-veraison stage where the total berry surface area is small (Figure 6). This means that development of conductive tissue should be the same in early stages of berry development, but is more variety dependent from veraison on. In Shiraz, which shows a decrease in hydraulic conductance for higher total berry surface area (Figure 6), a blockage of the xylem vessels by gels or solutes could be the case. This type of blockage has already been observed in post-veraison berries of Chardonnay (Choat et al., 2009).

It seems that changes in the berries contribute largely to the adaption of hydraulic conductance in Grenache since no correlation was found between the hydraulic conductance of the rachis with pedicels attached and the change in total berry surface area (Figure 6 B). However, a highly significant positive correlation between rachis hydraulic conductance and change in total berry surface area indicates an adaption in the rachis part of bunches of Grenache (Figure 6 C). Interestingly, the increase in pedicel weight per berry in Grenache (Figure 5 A) is slightly higher than the increase in rachis weight per berry (Figure 5 B). Therefore, there might not be a direct link between total growth of pedicels and rachis and the hydraulic conductance. Even though, the pedicel weight per berry was not much different at any time between the varieties (Figure 5 A), Grenache had the lowest pedicel weight per total berry surface area (Figure 5 C) since the berries of Grenache were significantly larger than the berries of Chardonnay and Shiraz (Figure 4 A). Hence, this might be a constraint for water flow. This assumption agrees with the increase in hydraulic resistivity of the pedicel part in grape bunches of Grenache during the course of development (Figure 7 B). A vertical separation of the rachis hydraulic conductance for the three varieties can also be seen in Figure 6 C. Grenache had a significantly larger rachis hydraulic conductance than Chardonnay. The statistics on the hydraulic resistivities of the rachis also show that the rachis resistivity of Grenache was significantly than the rachis resistivity of Chardonnay from 60 DAA onwards (Figure 8 B). The hydraulic conductance or hydraulic resistivity of rachis from Shiraz were intermediate. This separation corresponds with the separation in rachis weight between the varieties (Figure 5 B). Grenache had the highest rachis weight per berry, Shiraz was intermediate, and Chardonnay the lowest. The same is also true for the rachis weight per total berry surface area (Figure 5 D). In conclusion, the size of the rachis is correlated to the rachis hydraulic conductance in these three varieties. A similar results for petioles of Grenache and Chardonnay was found by Shelden (2008). In this study, a higher petiole hydraulic conductance in Grenache was related to a larger xylem vessel diameter in petioles of Grenache.

In previous studies, it was observed that the berries make up a larger hydraulic resistivity compared

to the pedicels (Choat et al., 2009; Tyerman et al., 2004) and that the rachis hydraulic resistivity was assumed to be close to zero (Tyerman et al., 2004). The results in Figure 7 B show, that pre-veraison berries of Grenache provide the major resistivity of those grape bunches. Also berries of Chardonnay dominated the total bunch resistivity at the end of ripening (Figure 7 C). However, the calculated berry hydraulic resistivity was comparably low for pre-veraison bunches of Chardonnay (Figure 7 A) and Shiraz (Figure 7 C), and post-veraison bunches of Grenache (Figure 7 B). A high hydraulic resistivity of the rachis in Chardonnay which corresponds to the rachis weight per berry, contradicts the assumption of a low hydraulic resistivity in this part of bunches (Tyerman et al., 2004).

One hypothesis that has been developed to explain why berries of Shiraz are susceptible to pre-harvest berry dehydration whereas berries of Chardonnay are not susceptible relies on the observation of a higher hydraulic conductance (lower hydraulic resistivity) in berries and bunches of Shiraz at the end of ripening compared to Chardonnay (Tilbrook & Tyerman, 2009; Tyerman et al., 2004). The hypothesis is that berries of Chardonnay become hydraulically isolated from the parent vine which prevents water backflow at times of high vine transpiration that could generate a very negative water potential in the plant. Berries that maintain a high hydraulic conductance at the end of ripening like in Sultana (Tilbrook & Tyerman, 2009) can prevent backflow by also maintaining a higher cell vitality compared to Chardonnay and Shiraz (Tilbrook & Tyerman, 2008). As the results in Figure 7 A show, berry hydraulic resistivity in Chardonnay did increase at the end of ripening, but there was also a very high variability. In Shiraz, the berry hydraulic resistivity was low for the last point of measurement, but equal or even higher than in Chardonnay for the second last point of measurement (Figure 7 C, Figure 8 A). This does not match with the onset of berry weight loss (Figure 4 A) in Shiraz at 95 DAA and in Chardonnay at 115 DAA, which also caused a significant increase in berry sugar concentration in Shiraz (Figure 4 C). Thus although similar observations to results by Tilbrook and Tyerman were made, results from this experiment do not fully support their hypothesis, mainly because of the timing of the change in hydraulic resistivity which seems to occur after berry weight loss has already occurred. Usually Chardonnay is regarded as a variety that is not affected by pre-harvest berry dehydration and the weight loss that was observed in this study occurred after the official harvest for this variety. Even for the last point of measurement the bunch hydraulic resistivity of Chardonnay was not significantly higher than in Shiraz (Figure 8 A), which might be due to the high variability.

A high variability in hydraulic conductance or resistivity was measured for bunches in general. This

could possibly be due to the terminal resistivity that berries provide in measurements of whole bunches or even due to differences in cell death within the berries (Fuentes et al., 2010). Bunches of Grenache had lower hydraulic resistivity than bunches of Chardonnay and Shiraz (Figure 8 A). Therefore, and according to Tilbrook's and Tyerman's hypothesis, it is surprising that no berry weight loss was observed in this variety (Figure 4 A). One could argue that the absence of weight loss could be a consequence of less cell death in this variety, however there was a relatively high degree of cell death observed in this variety at the end of ripening (Fuentes et al., 2010).

The measurement of flow rate into Grenache grape bunches at zero pressure showed a significant increase during the last four weeks of measurements (Figure 9 and insets). This indicates the presence of intact membranes in berries that can generate a negative water potential for water uptake. Berries of Grenache are not only larger than berries of Chardonnay and Shiraz, but also had a lower berry density, at least for pre-veraison berries (Figure 4 A, B). This could indicate a lower amount of cell material per berry and hence larger cells, which could result in different hydraulic properties. In Chardonnay and Shiraz the flow rate at zero pressure declined at the end of ripening. For the second last point of measurement there was still a slightly higher flow rate in Chardonnay compared to Shiraz which coincides with the onset of berry weight loss in Shiraz and still continuing berry weight increase in Chardonnay (Figure 4). Inflow into berries at zero pressure was also reported by Choat et al. (2009).

In conclusion, an adjustment of bunch hydraulic conductance to berry growth was detected in the isohydric variety Grenache which was not reported in the literature before. This also confirms the large differences in stomatal behaviour and petiole hydraulic conductance that have been detected between isohydric and anisohydric grape varieties (Schultz, 2003). The adaption seems to happen mainly in the berries and the rachis. However, it is not known whether changes in physical properties or for example changes in aquaporin expression are responsible for this behaviour. Therefore, a study of xylem integrity, vessel diameter, and aquaporin expression could explain the discovered differences. In addition, the hydraulic conductance of the rachis correlates with the rachis weight per berry. The rachis can provide a large resistivity as part of the bunch and should not be neglected.

The new findings reported here do not support the hypothesis of Tilbrook and Tyerman (2009) that a hydraulic isolation in grape berries of Chardonnay may prevent water backflow. The increase in hydraulic resistivity observed here in Chardonnay compared to Shiraz is late and after the onset of berry weight loss, and also not very strong. It is also difficult to reconcile the Tilbrook and Tyerman hypothesis with the observations made here for Grenache, which maintains a low hydraulic resistivity

late in ripening and did not show weight loss. The differences between varieties in the positive flow into bunches at zero pressure at the end of ripening raises the possibility that gradients developed in the berry are more important than the hydraulic resistivity. A detailed study of cell vitality and sites where membrane semi-permeability is maintained in post-veraison Grenache would offer new answers.

Chapter 3 – Water uptake and transpiration in grape berries

3.1 Introduction

Water relations of grape berries have been studied using dye uptake techniques and measurements of fruit volume changes in combination with phloem girdling (Creasy & Lombard, 1993; During et al., 1987; Greenspan et al., 1996; Greenspan et al., 1994; Lang & Thorpe, 1989).

A general decline of xylem water uptake at veraison was discovered (During et al., 1987). Greenspan et al. (1996; 1994) found that in post-veraison berries water uptake is mainly through the phloem to facilitate sugar accumulation. They also suggested that diurnal cycles of contraction and expansion in pre-veraison berries are due to transpiration and water backflow via the xylem to the parent vine. At the same time they assumed no water backflow in post-veraison berries since the amplitude of daily contraction and expansion were much smaller and the berries seemed hydraulically isolated from transpirational changes in the parent vine (Greenspan et al., 1996). However Lang and Thorpe (1989) saw the need for water backflow at late stages of ripening to explain the constant sugar uptake when berry growth already declined.

A net water loss from berries at late ripening stages which is called pre-harvest berry dehydration was properly investigated for the first time by McCarthy (1997, 1999). This net water loss can cause a berry weight loss of up to 30% (McCarthy, 1999; S. Y. Rogiers et al., 2006) at the end of ripening, and was attributed to a decline in phloem water uptake when sugar accumulation cease, since xylem was regarded as non-functional in post-veraison berries (McCarthy & Coombe, 1999). However, studies on calcium accumulation which is only transported via the xylem and dye uptake studies with a water potential gradient applied through a pressure membrane technique showed that the xylem is still functional in post-veraison berries (Bondada et al., 2005; Keller et al., 2006; S. Rogiers et al., 2000). S. Y. Rogiers et al. (2004) investigated if damage due to cracking of the cuticle might be the cause for the water loss at late ripening stages in Shiraz. They found that the transpiration was high in pre-veraison berries and then declined during the course of berry development. No increase was detected during the phase of pre-harvest berry dehydration. Therefore, they concluded that the net

water loss is rather caused by an imbalance between water uptake and continuing transpiration and that there was no disruption of the cuticle. In a later study by Greer and Rogiers (2009), an infra-red gas analyser was used to measure transpiration and respiration of potted Shiraz vines and the bunch water balance was measured by weighing bunches *in situ*. This study confirmed an imbalance between water uptake and water loss via transpiration at late ripening stages.

However, no comparative studies of berry transpiration have been reported between varieties that show differences in berry weight loss. Therefore, the aim of this study was to compare transpiration for berries of Chardonnay, Chenin Blanc, Grenache, Shiraz, and Sultana during the course of berry ripening. For this, a custom-made transpiration chamber was designed and constructed to measure water uptake and transpiration simultaneously.

3.2 Material and methods

3.2.1 Plant material

Vitis vinifera L. cultivars Chardonnay clone I10V1, Chenin Blanc, Grenache clone 137, Shiraz clone BVRC12, and Sultana were grown in the Coombe vineyard, 34° 58' S 138° 37' E, at the University of Adelaide Waite Campus in the Adelaide region (South Australia) on own roots. Chardonnay was planted in 2006 with a row spacing of 3 m, a vine spacing of 1.8 m, and flowering which was determined as 50% cap-fall across all vines in the row, occurred on the 24th of October in year 2011. Grenache was planted in 2002 with a row spacing of 3 m, a vine spacing of 1.8 m, and flowering occurred on the 28th of October in year 2011. Shiraz was planted in 1993 with a row spacing of 3 m, a vine spacing of 2.7 m, and flowering occurred on the 29th of October in year 2011. Chenin Blanc and Sultana were sourced from the variety collection block without information about the year when these plants have been planted. Moreover, most grape berries of Chenin Blanc were infected with *Botrytis cinerea* during the 2011-12 season. However, healthy berries were selected for the experiments during sampling.

3.2.2 Sampling regime

Parts of bunches of grapes were collected from the varieties Chardonnay, Chenin Blanc, Grenache, Shiraz, and Sultana at the Coombe vineyard seven times during the 2011-12 season to gather information on berry development and to measure the water relations of berries from these varieties

in an *in vitro* transpiration assay. Small parts (stem with several berries) were collected from five bunches per variety between 4pm and 5pm by immersing the bunches into purified (Milli-Q Plus; Merck Millipore, Billerica, MA, USA), degassed (1.0 x 5.5 Mini Module(TM); Membrana GmbH, Wuppertal, Germany) water and cutting the rachis using a pair of secateurs. The samples were immediately transferred to the laboratory for further processing.

3.2.3 Custom-made transpiration chamber

The custom-made transpiration chambers was constructed from a shallow, oblong 1.75 L PET container (Tellfresh®; The Decor Corporation Pty Ltd, Scoresby Vic, Australia), 20 x 50-mL falcon tubes, and 20 x 2-mL Eppendorf tubes.

A 20-mm drill bit was used to drill 4 x 5 holes lengthwise into the lid of the PET container. The screw-caps of all falcon tubes were removed, and each tube was cut into three pieces using a hacksaw: 1) a 15 mm long piece from the upper part with the screw thread, 2) a 45 mm long piece from the middle part, 3) a 35 mm long piece from the lower part with the conical base. In the centre of each screw-cap a 10.5 mm hole was drilled. Eight 5 mm holes were drilled into each of the 35-mm pieces just above the conical base as ventilation holes. The 15-mm pieces with the screw threads were glued on top of the PET container lid just over the holes using an instant adhesive (Loctite 401; Henkel Australia Pty. Ltd., Kilsyth Vic, Australia). On the lower side of the PET container lid, the 35-mm pieces with the conical base were glued. The 45-mm middle pieces were glued on top of the screw-caps with the holes as a socket for the Eppendorf tubes.

A 2 mm hole was drilled in the centre of each lid of the 2-mL Eppendorf tubes. For each tube, a 7 x 5 mm cylindrical rubber seal with a 2 mm hole in the centre was prepared using silicone impression material (Optosil-Xantopren; Heraeus Kulzer, Dormagen, Germany). After setting, the rubber seals were glued on top of the lids of the Eppendorf tubes whilst centring the 2-mm holes on top of each other. A short piece of 2-mm tubing as used to avoid clogging the holes. Finally, the Eppendorf tubes with the rubber seals were put together with the sockets to form the berry holder units.

Figure 10. Close-up views of the custom-made transpiration chamber: (A) Socket for the Eppendorf tubes; (B) Eppendorf tube with rubber seal; (C) Assembled berry holder unit; (D) Lid of the transpiration chamber with individual berry cells; (E) Complete transpiration chamber (upside down); (F) Transpiration chamber with one berry holder unit attached.

3.2.4 Transpiration assay

Transpiration and xylem water uptake of detached berries with the cut end of the pedicel in water was measured as total weight change of the berry plus water reservoir and the weight change of the water reservoir only, respectively. Therefor five individual berries with pedicels attached were cut

from the bunch samples with a fresh, sharp razorblade under purified, degassed water. A 40 mm long piece of PE tubing (2.00 x 1.00 mm PE Tubing, Adelab Scientific, Thebarton SA, Australia) with one end flared by a puncher was filled with purified, degassed water and attached to the pedicel. The berry with tubing attached was removed from the water, the pedicel tubing junction carefully dried with a piece of tissue, and then sealed with adhesive-free silicone glue (3140 RTV Coating; Dow Corning Corp., Midland, MI). Berry diameter, and height were measured using digital callipers (Digitronic Caliper MW110-DBL, Moore & Wright, Bowers Metrology Ltd., Bradford, West Yorkshire, UK). Curing took place over night while the berries could take up water from a reservoir through the tubing.

The next morning, two custom-made transpiration chambers were filled with 140 g of silica gel each as a drying agent. This guaranteed a constant low relative humidity of 20% in the chambers during the experiment which was carried out at 20°C (Temperature and Humidity Datalogger QP6014, DIGITECH, Roorkee, Uttarakhand, India). For each berry the Eppendorf tubes of the berry holder units were filled with 1.75 mL purified, degassed water. Afterwards the initial weights of the berry holder units and the berries with tubing attached were measured on a fine balance (OHAUS Corporation, Parsippany, NJ, USA), separately. The berries with tubing attached were connected to the water reservoir of the berry holder units, and the initial weight of each entire holder unit with berry attached was measured. The holder units with berries attached were connected to the transpiration chambers and the weight loss through transpiration was measured hourly for five consecutive hours.

After 24 hours the final weight was measured of the holder units with berries, the berry holder units without berries attached, and the berries with tubing attached. Berry sugar concentrations were determined using a digital refractometer (PR-101, ATAGO CO., LTD, Tokyo, Japan).

3.2.5 Data analysis and statistics

The MATLAB software (MathWorks, Natick, MA, USA) was used for batch processing of the raw data. For each berry, berry volume (Eq. 1), and berry surface area (Eq. 2-6) were calculated based on the berry diameter (d) and berry height (h) measurements (see chapter 2). A linear regression was fitted to the weight loss data from the five-hours repetitive measurement period using the statistical software R (R Core Team, 2012) to determine whether the berries show a constant transpiration rate.

The final transpiration rate was calculated over the 24-hours measurement period per berry surface area from the weight change of the entire berry holder units with berries attached. The weight

change of the berry holder units without berries over the 24-hours measurement period was used to determine the water uptake rate per berry surface area from the reservoir into the berry, and the weight change of the berries with tubing attached was used to calculate the ratio between rehydration and transpiration of the berries.
GraphPad Prism 5 for Windows (GraphPad Software, San Diego, California, USA) was used for the statistical tests (one-way ANOVA) and all figures have been created in this programme.

3.3 Results

3.3.1 Grape berry development

Berries of all five grape varieties showed a typical development pattern during the 2011-12 season. After initial rapid growth during the berry formation phase, berries started to get soft (onset of veraison) and changed colour between the second and third time of measurements at around 60 days after anthesis (DAA)(Figure 11). Berry sugar concentration was low and did not change much before that point, but started to increase when veraison occurred (Figure 11 B). At the third time of measurements berries of all varieties, except Chenin Blanc, had gone soft, and berries of Chardonnay, Shiraz, and Sultana had already changed colour. In Chardonnay and Grenache a lag phase was observed between the second and third time of measurements where berry growth came to a halt (Figure 11 A). This usually occurs before veraison. At the fourth time of measurements berries of Chenin Blanc had passed veraison as well. A second rapid growth phase followed after veraison before the berries reached maximum berry weight. Sultana had the largest berries, followed by Grenache, and Shiraz, whereas Chenin Blanc and Chardonnay had the smallest berries at the end of development. Berries of Chardonnay, Chenin Blanc, and Sultana reached a maximum sugar concentration of about 26°Brix (Figure 11 B). In Grenache a higher berry sugar concentration of about 28°Brix was measured at the end of development. A significant higher sugar concentration of about 33°Brix was measured in berries of Shiraz which coincided with a loss of berry weight between 115 and 128 DAA (Figure 11 A, B).

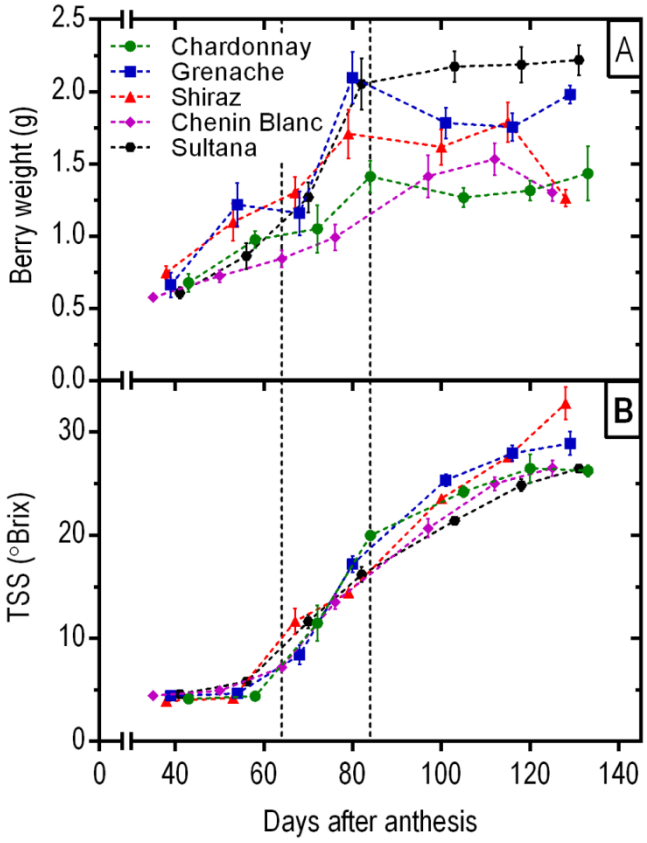

Figure 11. Developmental parameters of grape berries. Berry weight (A) and total soluble solids (B) of ripening grape berries of the *Vitis vinifera* L. cultivars Chardonnay (green solid circles), Grenache (blue solid squares), Shiraz (red solid triangles), Chenin Blanc (pink solid rhombus), and Sultana (black solid hexagon) during the 2011-12 season. The time period between the dashed lines corresponds to the time when the water uptake rate into berries came below the transpiration rate as measured in the *in vitro* transpiration assay. Means ± SEM, n = 5 at each time point.

3.3.2 Berry water relations

In vitro berry transpiration rate (Figure 12 A) normalised to berry surface area and water uptake (Figure 12 B) rate both declined during the course of berry development in all five varieties used in this study. Transpiration rate and water uptake rate were both the highest in Sultana at the first time of measurements. From the second time of measurements and when the berries passed through veraison both transpiration rate and water uptake rate declined exponentially. Berries of Sultana experienced the most rapid decline in transpiration rate and water uptake rate between the first and second time of measurements which resulted in the lowest transpiration rate and water uptake rate for berries of this variety after that time. In all other varieties, the largest decline of transpiration rate occurred between the second and third time of measurements around veraison (Figure 12 A). The water uptake rate continued to decline even after veraison and for early post-veraison berries (Figure

12 B). This brought about a change of net flux into berries from positive to negative as the water uptake rates became lower than the transpiration rates (Figure 12 C).

For pre-veraison and veraison berries the water uptake rate was larger than the transpiration rate, which caused an increase in berry weight during the experiment (Figure 12 C). Shortly after veraison, between 70-80 DAA, the water uptake rate dropped below the transpiration rate and a net water loss from the berries occurred during the experiment.

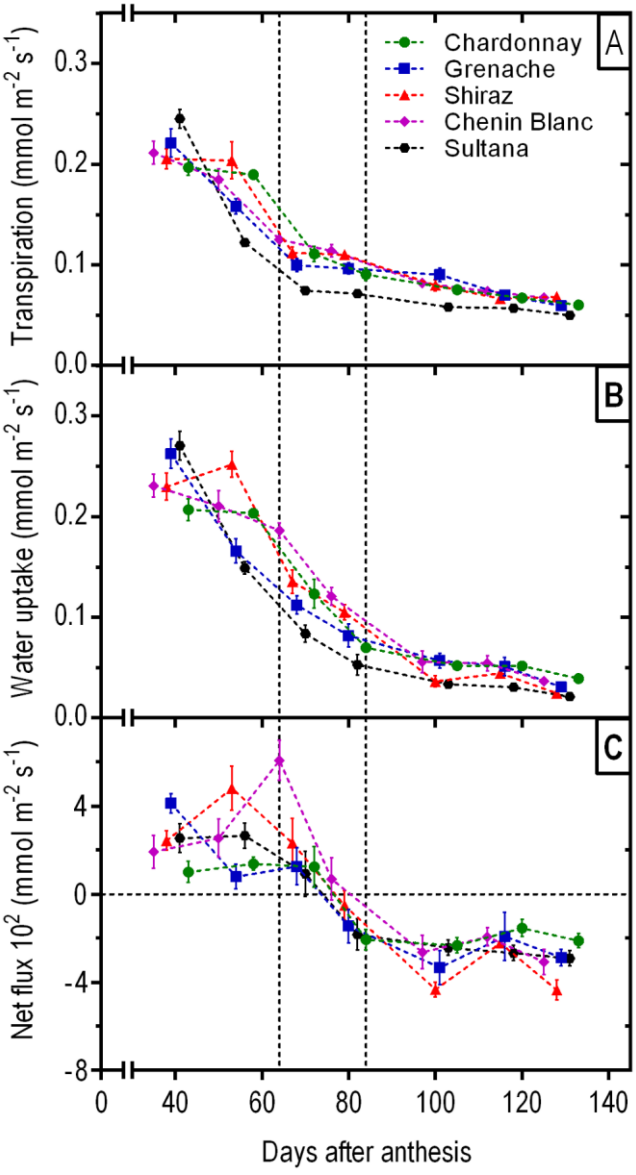

Figure 12. Water relations of grape berries. Transpiration rate (A), water uptake rate (B), and net flux rate (C), calculated as difference between water uptake rate and transpiration rate, of ripening grape berries of the *Vitis vinifera* L. cultivars Chardonnay (green solid circles), Grenache (blue solid squares), Shiraz (red solid triangles), Chenin Blanc (pink solid

rhombus), and Sultana (black solid hexagon) measured in the *in vitro* transpiration assay during the 2011-12 season. The time period between the dashed lines corresponds to the transitional phase of berry softening and colour change from veraison on. Means ± SEM, n = 5 at each time point.

Post-veraison berries of Sultana had always the lowest transpiration rate (Figure 13 A). This difference was significant or highly significant compared to Chenin Blanc and Grenache between 97-120 DAA, and highly significant compared to Chenin Blanc and Shiraz for the last time of measurement. At the same time this means that Chenin Blanc and Grenache had a higher transpiration rate between 97-120 DAA, and Chenin Blanc and Shiraz had a higher transpiration rate at the last time of measurement.

Post-veraison berries of Sultana also had the lowest water uptake rate (Figure 13 B). This difference was significant compared to Chenin Blanc at the second to last time of measurement, and significant or highly significant compared to Chardonnay and Chenin Blanc at the last time of measurement. Also Shiraz had a lower water uptake rate and this was significant compared to Chardonnay and almost significant compared to Chenin Blanc at the last time of measurement.

These differences in transpiration rate and water uptake rate lead to a higher net water loss in Shiraz between 97-105 DAA and 125-133 DAA (Figure 13 C). At the last time of measurement berries of Shiraz had a significant two-fold higher net water loss compared to berries of Chardonnay.

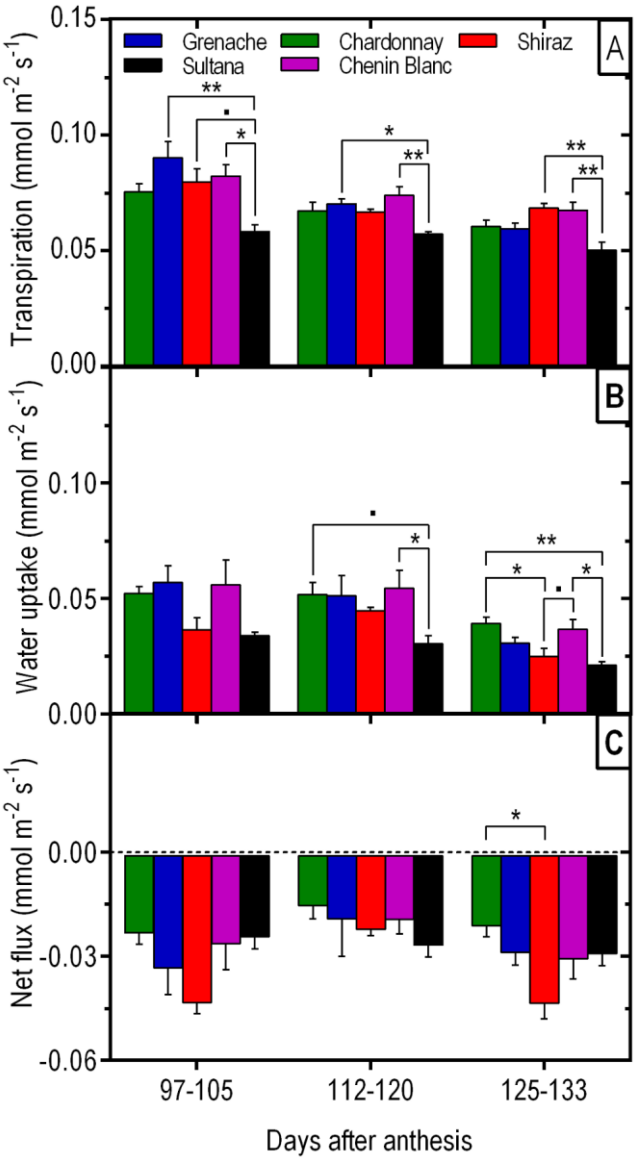

Figure 13. Varietal comparison of water relations in post-veraison grape berries. Comparison of transpiration rate (A), water uptake rate (B), and net flux rate (C) , calculated as difference between water uptake rate and transpiration rate, between post-veraison grape berries of the *Vitis vinifera* L. cultivars Chardonnay (green bars), Grenache (blue bars), Shiraz (red bars), Chenin Blanc (purple bars), and Sultana (black bars) measured in the *in vitro* transpiration assay during the 2011-12 season. Means ± SEM, n = 5 at each time point. Asterisks indicate statistical significant differences within a group according to one-way ANOVAs; a dot denotes almost significant differences (˙ $p \leq .100$; * $p \leq .050$, ** $p \leq .010$, *** $p \leq .001$).

3.4 Discussion

A general decline in transpiration and water uptake via the xylem was observed in berries of all five grape varieties in this study (Figure 12 A, B). This corresponds with the previous reports of berry

water relations from the literature (Greer & Rogiers, 2009; Keller et al., 2006; Lang & Thorpe, 1989; Palliotti & Cartechini, 2001; S. Y. Rogiers et al., 2004). However, in this study grape berry water uptake and transpiration were measured at the same time due to the use of a custom-made transpiration chamber (Figure 10). In previous studies, transpiration was measured on detached fruits with no water uptake (S. Y. Rogiers et al., 2004). Water uptake that was measured in this experiment can occur only via the xylem since the phloem becomes non-functional when berries were excised from bunches. Fishman et al. (2001) argues that separate studies on phloem and xylem water uptake introduce a significant error. However, this study aims to investigate at which stages of development the possible xylem water uptake can balance water loss via transpiration.

The result in figure Figure 12 A shows no large differences in berry transpiration between the varieties Chardonnay, Chenin Blanc, Grenache, and Shiraz at any time of development. Moreover, the differences become smaller after veraison. In post-veraison berries only significant differences were found compared to Sultana (Figure 13 A). Berry transpiration in the table grape variety Sultana was highest for the first point of measurement when berries are in a pre-veraison stage and then dropped below the transpiration rate of the other varieties. This different behaviour could be due to differences in the cuticle which serves as a barrier against water loss. Compared to Shiraz which is reported to undergo a decrease in wax per berry surface area during veraison and then remains stable (S. Y. Rogiers et al., 2004), berries of Sultana showed no changes in wax amount per berry surface area during berry development (Radler, 1965; S. Y. Rogiers et al., 2004). The decrease in wax per berry surface area in Shiraz does match with the decline in berry transpiration, but S. Y. Rogiers et al. (2004) assume that a decrease in transpiration could be due to a change in composition or structure of the waxy cuticle. Interestingly, they measured 1.1 to 1.2 µg mm^{-2} of total wax amount on Shiraz berries from 35 to 50 DAA which declined to 0.2 to 0.4 µg mm^{-2} at 90 DAA (S. Y. Rogiers et al., 2004). Therefore the wax amount in berries of Shiraz is similar to berries of Sultana (1 µg mm^{-2}) (Radler, 1965) until 90 DAA and is lower afterwards. This higher amount of wax per surface area in Sultana could cause the observed lower berry transpiration (Figure 12 A).

Weight change due to fruit respiration was not taken into account in this study since respiration accounts only for a fraction of the berry weight change. The lowest measured weight loss at the end of berry ripening was 45 to 62 mg per berry and day, whereas the average berry respiration is assumed to be as low as 1.9 mg CO_2 per berry and day (Niimi & Torikata, 1979).

The result in Figure 12 B demonstrates a decline in water uptake via the xylem at veraison, but ongoing water uptake well into ripening. This confirms that the xylem remains functional in post-

veraison berries (Bondada et al., 2005). Also a larger variability of water uptake was discovered between the varieties (Figure 12 B). Similar to the transpiration, berries of Sultana had a high water uptake rate at the first point of measurements and then dropped below the other varieties. This demonstrates a functional connection between water uptake and water loss via transpiration. After veraison, Shiraz had a low water uptake compared to Chardonnay, Chenin Blanc, and Grenache (Figure 13 B). A lower uptake could be due to a higher hydraulic resistivity which is not indicated by the results in Chapter 2, or due to a lower driving force for water uptake. This lower driving force for water uptake could be due to the observed decrease in living tissue in post-veraison berries of Shiraz (Tilbrook & Tyerman, 2008). However, a high amount of living tissue and a good hydraulic connection has been reported for Sultana (Tilbrook & Tyerman, 2008, 2009) and this should result in a better ability for water uptake which is not confirmed by the results in figure Figure 12 B. However, Chenin Blanc which is reported to have a high percentage of living tissue close to harvest (Fuentes et al., 2010) shows a high water uptake even after veraison (Figure 13 B).

Finally, balances between water uptake and water loss were calculated and matched the measured changes in berry weight (Figure 12 C). For pre-veraison berries of all varieties more water uptake than water loss was observed. This uptake could be due to rehydration or due to ongoing growth of the berries during the experiment. However, any rehydration should have already occurred during the over-night curing period for the glue. Therefore, this results support the hypothesis that xylem water uptake covers transpiration during the pre-veraison stage and also supplies water for berry growth (Greenspan et al., 1994). Shortly after veraison the water balance turns into a net water loss. The decline in xylem water uptake is usually associated with an increase in phloem water supply to facilitate sugar accumulation (Lang & Thorpe, 1989). Interestingly, sugar accumulation commenced before the change in the berry water balance (Figure 11 B, Figure 12 C). When comparing the water balance of all berries after veraison (Figure 13 B), Shiraz had always a high net water loss. For the last point of measurement the water loss from berries of Shiraz was twice the amount of water loss from berries of Chardonnay. At the same time a drop in berry fresh weight was observed in attached Shiraz berries (Figure 11 A). The transpiration assay suggests that the higher net water loss from berries of Shiraz was caused by a lower water uptake compared to the other varieties, than by higher transpiration. If phloem water uptake into post-veraison berries is similar in Chardonnay and Shiraz, a net water loss could be due to a lower xylem water uptake. However, caution has to be taken with these results since the phloem function was disrupted and no water potential difference in the plants had influence on water flow characteristics in this experiment.

In conclusion, water loss via transpiration when measured under identical VPD conditions is very similar in all five varieties used in this study. However, berries of Sultana have a lower transpiration and water uptake from veraison on. In Shiraz compared to Chardonnay, a two-fold higher net water loss from post-veraison berries was rather due to a lower water uptake capacity than to higher transpiration. It remains to be investigated how the phloem balances the transpiration after veraison and what influence differences in hydraulic conductance and water potential in the berries have on transpiration and water uptake.

Chapter 4 – Model of field water relations

4.1 Introduction

Grape berry growth depends on berry water relations during the course of development (Coombe & McCarthy, 2000). In different studies it was observed that pre-veraison berries were more sensitive to water stress than post-veraison berries (Creasy & Lombard, 1993; Greenspan et al., 1996; Smart, Turkingt.Cr, & Evans, 1974). A larger amplitude in daily berry expansion and contraction was observed by Greenspan et al. (1994) in pre-veraison berries compared to post-veraison berries. Studies on dye uptake into ripening grape berries also suggested a reduction in xylem water uptake after veraison which brings about the need for a larger phloem water import (Creasy et al., 1993; During et al., 1987). From these observations it was concluded that berry water uptake for pre-veraison berries must be mainly via the xylem whereas in post-veraison berries sugar and water uptake is facilitated via the phloem.

Studies using girdling techniques to disrupt the phloem flow into the berries were carried out to assess the contribution of the xylem and the phloem water uptake on berry water content, separately (Creasy & Lombard, 1993; Greenspan et al., 1994; S. Rogiers et al., 2000). The method to treat fruit growth as a balance between xylem, phloem, and transpiration flow presented by Lang (1990) was later used to calculate the cumulative flow rates of the xylem, phloem, and transpiration from girdling experiments. However, Fishman et al. (2001) criticise that these techniques can introduce errors in the estimation of xylem and phloem flows since they are tightly linked.

A detailed model to simulate the growth of peach fruit (*Prunus persica* L. cultivar Batsch) was introduced by Fishman and Genard (1998). They used this model to assess fruit growth as a function of diurnal fresh and dry matter accumulation and loss via transpiration and respiration. A different model was used by Dreier, Stoll, and Ruffner (2000) to investigate the relation between water uptake into grape berries and sugar accumulation.

The current opinion on grape berry water relations is that berry volume growth in pre-veraison grape berries depends both on xylem and phloem water uptake, but the main source would be the xylem (Coombe & McCarthy, 2000). At veraison xylem flow decreases and increased phloem water import facilitates sugar accumulation. When maximum sugar concentration is reached at the end of

ripening, phloem inflow decreases again. An imbalance between low xylem and phloem inflow at the end of ripening and continuing transpiration could lead to a net berry weight loss as observed in the variety Shiraz (McCarthy, 1999; McCarthy & Coombe, 1999). Greer and Rogiers (2009) found that the water uptake into post-veraison berries of the variety Shiraz is not sufficient to balance the water loss via transpiration. However, it is not clear why only some varieties show a net water loss at the end of ripening and whether the xylem or phloem fails to balance transpiration at the end of ripening. Furthermore, a strong influence of extreme climate events like heat-waves during late ripening stages on grape berry water relations was predicted by McCarthy (1999). Clearwater et al. (2012) showed that a pre-harvest 'shrivel' disorder in ripening kiwifruit berries (*Actinidia chinensis* var. *chinensis* 'Hort16A') that was observed in California, but not in New Zealand, was due to a net water loss from the fruits under higher vapour pressure deficit conditions in California compared to New Zealand. They also observed that the fruit water supply during ripening was facilitated by the xylem and phloem to almost the same extent. This contradicts the current opinion on grape berry water relations which assumes a decline in xylem water supply after veraison (Coombe & McCarthy, 2000).

Hence this Chapter will define a simple model to study grape berry water relations during the post-veraison ripening phase in different varieties and under different climate conditions. The aim is to integrate the measured data of xylem water uptake, and transpirational water loss as a function of climate together with an estimate of phloem water uptake as a function of berry sugar accumulation to establish a best fit to observed grape berry growth in the field for the varieties Chardonnay, Grenache and Shiraz. Data obtained from the *in vitro* transpiration assay was used to calculate xylem water uptake, and water loss via transpiration as a function of vapour pressure deficit which was monitored in the field during the season. Phloem water uptake was estimated as a function of sugar accumulation in the berries, which was measured in the field, and fitted together with xylem water uptake and transpirational water loss to the field data of berry weight change under the assumption of a range of different constant sugar concentrations in the phloem sap. Estimated phloem sap concentrations were derived from the best fit between simulated and measured berry weight changes during ripening. Moreover, testing the model on a modified climate without heat-waves at the end of the season will give information on the sensitivity of grape berry water relations to extreme climate events.

4.2 Material and methods

4.2.1 Plant material

Vitis vinifera L. cultivars Chardonnay clone I10V1, Grenache clone 137, and Shiraz clone BVRC12 were grown in the Coombe vineyard, 34° 58' S 138° 37' E, at the University of Adelaide Waite Campus in the Adelaide region (South Australia) on own roots. Chardonnay was planted in 2006 with a row spacing of 3 m, a vine spacing of 1.8 m, and flowering which was determined as 50% cap-fall across all vines in the row, occurred on the 24th of October in year 2011. Grenache was planted in 2002 with a row spacing of 3 m, a vine spacing of 1.8 m, and flowering occurred on the 28th of October in year 2011. Shiraz was planted in 1993 with a row spacing of 3 m, a vine spacing of 2.7 m, and flowering occurred on the 29th of October in year 2011.

4.2.2 Sampling regime

Data that were used in this chapter to fit the model on grape berry water relations originate from the experiments on grape bunch hydraulic conductance in Chapter 2 and the *in vitro* transpiration assay in Chapter 3. For each of these experiments shoots with bunches of grapes and parts of grape bunches were collected from the varieties Chardonnay, Grenache, and Shiraz at the Coombe vineyard seven times each during the 2011-12 season. The sampling started from a pre-veraison, pea-sized berry stage and was carried out until late harvest.

From the experiment on grape bunch hydraulic conductance information on berry weight, berry surface area, and total soluble solids in berries were used for the model. The same information was also collected for berries used in the *in vitro* transpiration assay, but due to the lower number of samples there was more variability in the data. Therefore, these data were used from the experiment on hydraulic conductance.

Information on berry surface conductance and berry xylem water uptake were used from the *in vitro* transpiration assay.

Since transpiration and xylem water uptake depend on the climate, temperature and relative humidity were monitored inside the canopy of the vineyard.

4.2.3 Model constants and variables

In Table 2 all constants and variables of the model below are listed. Units and a brief description are given and values are assigned to all constants.

Table 2. Constants and variables for the model of field water relations of grape berries.

Variable/ constant	Value	Units	Description
$A_{f\_chamber}$		m^2	Berry surface area of berries in the transpiration assay
A_{f_field}		m^2	Berry surface area of berries measured in the hydraulics experiment
W_{f_field}		g	Berry fresh weight of berries measured in the hydraulics experiment
W_{f_model}		g	Calculated berry fresh weight from the model
S_{f_field}		$g\ s^{-1}$	Calculated sugar content (sucrose equivalent) of berries measured in the hydraulics experiment
TTS_{f_field}		°brix	Total soluble solids of berries measured in the hydraulics experiment
$E_{f\_chamber}$		$g\ s^{-1}$	Berry transpiration rate measured in the transpiration assay
E_{f_field}		$g\ s^{-1}$	Calculated berry transpiration rate from the model in the field
g_f		$m\ s^{-1}$	Berry surface conductance measured in the transpiration assay
R	$8.3144621*10^{-3}$	$m^3\ kPa\ K^{-1}\ mol^{-1}$	Gas constant
M_W	18.02	$g\ mol^{-1}$	Molecular mass of water
M_S	342.30	$g\ mol^{-1}$	Molecular mass of sucrose
VPD		kPa	Vapour pressure deficit
$VPD_{chamber}$	1.9226	kPa	Vapour pressure deficit in the transpiration assay
VPD_{field}		kPa	Vapour pressure deficit in the field
$VPD_{modified}$		kPa	Modified vapour pressure deficit with no heat-waves before harvest
T		K	Temperature
$T_{chamber}$		K	Temperature in the transpiration assay
T_{field}		K	Temperature measured in the field
$T_{modified}$		K	Modified temperature in the field with no heat-waves before harvest
H_f	100	%	Relative humidity fruit
H_a		%	Relative humidity transpiration chamber/in the field
K_{uptake}		$mmol\ kPa^{-1}\ m^{-2}\ s^{-1}$	Xylem water uptake conductance
$U_{X\ chamber}$		$mmol\ m^{-2}\ s^{-1}$	Xylem water uptake rate in the transpiration assay
U_{X_model}		$g\ s^{-1}$	Calculated xylem water uptake rate from the model in the field
C_{P_model}	0.5 – 1.5	$mol\ L^{-1}$	Calculated phloem sap sugar concentration
U_{P_model}		$g\ s^{-1}$	Calculated phloem water uptake rate from the model in the field

4.2.4 Berry weight and surface area

Berry weight and surface area data were obtained from data collected for the experiment on grape bunch hydraulic conductance (Chapter 2). Monotonic splines were fitted to the data by using the function splinefun (method = monoH.FC) in the programme R (R Core Team, 2012). Concurrently, the spline functions of each data set were used to calculate the berry fresh weight (W_{f_field}) and the berry surface area (A_{f_field}) at any time between the 28th of December 2011 and 28th of February 2012.

4.2.5 Berry sugar content

Data on total soluble solids were used from the experiment on grape bunch hydraulics to estimate the equivalent amount of sucrose in berries ($1°brix \approx \frac{1\ g\ sucrose}{100\ g\ solution}$). It is acknowledged that the berry sap contains mainly glucose and fructose (Wada, Shackel, & Matthews, 2008), but a simplification seemed to be reasonable to estimate the berry sugar content for phloem fluxes in the model. Therefore, the sugar content (sucrose equivalent) of ripening berries (S_{f_field} in gram sucrose) was estimated as the product of total soluble solids (TTS_{f_field} in °brix) and 70% of the berry weight (W_f in gram) divided by 100. The scaling factor of 70% of the berry fresh weight was introduced to account for the water content in berries which serves as solvent for the solutes (i.e. sucrose equivalent) (M. Bonada, personal communication, September 21, 2012).

$$S_{f\_field}(t) = \frac{TTS_{f\_field}(t)}{100} \times 0.7 \times W_{f\_field}(t) \qquad 1)$$

Monotonic splines were fitted to the data of berry sugar content by using the function splinefun (method = monoH.FC) in the programme R and these functions were used to calculate the berry sugar content at any time between the 28th of December 2011 and 28th of February 2012.

4.2.6 Berry surface conductance and xylem water uptake conductance

The berry surface conductance was calculated from the data on berry transpiration rate ($E_{f\_chamber}$ in g s^{-1}) from the *in vitro* transpiration assay in chapter 3. During the transpiration assay the

temperature of the custom-made transpiration chambers were kept at 20°C and at a relative humidity of 20% by using silica gel as a drying agent. Therefore, the formula presented by Fishman and Genard (1998) could be used to calculate the fruit surface conductance (g_f in m s^{-1}) for grape berries (Lescourret, Genard, Habib, & Fishman, 2001).

$$g_f = \frac{E_{f\_chamber} \times R \times T_{chamber}}{A_{f\_chamber} \times M_W \times VPD_{chamber}} \quad 2)$$

The formula requires the berry surface area ($A_{f\_chamber}$ in m^2) through which the transpiration occurs, the gas constant R (8.3144621*10^{-3} m^3 kPa K^{-1} mol^{-1}), and the molecular mass of water M_W (18 g mol^{-1}). According to a formula by (Fishman & Genard, 1998), the vapour pressure deficit (VPD in kPa) was estimated by an exponential function, the temperature (T in K) and the difference in relative humidity ($H_f - H_a$) between the fruit ($H_f \approx 100\%$) and the transpiration chamber ($H_a \approx 20\%$).

$$VPD = 0.008048 \times e^{0.0547 \times (T - 273.15)} \times (H_f - H_a) \quad 3)$$

To estimate xylem water uptake under field conditions, a hypothetical xylem water uptake conductance (K_{uptake} in mmol kPa^{-1} m^{-2} s^{-1}) was calculated from the xylem water uptake rate ($U_{X\_chamber}$ in mmol m^{-2} s^{-1}) and vapour pressure deficit ($VPD_{chamber}$ in bar) measured in the transpiration assay. This xylem water uptake conductance is not equal to the hydraulic conductance measured for xylem water uptake in Chapter 2, since K_{uptake} was calculated on the assumption that VPD is the sole gradient for water uptake into the berries; all other gradients inside the berry (e.g. osmotic potential) were neglected. However, this simplification should reflect the observation by Clearwater et al. (2012) that the xylem in ripening kiwifruit berries was responsive to changes in VPD.

$$K_{uptake} = \frac{U_{X\_chamber}}{VPD_{chamber}} \quad 4)$$

Finally, a nonlinear model (nls) in the programme R was used to fit one phase exponential decay functions to the data of grape berry surface conductance and berry xylem water uptake conductance

for berries from veraison till late harvest. The parameter $y0$ is the upper limit, p is the lower asymptote, and k is the rate constant. For the time x in seconds midnight of the 29th of November 2011 was set as point zero.

$$y = (y0 - p) \times e^{(-k \times x) + p} \quad 5)$$

4.2.7 Climate

The temperature and relative humidity were measured inside the canopy of the vineyard at 15 minute intervals using sensors and a data logging system (Measurement Engineering Australia, Magill, SA, Australia). Subsequently, the vapour pressure deficit was calculated according to the formula by Fishman and Genard (1998) using the measured field temperature (T in K) and relative humidity (H_a) (see equation 3).

To model the berry water relations under conditions without heat-waves at the end of ripening, the climate (VPD data) from the 14th of February to 28th of February was replaced by iterations of the climate from the 6th of February to the 13th of February. Thus, two data sets for VPD (VPD_{field} and $VPD_{modified}$) were used to calculate xylem water uptake (U_{X_model}) and transpiration (E_{f_model}).

$$U_{X\_model}(t) = K_{uptake}(t) \times 10^{-3} \times M_W \times VPD_{field/modified}(t) \times A_{f\_field} \quad 6)$$

$$E_{f\_model}(t) = \frac{A_{f\_field}(t) \times M_W \times VPD_{field/modified}(t)}{g_f \times R \times T_{field/modified}(t)} \quad 7)$$

4.2.8 Model of grape berry water relations

Similar to the model by Fishman and Genard (1998) for fruit growth of peach, the simulated change in total berry weight over time ($W_{f\_model}(t)$) depends on the water uptake via the xylem ($U_{X\_model}(t)$), the water uptake via the phloem ($U_{P\_model}(t)$), the sugar accumulation ($S_{f\_field}(t)$), and water loss via transpiration ($E_{f\_model}(t)$). Respiratory losses from berries of 1.9 mg CO_2 per berry/day (Niimi & Torikata, 1979) were neglected in this simplified model.

$$W_{f_{model}}(t) = U_{X\_model}(t) + U_{P\_model}(t) + S_{f\_field}(t) - E_{f\_model}(t) \quad 8)$$

To calculate the water uptake rate via the phloem ($U_{P\_model}(t)$), a constant sugar concentration (C_{P_model}) was assumed in the phloem. Hence, the water uptake via the phloem would be a function of the sugar accumulation and the phloem sap concentration.

$$U_{P\_model}(t) = \frac{S_{f\_field}(t) \times 1000}{M_S \times C_{P\_model}} \quad 9)$$

Therefore, the change in total berry weight over time could be calculated as follows:

$$W_{f\_model}(t) = U_{X\_model}(t) + S_{f\_field}(t) \times (1 + \frac{1000}{M_S \times C_{P\_model}}) - E_{f\_model}(t) \quad 10)$$

A range of phloem sap concentrations (C_{P_model}) from 0.5 molar sucrose to 1.5 molar sucrose was used to calculate berry weight as a function of time from the 28th of December to the 28th of February using the model. Therefore, the change in berry fresh weight and the change in berry sugar content were calculated for every day from the spline functions. The transpiration rates and xylem water uptake rates were computed for the 15 minute intervals of VPD measurements using the nls models. A linear interpolation was performed on these rates over time using the approxfun function (R Core Team, 2012). To derive the total daily amount of transpired water and water taken up via the xylem, these functions were integrated by using the integrate function (R Core Team, 2012), which is based on a Gauss–Kronrod quadrature, with subdivisions of 10^5 subintervals.

To find the appropriate phloem sap concentration which brings the modelled berry weight kinetics as close as possible to the measured berry weight, the residual sum of squares was calculated for each concentration. The smallest residual sum of squares indicated the best fit.

$$W_{f\_field} \approx W_{f\_model}$$

Finally, xylem water uptake and transpiration were calculated to the measured climate in the vineyard and for the modified climate without the heat-waves in the second half of February 2012. These data, the sugar accumulation and the phloem water uptake, which is assumed not to be influenced by the climate (i.e. transpiration) in this model, were used to compute the total berry weight change for the two climatic conditions.

4.3 Results

A monotonic spline function was fitted to the measured berry fresh weight data (see chapter 2) for grape berries of the varieties Chardonnay (Figure 14 A), Grenache (Figure 14 B), and Shiraz (Figure 14 C) to be able to calculate changes in berry weight ($W_{f\_field}(t)$) as a function of time. Since this model of berry water relations addresses changes during the ripening phase of grape berries, only data from veraison till late harvest were used. Between the 14th and the 28th of December, berry weight change came to a halt which is usual before veraison. Therefore, it was estimated that veraison occurred around the 28th of December. The increase in sucrose content of berries from that day also indicated veraison (Figure 15). Afterwards, berry weight increase rapidly during the berry ripening phase until a significant berry weight loss of 10% in Chardonnay and 21% in Shiraz before harvest (Figure 14 A, C). In Grenache, no significant berry weight loss was observed (Figure 14 B).
A similar pattern was observed for the change in berry surface area during the course of grape berry ripening (Figure 14 D-F). The monotonic spline functions that were fitted to the measured berry surface area data were used to calculate transpiration and xylem water uptake as a function of changing berry size from veraison till late harvest under different climate conditions.

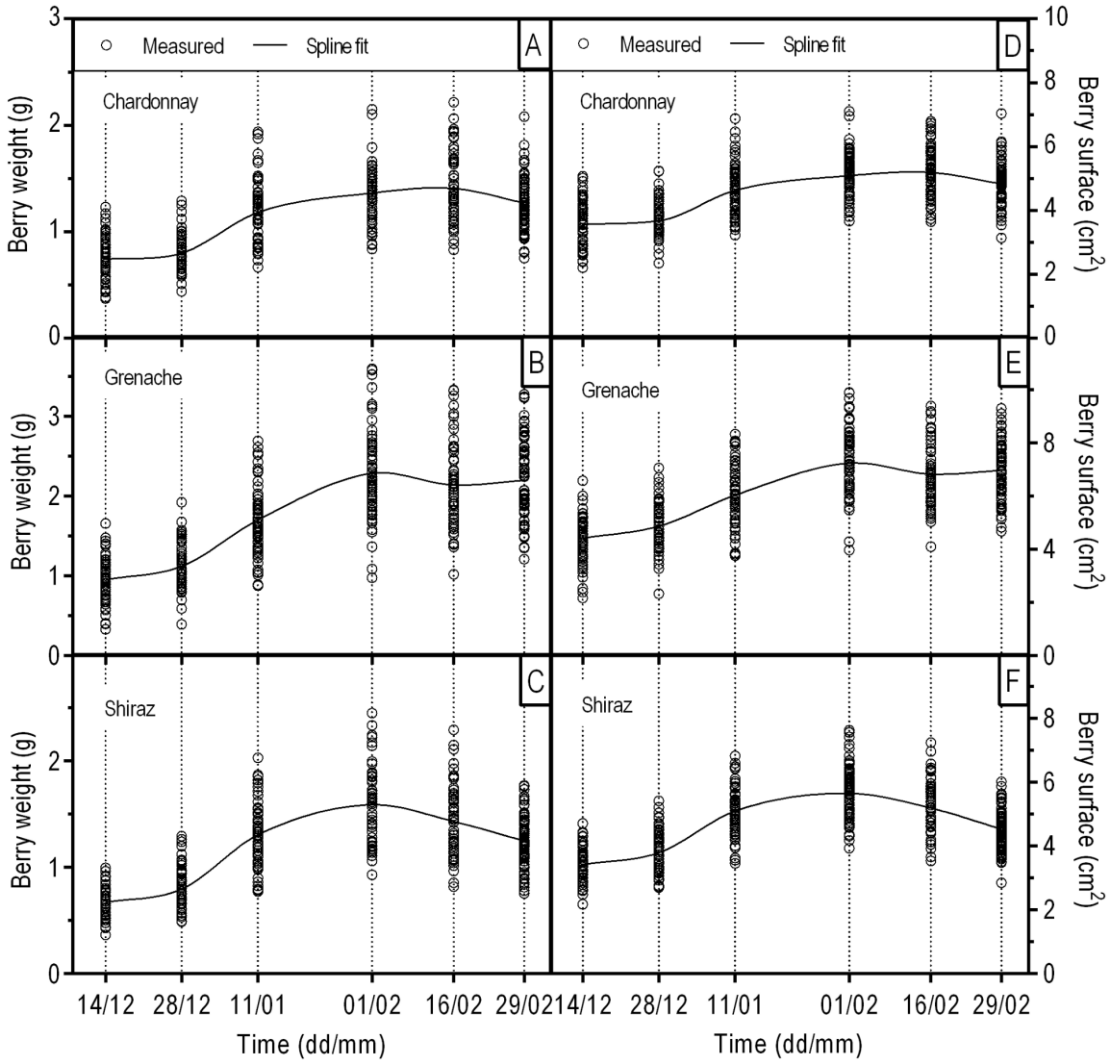

Figure 14. Spline fit of berry weight and surface area. Measured (open circles) and spline fitted (solid lines) berry weight (A-C) and berry surface area (D-F) of late pre-veraison, veraison, and post-veraison grape berries of the *Vitis vinifera* L. cultivars Chardonnay (A, D), Grenache (B, E), and Shiraz (C, F) during the 2011-12 season. Flowering occurred on the 24th of October 2011 for Chardonnay, on the 28th of October 2011 for Grenache and on the 29th of October 2011 for Shiraz. Times when measurements have been carried out in the field are shown as dotted vertical lines. Splines were fitted using the R (R Core Team, 2012) function splinefun (method = monoH.FC). Berry n = 75 (5 per 15 bunches of grapes) at each time point.

The sugar content (in sucrose equivalents) of the grape berries didn't change significantly between the 14th and 28th of December for all three varieties (Figure 15). A rapid increase in sugar content after the 28th of December indicates that veraison occurred at that time. In Chardonnay, the sugar content per berry increased until the 16th of February and dropped for about 2% during the following two weeks afterwards (Figure 15 A). Meanwhile, the sugar content of berries from Grenache and Shiraz continued to increase for about 3% (Figure 15 B, C).

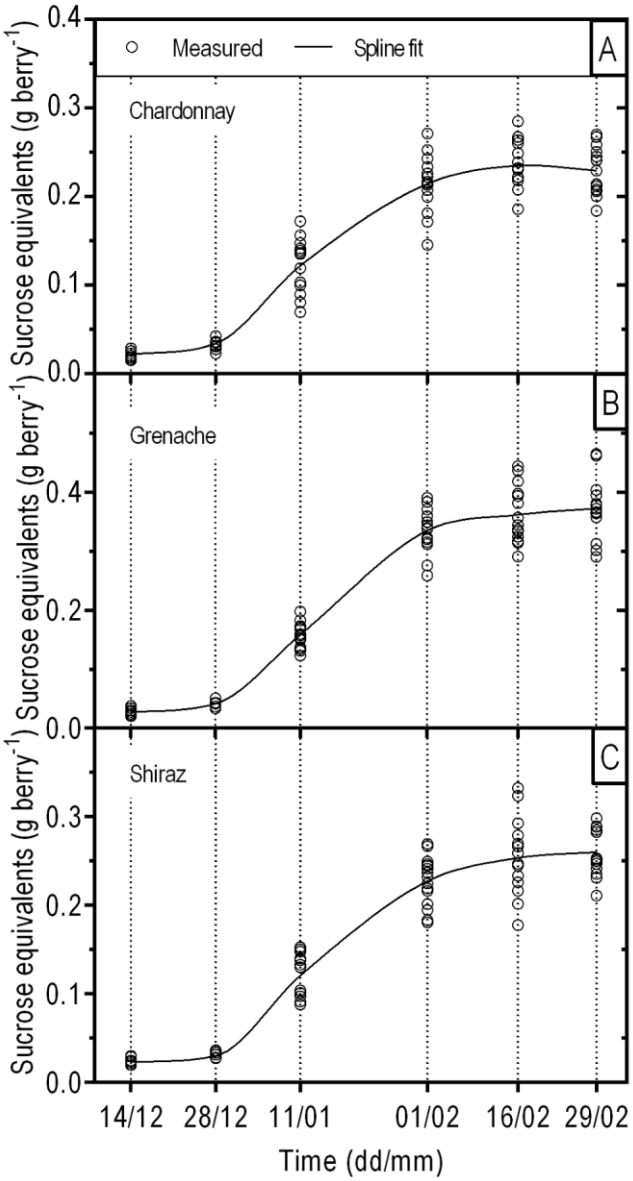

Figure 15. Spline fit of berry sucrose equivalents. Measured (open circles) and spline fitted (solid lines) berry sugar content (as sucrose equivalents) of late pre-veraison, veraison, and post-veraison grape berries of the *Vitis vinifera* L. cultivars Chardonnay (A), Grenache (B), and Shiraz (C) during the 2011-12 season. The sugar content was calculated as sucrose from measurements of total soluble solids (1 brix = 1 g sucrose / 100g of solution) and the berry fresh weight. A static dry weight of 30% per berry was assumed. Flowering occurred on the 24th of October 2011 for Chardonnay, on the 28th of October 2011 for Grenache and on the 29th of October 2011 for Shiraz. Times when measurements have been carried out in the field are shown as dotted vertical lines. Splines were fitted using the R (R Core Team, 2012) function splinefun (method = monoH.FC). Sample n = 15 (5 berries per bunch in one mixed samples = 75 berries in total) at each time point.

Grape berry surface conductance (g_f) and xylem water uptake conductance (K_{uptake}) were described

by one phase exponential decay functions for late pre-veraison till late harvest grape berries of the varieties Chardonnay, Grenache, and Shiraz (Figure 16). The parameters for each function are shown in Table 3.

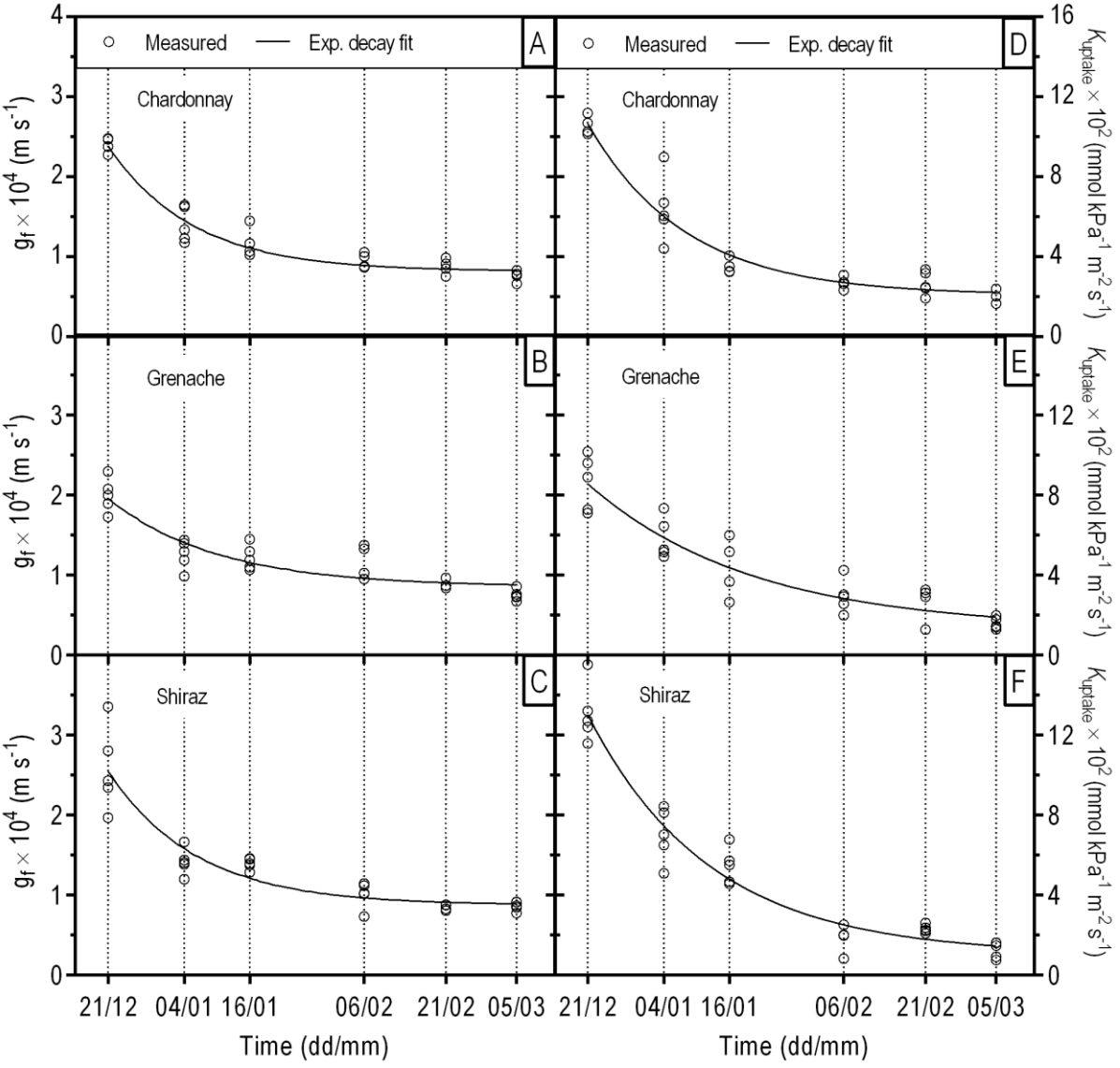

Figure 16. One phase exponential decay fit of grape berry surface conductance and xylem water uptake conductance. Measured (open circles) and one phase exponential decay fitted (solid lines) grape berry surface conductance (A-C) and grape berry xylem water uptake conductance (D-F) for late pre-veraison, veraison, and post-veraison grape berries of the *Vitis vinifera* L. cultivars Chardonnay (A, D), Grenache (B, E), and Shiraz (C, F) during the 2011-12 season. Flowering occurred on the 24th of October 2011 for Chardonnay, on the 28th of October 2011 for Grenache and on the 29th of October 2011 for Shiraz. Times when measurements have been carried out in the field are shown as dotted vertical lines. In R (R Core Team, 2012) a nonlinear model (nls) was used to fit a one phase exponential decay $\boldsymbol{y} = (\boldsymbol{y0} - \boldsymbol{p}) \times \boldsymbol{e}^{(-\boldsymbol{k} \times \boldsymbol{x})} + \boldsymbol{p}$ to the measured data. Y0 is the upper limit, p is the lower asymptote, and k is the rate constant. Berry n = 4-5 berries at each time point.

Table 3. Parameters of the one phase exponential decay functions $\boldsymbol{y = (y0 - p) \times e^{(-k \times x)} + p}$ fitted to the data of grape berry surface conductance, and xylem water uptake conductance (see Figure 16). Y0 is the upper limit, p is the lower asymptote, and k is the rate constant. The time (x) refers to the 29th of November as point zero. Asterisks indicate statistical significant parameters; a dot denotes an almost significant parameter (˙ p ≤ .100; * p ≤ .050, ** p ≤ .010, *** p ≤ .001).

Variety	Parameter	y0	p	k
Chardonnay	Surface conductance	7.342×10^{-4} ***	8.193×10^{-5} ***	7.491×10^{-7} ***
Chardonnay	Uptake conductance	3.128×10^{-1} ***	2.094×10^{-2} ***	6.443×10^{-7} ***
Grenache	Surface conductance	4.147×10^{-4} ***	8.550×10^{-5} ***	5.738×10^{-7} ***
Grenache	Uptake conductance	1.635×10^{-1} ***	1.300×10^{-2} ˙	3.819×10^{-7} ***
Shiraz	Surface conductance	7.390×10^{-4} ***	8.762×10^{-5} ***	7.138×10^{-7} ***
Shiraz	Uptake conductance	3.295×10^{-1} ***	1.045×10^{-2} *	5.153×10^{-7} ***

Differences were observed when comparing the functions for of grape berry surface conductance and xylem water uptake conductance for all three varieties over time (Figure 17).

Late pre-veraison berries of Shiraz had the highest surface conductance and uptake conductance, whereas berries of Chardonnay had the lowest surface conductance and uptake conductance at that stage; Grenache was intermediate. The surface conductance of Chardonnay and Shiraz declined almost at the same rate over time, whereas the rate change was smaller in Grenache (Table 3, Figure 17 A). For the water uptake conductance the change in rate was different for all three varieties (Figure 17 B). At the end of ripening berries of Chardonnay had the highest xylem water uptake conductance followed by Grenache, and Shiraz which had the lowest xylem water uptake conductance.

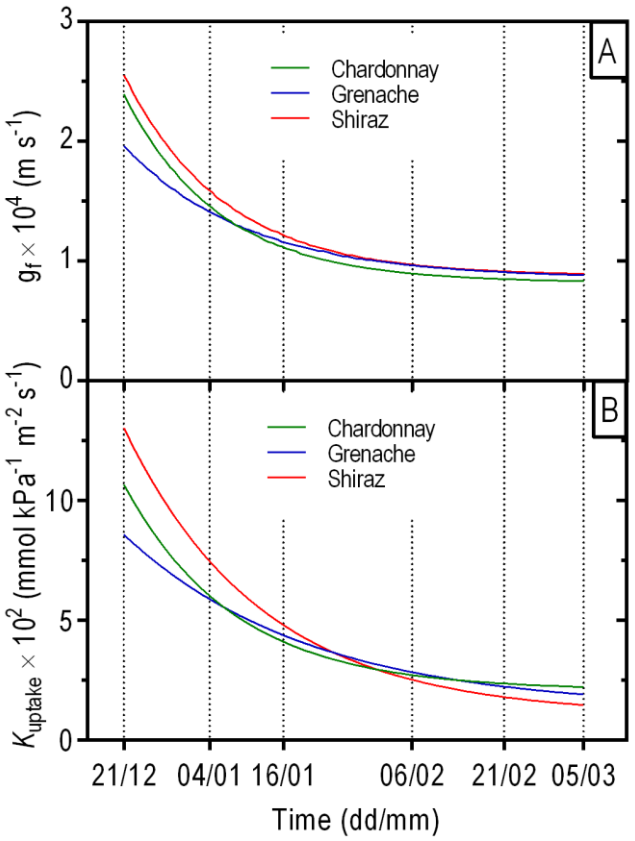

Figure 17. Varietal comparisons of grape berry surface conductances and xylem water uptake conductances. Comparison of the one phase exponential decay fitted grape berry surface conductance (A) and grape berry xylem water uptake conductance (B) between the *Vitis vinifera* L. cultivars Chardonnay (green solid line), Grenache (blue solid line), and Shiraz (red solid line) for ripening grape berries during the 2011-12 season. Flowering occurred on the 24th of October 2011 for Chardonnay, on the 28th of October 2011 for Grenache and on the 29th of October 2011 for Shiraz. Times when measurements have been carried out in the field are shown as dotted vertical lines. For details see Figure 16.

Changes in temperature, and relative humidity were monitored inside the canopy of the vineyard to calculate changes in vapour pressure deficit which influence berry transpiration on water uptake (Figure 18). The temperature (Figure 18 A) and relative humidity (Figure 18 B) showed an inverse correlated pattern as expected. At the end of December 2011, the second half of January 2012, and the second half of February 2012 were dominated by heat-waves causing spikes in vapour pressure deficit (Figure 18 C).

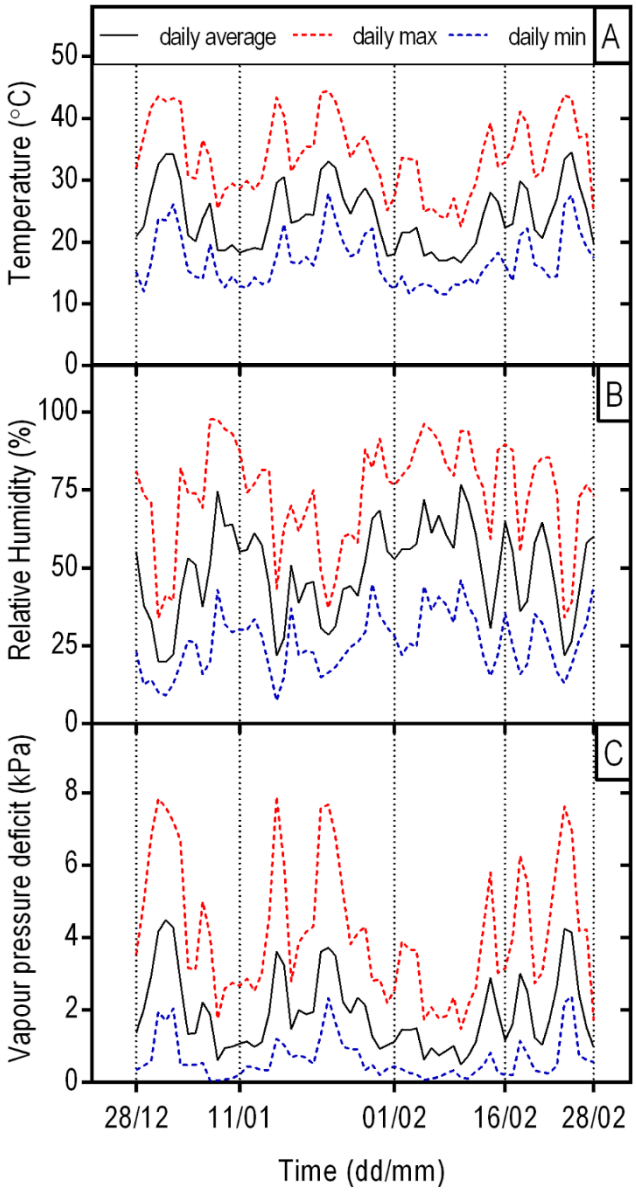

Figure 18. Climate records from the vineyard. Temperature (A), relative humidity (B), and vapour pressure deficit (C) inside the canopy of the vineyard at the University of Adelaide Waite Campus in the Adelaide region (South Australia) during the 2011/12 season. The daily average, daily max, and daily min all refer to a 24-hours period from midnight to midnight. Times when measurements have been carried out in the field are shown as dotted vertical lines.

Since water uptake via the xylem and water loss via transpiration were calculated from the results of the *in vitro* transpiration assay in Chapter 3, the water uptake via the phloem had to be estimated based on the sugar accumulation rate in the grape berries (Figure 19). In the simplified model berry weight change would be due to the sum of water uptake via the xylem and phloem minus water loss via transpiration, plus the change in dry matter due to sugar accumulation. For this model it was assumed that the sugar concentration in the phloem is constant over time. Therefore the phloem water uptake rate depends on the sugar accumulation rate. A range of estimated sugar

concentrations in the phloem from 0.5 molar to 1.5 molar sucrose were used to model the change in berry weight of Chardonnay, Grenache and Shiraz. The best fit between the estimated berry weight and the measured berry weight as a function of time was determined by calculating the residual sum of squares. In Chardonnay (Figure 19 A) the smallest residual sum of squares was achieved at 1 molar sucrose, in Grenache (Figure 19 B) at 0.8 molar sucrose, and in Shiraz (Figure 19 C) at 1.2 molar sucrose in the phloem sap. The modelled berry weight change always predicted a higher berry weight loss at the end of ripening.

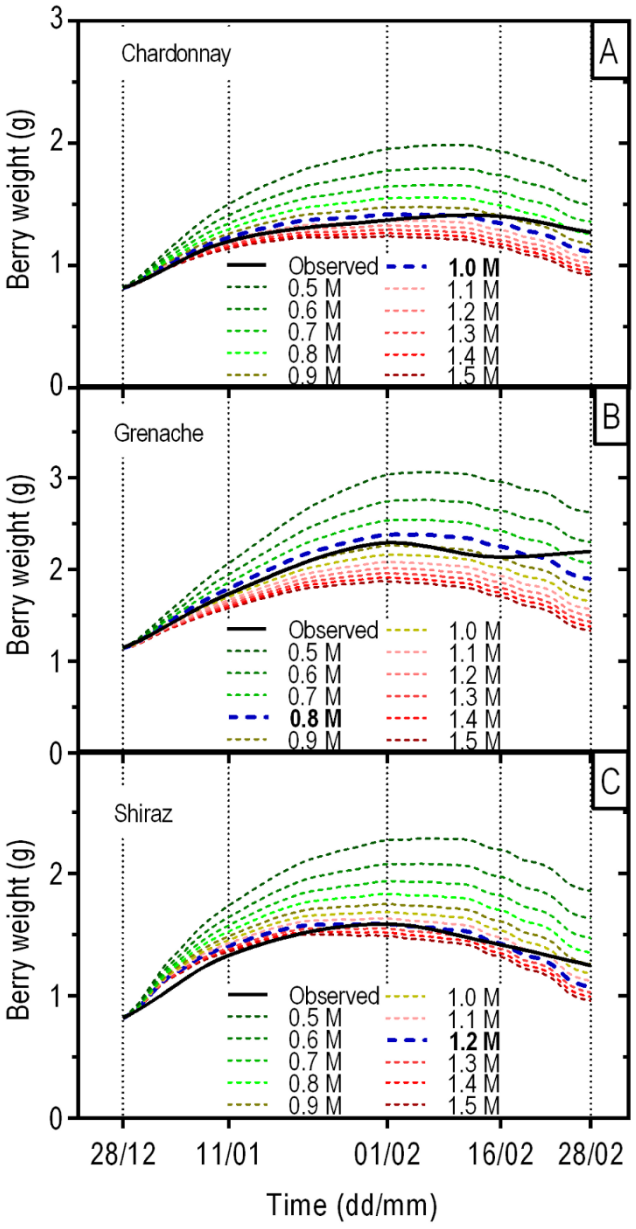

Figure 19. Modelled phloem sap sucrose concentrations. Measured (solid black line) and estimated (dashed lines) berry weight change by integration of Equation 14 from veraison till late harvest for grape berries of the *Vitis vinifera* L. cultivars Chardonnay (A), Grenache (B), and Shiraz (C) during the 2011-12 season. Berry weight change was estimated

as a function of xylem and phloem water uptake, sugar accumulation, and water loss via transpiration. Xylem water uptake and transpiration were calculated in relation to vapour pressure deficit, and phloem water uptake was estimated for a range of sucrose concentration in the phloem sap from 0.5 molar to 1.5 molar. The best fit (dashed blue line) between the observed berry weight change and the estimated change with the smallest residual sum of squares was at 1.0 M sucrose for Chardonnay, 0.8 M sucrose for Grenache, and 1.2 M sucrose for Shiraz. Times when measurements have been carried out in the field are shown as dotted vertical lines.

The flux rates for the phloem water uptake and the sum of xylem water uptake minus transpiration, and the net rate change of grape berry water content are shown in Figure 20 B-D. These fluxes were calculated both for a field climate that was measured inside the canopy of the vineyard, and for a modified climate where the heat-waves of the second half of February 2012 were replaced by the climate between the 6th till 13th February 2012. The vapour pressure deficit for the measured field climate and the modified climate are shown as solid orange line and a dashed orange line, respectively (Figure 20 A). The estimated flux rates in Chardonnay (Figure 20 B) were significantly smaller compared to Grenache and Shiraz (Figure 20 C, D). A negative phloem water uptake was estimated in Chardonnay for the last two weeks (Figure 20 B). In Grenache the phloem water uptake rate was two-fold higher compared to Shiraz and at the end of ripening berries of Grenache maintained a small phloem water uptake rate whereas the phloem water uptake rate in Shiraz tapered to almost zero. The net flux of xylem water uptake minus water loss due to transpiration was positive until around the 11th of January 2012 for all three varieties. Afterwards, transpiration exceeded xylem water uptake. The heat-waves between the 11th of January 2012 and the 1st of February 2012 did not influence the net flux of xylem water uptake minus transpiration significantly. However, the heat-waves during the second half of February caused a large imbalance between xylem water uptake and transpiration (black line). Using the modified climate without the heat-waves removed this imbalance (blue line). Until the second week of February, phloem water uptake balanced the net flux of xylem water uptake minus transpirational loss, but was too small at the end of ripening. Therefore, the rate of change of berry water became negative from the beginning of February (red line). The modified climate was able to remove the large loss of berry water at the end of the season (green line).

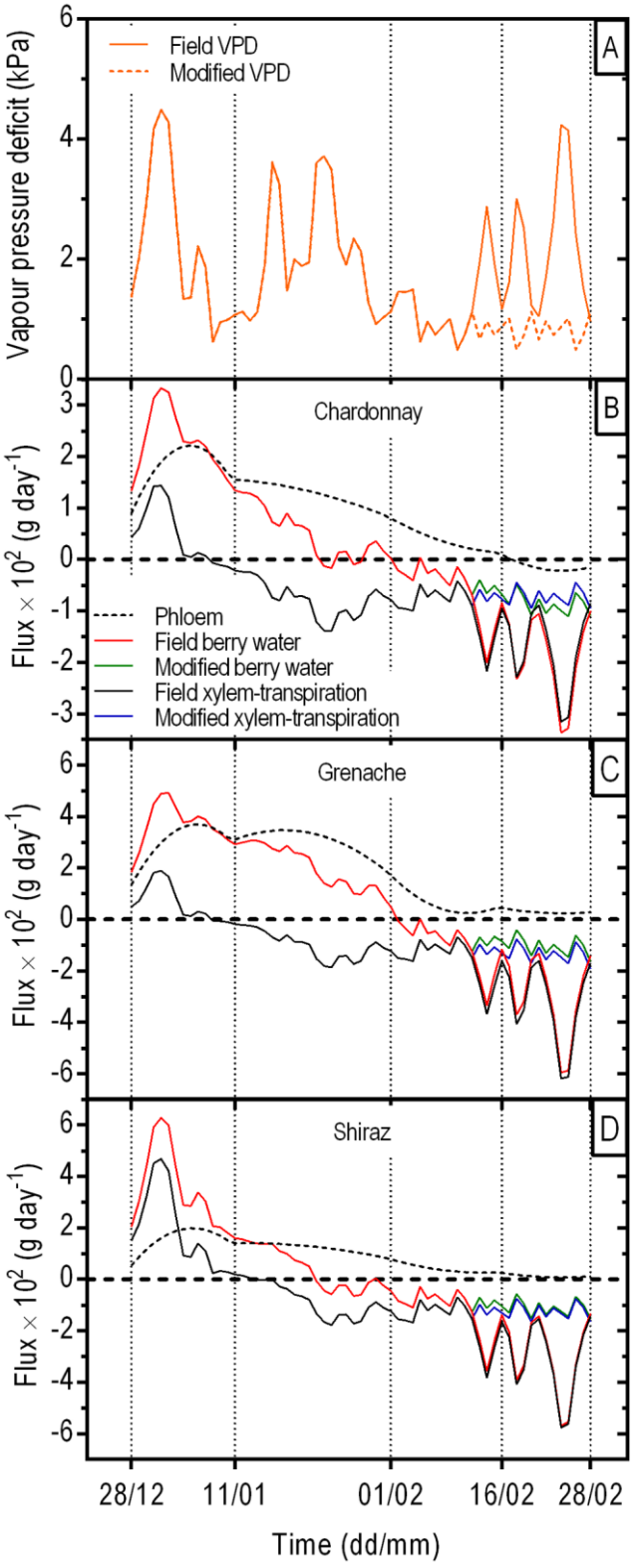

Figure 20. Comparison of the estimated water fluxes in and out of grape berries and grape berry water content change rate for the measured field climate and a modified climate (A) from veraison till late harvest for grape berries of the *Vitis vinifera* L. cultivars Chardonnay (B), Grenache (C), and Shiraz (D) during the 2011-12 season. Grape berry water relations were estimated for measured vapour pressure deficit (VPD) changes inside the canopy (solid orange line)

during the 2011/12 season and for a modified climate (dashed orange line) where heat-waves at the end of the end of the season (14/02/2012-28/02/2012) were replaced by the moderate climate from the 6th of February 2012 till the 13th of February 2012. Xylem water uptake rates and transpiration rates were calculated in relation to changes in VPD. Net xylem-transpiration water uptake rates were computed for the field climate (solid black line) and for the modified climate (solid blue line). Phloem water uptake rates (black dashed line) are shown as a function of measured sucrose accumulation rate in the berry over best fit estimated sucrose concentration in the phloem sap (see Figure 19: Chardonnay 1 M sucrose, Grenache 0.8 M sucrose, Shiraz 1.2 M sucrose). Grape berry water content change rates were calculated for the field climate (solid red line) and for the modified climate (solid green line) as a sum of xylem and phloem water uptake rate minus transpiration. Times when measurements have been carried out in the field are shown as dotted vertical lines.

When comparing the berry weight loss at the end of ripening between the model for the measured climate in the vineyard and the modified climate without the heat-waves, a large reduction in berry weight loss was observed (Figure 21). In Chardonnay (Figure 21 A) the difference was 11%, in Grenache (Figure 21 B) the difference was 12%, and in Shiraz (Figure 21 C) the difference was 19%. Therefore Shiraz benefited the most from the modified climate.

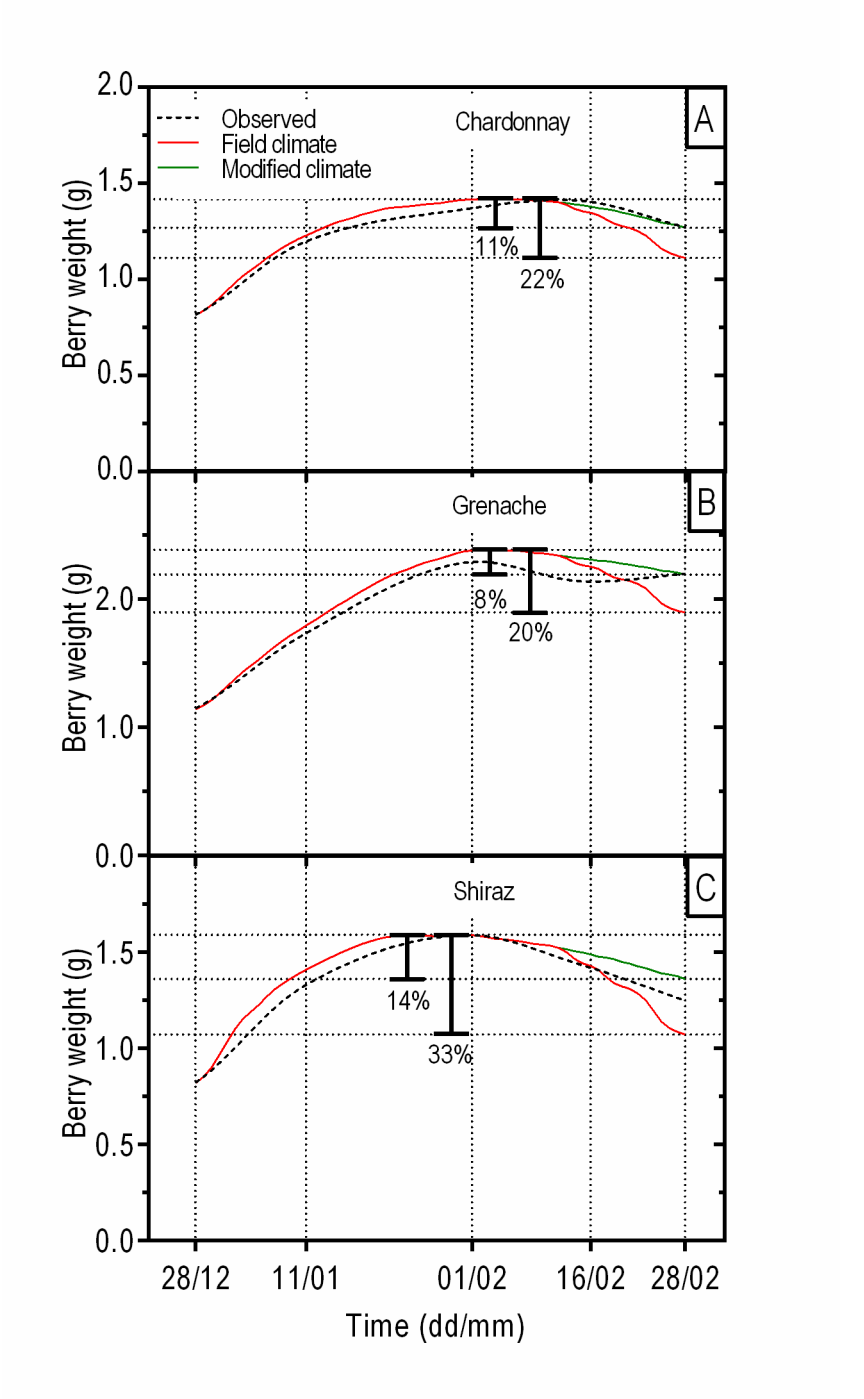

Figure 21. Berry weight loss estimated by the model at different climate conditions. Comparison of the estimated grape berry weight as a function of time for the measured field climate (solid red line) and a modified climate (solid green line) from veraison till late harvest for grape berries of the *Vitis vinifera* L. cultivars Chardonnay (A), Grenache (B), and Shiraz (C) during the 2011-12 season; see Figure 20 for the measured and modified climate. The actual measured berry weight as a function of time is shown as a black dashed line and the percentage of berry weight loss is written under the scale bars. Times when measurements have been carried out in the field are shown as dotted vertical lines.

4.4 Discussion

A high sensitivity of grape berry water relations heat-waves at the end of berry ripening was observed according to the estimated net flux rates in the model (Figure 20). This supports the hypothesis of McCarthy (1999), that a net water loss from berries at the end of ripening might be due to high evaporative conditions. A similar observation was also made by Clearwater et al. (2012) in kiwi fruit.

Just after veraison there was still more xylem inflow into berries than water loss via transpiration (Figure 20 B-D). When the net xylem-transpiration flux rate became negative, berry growth was sustained by water uptake via the phloem. In this model the phloem water uptake was estimated from the sugar accumulation profile during berry ripening. For this purpose a fixed sugar concentration was assumed and the required phloem sap concentration for sugar was calculated to gain enough phloem water uptake to balance transpiration and fruit growth together with the xylem water uptake. A constant sugar concentration in the phloem of grapevine was reported by Zhang et al. (2006), and a strong relation between sugar accumulation and transpiration was assumed by Dreier et al. (2000). The estimated sugar concentrations of 1 molar sucrose in Chardonnay, 0.8 molar sucrose in Grenache, and 1.2 molar sucrose in Shiraz in the phloem of berries (Figure 19) are significantly higher than the reported concentration of 0.05 molar reported by Zhang et al. (2006) for the grape variety Kyoho (*Vitis vinifera* × *Vitis labrusca* L.). However, a sucrose concentration of 0.05 molar seems to be very low in comparison to other reported concentrations in the literature, e.g. 0.25 molar in wheat (Hayashi & Chino, 1986), 0.3 molar in *Ricinus* (Hall & Baker, 1972), and even 0.9 molar in maize (Ohshima, Hayashi, & Chino, 1990). The high sucrose concentrations that were calculated from this model might be due to the assumption that there is no water recycling for sugar accumulation. Choat et al. (2009) suggested that phloem water recycling back to the parent plant may facilitate sugar accumulation at lower phloem sap concentrations. Moreover, xylem water uptake could be overestimated in this model, since it was calculated based on results from the *in vitro* transpiration assay, where a water potential of almost zero in the water reservoir did not reduce the xylem water uptake rate, as a negative water potential usually would do in a plant. This would lead to a lower demand of phloem water inflow in this model with the result of higher estimates for phloem sap concentrations. According to the model, the phloem water uptake contributes the most to the total balance in berries of Grenache, and the least in berries of Shiraz (Figure 20). In Chardonnay the model even predicts a negative phloem flow rate at the end of ripening (Figure 20 A)

which is due to the decrease in sucrose equivalent per berry (Figure 15). This negative flow will be rather an artefact of the model, but a decrease of sucrose equivalent per berry could be due to respiratory losses. Berry respiration was not included in this model since it is comparably small (Niimi & Torikata, 1979). At the end of ripening some phloem inflow still continued in Grenache, whereas the phloem inflow in Shiraz tapered almost to zero (Figure 20 B, C). Therefore, water loss via transpiration seems to be less balanced by phloem inflow in Shiraz compared to Grenache.

Changes in transpiration due to changes in vapour pressure deficit have to be mostly balanced by xylem water uptake when the phloem sap concentration is assumed to be constant. The reduction of xylem water uptake at veraison which causes a negative net xylem-transpiration flow rate has been reported in the literature before (Greenspan et al., 1994). Heat waves during the second half of January were balanced by xylem water uptake (Figure 20). However, significant spikes were observed in the net xylem-transpiration water flux during the heat-waves of the second half of February. Hence, the declining xylem water uptake was not able to balance transpiration at that stage. In combination with the declining phloem water uptake at the end of ripening this causes a net water loss from the berries of all three varieties in the model. These observations align with results from a study on water relations of kiwi fruit in two different climatic environments (Clearwater et al., 2012). A net water loss from kiwi fruits was observed in California at the end of ripening, whereas fruits of the same variety developed normally in New Zealand. The study revealed that the higher evaporative demand in California caused the observed net water loss. Therefore, these results support our findings. The net berry weight loss in the model was always larger than the berry weight loss observed for these varieties in the field (Figure 19). Therefore, the model either underestimates the xylem or phloem inflow, or overestimates the loss via transpiration. It is most likely that transpiration is overestimated since the complex architecture of bunches should cause a lower transpiration per berry compared to the measurements on single berries, particularly in the variety Chardonnay where berry-to-berry contact should result in a lower total transpiration due to a reduced surface area. Calculations of xylem water uptake should also be looked at with caution, since this model calculates a uptake conductance based on VPD, which means transpiration, as solely driving force for xylem water uptake. Indeed, xylem water uptake was shown to be sensitive to changes in VPD (Clearwater et al., 2012), but there is certainly not a linear link between them and osmotic gradients should also contribute as a gradient for xylem water uptake.

Interestingly, the modified climatic conditions at the end of berry development without the heat waves reduced the predicted berry weight loss significantly (Figure 20Figure 21). The largest reduction in

berry weight loss was observed in Shiraz (Figure 21 C). This might be due to the fact that in Shiraz xylem water uptake had a larger contribution to the berry water relations than in Chardonnay and Grenache.

In conclusion, xylem water uptake can balance spikes in transpiration due to heat waves during early stages of berry ripening. During late stages of berry ripening both xylem and phloem water uptake decline and therefore the berries become more vulnerable to water loss due to enhanced evaporative conditions. Especially in Shiraz, the relation between xylem water uptake and transpiration is very sensitive to heat events. These findings match with the results from a study on kiwi fruit water relations at low and high evaporative conditions (Clearwater et al., 2012). Moreover, the results show that xylem water uptake still has a major contribution to berry water uptake after veraison which questions the conclusions of studies that found a decline in the contribution of xylem to berry water status after veraison (Creasy & Lombard, 1993; Greenspan et al., 1996; Greenspan et al., 1994). The main weaknesses of this model are that phloem flow could not be measured directly as well as the phloem sap concentration. Moreover, xylem water uptake might have been overestimated as well as transpiration in the field since experiments were performed on excised berries. Weight changes due to respiration were also not included in this model. To improve this model that is based on many assumptions such as a constant phloem sugar concentration during grape berry development, a direct measurement of phloem sap concentration and fluxes via the xylem and phloem would be required. Measurement of transpiration and respiration on intact grape bunches in the vineyard would improve the understanding of the effect of grape bunch architecture on transpiration.

Chapter 5 – General Discussion and Conclusions

5.1 Introduction

The aim of this research was to investigate the water relations of grape berries from different varieties in relation to pre-harvest berry dehydration. Pre-harvest berry dehydration occurs due to a net water loss from the berries in some grape varieties at the end of the season and is also often referred to as berry shrivel in the literature (Krasnow et al., 2010). Australia's most popular red grape variety Shiraz is very susceptible to this disorder (McCarthy, 1999). Much research has already been done on water relations of grape berries during grape berry development, but so far no firm conclusions can be drawn about why berries of the variety Shiraz are more susceptible than berries of other varieties.

It is assumed, that xylem water uptake is predominant in pre-veraison grape berries, whereas phloem provides the bulk water after veraison to facilitate sugar accumulation (Greenspan et al., 1994; Lang & Thorpe, 1989). Dye studies suggested that the xylem becomes non-functional in post-veraison grape berries (Creasy et al., 1993; During et al., 1987), but later studies found that the xylem remains functional in post-veraison berries (Bondada et al., 2005; S. Rogiers et al., 2000; S. Y. Rogiers et al., 2001). It was also hypothesised that a decline in phloem inflow at the end of ripening could cause the net water loss observed in berries of Shiraz (McCarthy & Coombe, 1999). Studies on xylem and phloem inflow, and water loss via transpiration suggested that the water loss is due to an imbalance between water uptake and water loss (Greer & Rogiers, 2009; S. Y. Rogiers et al., 2004). However, a detailed study on the contribution of each of the components to the net water balance of grape berries and comparison of varieties that differ in weight loss late in ripening has not been done.

Studies on hydraulic properties of grape bunches and berries have found a general increase in hydraulic resistance in particular in the berries during the course of ripening (Choat et al., 2009; Tilbrook & Tyerman, 2009; Tyerman et al., 2004). It was suggested that a net water loss from grape berries at the end of ripening could be also due to water backflow from the berries to the parent vine via the xylem (Tilbrook & Tyerman, 2009). A higher hydraulic resistance was measured in Chardonnay compared to Shiraz. Therefore, it was hypothesised that an increase in resistance could

prevent water loss from the berries. In cases like Sultana which has a low resistance, higher cell vitality at the end of ripening could generate a water potential gradient which helps to withstand the negative water potential in the plant under conditions with high transpiration (Tilbrook & Tyerman, 2008, 2009). A general study on cell vitality of grape berries from 22 different varieties found a correlation between cell death and pre-harvest berry dehydration (Fuentes et al., 2010).

To test these hypotheses on different varieties a range of experiments were performed in this study. The hydraulic properties of grape bunches from the varieties Chardonnay, Grenache, and Shiraz were investigated down to the individual parts of grape bunches. Berries from the same varieties plus berries from the varieties Chenin Blanc and Sultana were studied in an *in vitro* transpiration assay to measure xylem water uptake and transpiration. Finally, a model was created to integrate phloem water uptake and compare the different varieties.

5.2 Discussion

Measuring the hydraulic conductance of grape bunches during the course of development showed that not all varieties decreased hydraulic conductance over time as found for Chardonnay and Shiraz (Choat et al., 2009; Tyerman et al., 2004). Grape bunches of the variety Grenache adapted their hydraulic properties according to berry growth (Figure 6). This falls in line with previous observations that Grenache is a more conservative water user than Chardonnay and Shiraz and with a larger leaf area specific hydraulic conductivity (Schultz, 2003). Indeed, grape bunches of Grenache had a higher hydraulic conductivity compared to Chardonnay and Shiraz (Figure 8 A). Interestingly, berries of Grenache did not show any higher water uptake via the xylem in the transpiration assay compared to Chardonnay (Figure 12) and nor were they more prone to berry shrinkage. Therefore, the driving force for water uptake must have been lower in Grenache. This could be due to a loss of intact cell membranes that can generate a negative water potential gradient. A study on cell vitality in ripe berries of different varieties showed that berries of Grenache had indeed low cell vitality. At the same time, an inflow of water at zero pressure into post-veraison bunches of Grenache suggest that there must have been a large enough negative water gradient in combination with high hydraulic conductivity to cause water uptake (Figure 9). Berries of the variety Sultana, which has a high hydraulic conductivity (Tilbrook & Tyerman, 2009) also only showed a higher water uptake before veraison (Figure 12). After veraison, water uptake via the xylem and transpiration dropped below the other varieties which were used in the transpiration assay. Therefore, high hydraulic conductivity is not necessarily coupled to higher water uptake. When comparing the balance between water uptake

via the xylem and water loss via transpiration, an imbalance was observed in berries of Shiraz (Figure 13). Especially in comparison with berries of Chardonnay, berries of Shiraz had a substantial higher net water loss. The model on grape berry water relations showed finally that due to this imbalance, berries of Shiraz are much more susceptible to heat wave events than berries of Chardonnay and Grenache (Figure 20Figure 21). Running the model on a modified climate without heat waves at the end of ripening caused a significant reduction in the estimated berry weight loss (Figure 21). This sensitivity only occurs later in ripening as a consequence of diminished phloem inflow. These results align with results on kiwi fruit water relations by Clearwater et al. (2012). They found that a net water loss from kiwi fruits before harvest in California compared to a normal development in New Zealand is caused by a larger vapour pressure deficit due to a different climate in California.

The results from the study on grape bunch hydraulics show no significant difference between the hydraulic conductance or hydraulic resistance between Chardonnay and Shiraz (Figure 7Figure 8). At the end of ripening, there was an increase of hydraulic resistance in Chardonnay, but due to high variability this resistance was not significantly higher compared to Shiraz. Therefore, the hypothesis that a higher hydraulic resistance in Chardonnay prevents backflow is not supported by the results reported here (Tilbrook & Tyerman, 2009).

5.3 Conclusions and Future Directions

This study shows once more that there are large differences between varieties which should not be neglected when investigating water relations of grapes. In particular the very much different behaviour of Grenache leads to new questions. Apparently, the high hydraulic conductivity balances the loss in cell vitality to maintain the ability for water uptake in post-veraison grape berries. However, this would also make these berries highly susceptible to water backflow. Possibly, the parent plant itself in Grenache prevents the occurrence of very negative leaf water potentials due to its isohydric behaviour. In future research this question should be addressed and also a study on structural changes in the rachis and pedicel of grape bunches would give new knowledge on how Grenache adapts its hydraulic conductance. Research on the expression of aquaporins in different parts of the grape bunch could give more information about the cause of changes in hydraulic conductance. The different behaviour in hydraulic properties between Chardonnay and Shiraz should be tested again since this study does not suggest large differences.

Apparently, transpiration of grape berries in different varieties is very similar. A higher net water loss

from berries of Shiraz in the transpiration assay was rather due to a lower water uptake via xylem compared to the other varieties. This resulted in a higher susceptibility of Shiraz to heat waves as demonstrated in the model on grape berry water relations. To improve the model and include more parameters, *in vivo* measurements of xylem water uptake and transpiration are required along with information on sugar concentration in the phloem sap to allow prediction of phloem flux rates.

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