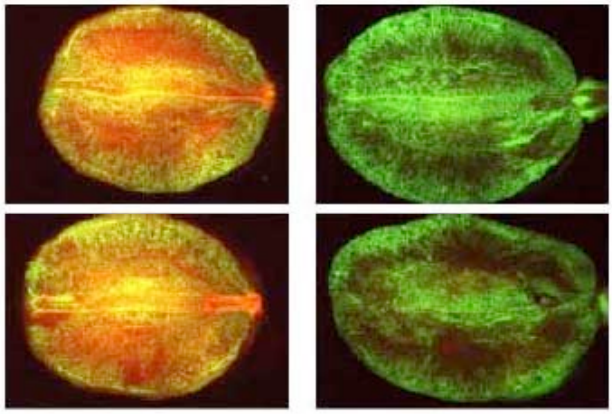
Characterising weight loss in *Vitis vinifera* Shiraz berries at sub-optimal maturity



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Contents

Appendices

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

Tilbrook J, Tyerman SD (2006) Water, sugar and acid: how and where they come and go during berry ripening. *In* Australian Society of Viticulture and Oenology: Finishing the job-optimal ripening of Cabernet Sauvignon and Shiraz. pp 4-12 Openbook Australia.

Tilbrook J, Tyerman SD (2008) Cell death in grape berries: varietal differences linked to xylem pressure and berry weight loss. *Functional Plant Biology* 35, 173-184.

Tilbrook J, Tyerman SD (2009) Hydraulic connection of grape berries to the vine: varietal differences in water conductance into and out of berries, and potential for backflow. *Functional Plant Biology* 36, 541-550.

Abstract

Post-veraison and prior to reaching harvest maturity, *Vitis vinifera* cv Shiraz berries lose weight where other varieties such as Chardonnay and Thompson Seedless do not. The berry weight loss occurs in the later stages of ripening from 90-100 days after anthesis. This defines a third phase of development in addition to berry formation and berry expansion. Berry weight loss is due to net water loss, but the component water flows through different pathways have remained obscure.

A method of direct measurement was developed using a pressure probe to measure the pedicel xylem hydraulic conductance of single detached berries through development. The probe measured the pressure developed in the xylem of non-transpiring berries. Pre-veraison, negative xylem pressures of -0.2 to -0.1 MPa were measured, increasing to around zero between veraison and 90 days after anthesis. The pressures around zero were maintained until harvest when the berry juice osmotic potential was around -3 MPa for Chardonnay and -4 MPa for Shiraz. Since cell turgor is low in the berry, this indicates that the juice osmotic potential is not translated into negative xylem pressure. It may suggest that the reflection coefficient of cell membranes surrounding the berry xylem in both varieties changes from close to 1 pre-veraison, to about 0.1-0.2 at veraison and decreases to 0 at harvest.

Both varieties showed a ten-fold reduction in hydraulic conductance from veraison to full ripeness. Shiraz had conductances that were two to five fold larger than Chardonnay, and maintained higher conductance from 90 days after anthesis, the period where berry weight loss occurred. In both varieties the hydraulic conductance reduced in the distal and proximal portions of the berries from veraison.

Focusing on xylem hydraulic conductance into and out of berries from 105 days after anthesis and during berry weight loss in Shiraz, significant varietal differences in xylem hydraulic conductance were found. Both varieties showed flow rectification such that conductance for inflow was higher than conductance for outflow. For flow in to the berry, Chardonnay had 14% of the conductance of Shiraz. For flow out of the berry Chardonnay was 4% of the conductance of Shiraz. From conductance measurements for outflow from the berry and stem water potential measurements, it was calculated that Shiraz could lose about 7% of berry volume per day, consistent with rates of berry weight loss.

Using a XYL'EM™ flowmeter, flow rates of water under a constant pressure into berries on detached bunches of these varieties are similar until 90-100 days after anthesis. Shiraz berries then maintain constant flow rates until harvest maturity while Chardonnay inflow tapers to almost zero. Thompson Seedless maintains high xylem inflows. These data are consistent with single berry measurements with the pressure probe.

A functional pathway for backflow from the berries to the vine via the xylem was visualised with Lucifer Yellow CH loaded at the cut stylar end of berries on potted vines. Transport of the dye out of the berry xylem ceased prior to 97 days after anthesis in Chardonnay, but was still transported into the torus and pedicel xylem of Shiraz at 118 days after anthesis. Xylem backflow could be responsible for a portion of the post-veraison weight loss in Shiraz berries. These data provide evidence of varietal differences in hydraulic connection of berries to the vine that we relate to cell vitality in the mesocarp. The key determinates of berry water relations appear to be maintenance or otherwise of semi permeable membranes in the mesocarp cells and control of flow to the xylem to give variable hydraulic connection back to the vine.

Because of the very negative osmotic potential of the cell sap, the maintenance of semipermeable membranes in the berry is required for the berry to counter xylem and apoplast tensions that may be transferred from the vine. The transfer of tension is determined by the hydraulic connection through the xylem from the berry to the vine, which changes during development. We assess the membrane integrity of the three varieties, Shiraz, Chardonnay and Thompson Seedless throughout development using the vitality stains, fluorescein diacetate and propidium iodide, on fresh longitudinal sections of whole berries. The wine grapes, Chardonnay and Shiraz, maintained fully vital cells after veraison and during berry expansion, but began to show cell death in the mesocarp and endocarp at or near the time that the berries attain maximum weight. This corresponded to a change in rate of accumulation of solutes in the berry and the beginning of weight loss in Shiraz, but not in Chardonnay. Continuous decline in mesocarp and endocarp cell vitality occurred for both varieties until normal harvest dates. Shiraz grapes classified as high quality and sourced from a different vineyard also showed the same death response at the same time after anthesis, but they displayed amore consistent pattern of pericarp cell death. The table grape, Thompson Seedless, showed near to 100% vitality for all cells throughout development and well past normal harvest date, except for berries with noticeable berry collapse that were treated with gibberellic acid. The high cell vitality in Thompson Seedless berries corresponded to negative xylem pressures that contrasted to the slightly positive pressures for Shiraz and Chardonnay. I hypothesise that two variety dependent strategies exist for grapevine berries late in development: (1) programmed cell death in the pericarp and loss of osmotically competent membranes that requires concomitant reduction in the hydraulic conductance via the xylem to the vine; (2) continued cell vitality and osmotically competent membranes that can allow high hydraulic conductance to the vine.

Weight loss in Shiraz berries before harvest maturity for winemaking has, to date, not been manipulable by viticultural practices such as irrigation. This work shows that foliar application of molybdenum to Shiraz vines changed the time course of berry weight accumulation regardless of the timing of the application in two vineyards over two seasons. Molybdenum treatment delayed the transition of berries from *phase* 2 (berry weight accumulation) to *phase* 3 (weight loss) of development for 2 to 7 days. It also slowed sugar accumulation relative to berry weight accumulation in *phase* 2. Allometric analysis of abscisic acid content of berries relative to weight accumulation in *phase* 2 and *phase* 3 showed no significant differences. Fruit yields from molybdenum treated and control vines were not significantly different when harvested at the same ºBrix rather than the same day after anthesis. Pruning weights of treated vines were significantly higher than control vines, suggesting increased vigour related to increased availability of the molybdoenzyme nitrate reductase, and therefore increased potential to reduce nitrate for assimilation. Wine made from fruit of treated vines contained five times higher molybdenum than wines made from control fruit, but were still at levels safe for human consumption. Sensory analysis of wines made from molybdenum treated and control fruit indicate that organoleptic differences may be perceived in the wines because of molybdenum treatment.

In summary, significant varietal differences were found in how berries isolate from the vine, with strong evidence that weight loss from Shiraz berries is caused by xylem backflow to the vine, perhaps associated with changes in aquaporin or cell membrane function in xylem associated tissue. Differences were also found in cell vitality and membrane competence across the endocarp and mesocarp of berries through development, with distinct varietal differences between the wine varieties Shiraz and Chardonnay, and the table grape Thompson Seedless. The kinetics of berry weight accumulation in Shiraz is altered by the foliar application

of molybdenum to vines at anthesis and capfall, but molybdenum may affect the organoleptic qualities of wine made from the fruit.

Thesis declaration

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 contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. The author acknowledges that copyright of the published works contained within this thesis (as listed below) resides with the copyright holders of those works.

The author was unable to work over the 2003-2004 season due to illness, however, using the author's method, hydraulic data was collected by Lukasz Kotula, a student visiting from the Department of Plant Ecology, University of Bayreuth. This data is clearly identified.

Professor Steve Tyerman provided expert and technical advice when required, and editorial advice on drafts of papers and thesis.

This thesis contains published work:

Chapter 2:

This chapter is the original work of the author. The data presented forms part of a published paper:

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

Chapter 3:

Awarded "Best paper of 2008 by an early career researcher" by the Australian Society of Plant Scientists and Functional Plant Biology, July 2009.

Tilbrook J and Tyerman SD (2008) Cell death in grape berries: varietal differences linked to xylem pressure and berry weight loss. *Functional Plant Biology* 35, 173-184.

Chapter 4:

Tilbrook J and Tyerman SD (2009) Hydraulic connection of grape berries to the vine: varietal differences in water conductance into and out of berries, and potential for backflow. *Functional Plant Biology* 36, 541-550.

Chapter 6:

The summary figure is modified from peer reviewed Proceedings.

Tilbrook J and Tyerman SD (2006) Water, sugar and acid: how and where they come and go during berry ripening. In *Australian Society of Viticulture and Oenology: Finishing the job-optimal ripening of Cabernet Sauvignon and Shiraz* pp 4-12, Openbook Australia.

Other Publications resulting from this work or published during candidature:

Accepted for publication:

This paper is the result of experiments planned by the author to explore varietal differences in loss of cell vitality during berry development.

Fuentes S, Sullivan W, **Tilbrook J**, Tyerman S (manuscript accepted November 2009) A novel analysis of grapevine berry tissue vitality and morphology demonstrates a variety dependent correlation between tissue vitality and berry shrivel. *Australian Journal of Grape and Wine Research*.

Published during PhD candidature:

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

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