



RESEARCH PAPER

Functional xylem in the post-veraison grape berry

Bhaskar R. Bondada*, Mark A. Matthews and Kenneth A. Shackel†

University of California, Department of Viticulture and Enology, One Shields Avenue, Davis, CA 95616, USA

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Abstract

A number of studies have shown a transition from a primarily xylem to a primarily phloem flow of water as fleshy fruits develop, and the current hypothesis to explain this transition, particularly in grape (*Vitis vinifera* L.) berries, is that the vascular tissue (tracheids) become non-functional as a result of post-veraison berry growth. In most studies, pedicels have been dipped in a vial containing an apoplastic dye, which was taken up into the entire peripheral and axial xylem vasculature of pre-veraison, but not post-veraison berries. The pressure plate/pressure membrane apparatus that is commonly used to study soil moisture characteristics was adapted and the pre- to post-veraison change in xylem functionality in grape berries was re-evaluated by establishing a hydrostatic (tension) gradient between the pedicel and a cut surface at the stylar end of the berry. Under the influence of this applied hydrostatic gradient, movement of the apoplastic tracer dye, basic fuchsin, was found in the pedicel and throughout the axial and peripheral xylem of the berry mesocarp. A similar movement of dye could be obtained by simply adjoining the stylar cut surface to a dry, hydrophilic wicking material. Since both pre- and post-veraison berries hydrate when the pedicel is dipped in water, it is hypothesized that the absence of dye movement into the vasculature of post-veraison berries indicates not a loss of xylem function, but rather the loss of an appropriate driving force (hydrostatic gradient) in the berry apoplast. Based on this hypothesis, and the substantial decrease in xylem flows that occur in intact grape berries at veraison, it is suggested that there may be significant changes in the pattern of solute partitioning between the fruit symplast and apoplast at veraison. It is further suggested that diurnal patterns in symplast/apoplast solute partitioning in grapes and other fleshy fruit, may explain the observed

minimal xylem contribution to the water budgets of these fruits.

Key words: Apoplast, hydrostatic gradient, symplast, vascular transport.

Introduction

The water required for enlargement and transpiration of most shoot organs generally enters the organ via the xylem. In fleshy fruits, both xylem and phloem participate in delivering water, depending upon the developmental stage of the fruit. In some young fruit (e.g. in platyopuntias, Nobel and De la Barrera, 2000) the phloem is the dominant source, and in other fruit such as the tomato (Ho *et al.*, 1987), apple (Lang and Ryan, 1994; Drazeta *et al.*, 2004), kiwifruit (Dichio *et al.*, 2003), and grape (Greenspan *et al.*, 1994), there is a clear reduction in the proportion of water entering the fruit via the xylem as the fruit matures. Growth of many fleshy fruit, including *Prunus* sp. and *Vitis* sp., exhibit a double sigmoid pattern (Coombe, 1976) in which there are two periods of growth separated by a lag phase, and in grape (*Vitis vinifera* L.) the transition from the lag phase (Stage II) to the second growth phase (Stage III) is rapid, occurring over 1 or 2 d (Coombe and Bishop, 1980; Matthews *et al.*, 1987). The rapid transition from a significant to a minimal xylem contribution to fruit water balance at the beginning of Stage III in grape makes this fruit particularly useful for testing hypotheses about the physiological interaction of phloem and xylem flows during the development of fleshy fruits, since anatomical changes would presumably require more than 1 or 2 d to be of significance. The regulation of the resumption of growth in Stage III is not well understood, although it is clear that the onset of Stage III coincides approximately with increases in sugar import (Coombe and Bishop, 1980) and in plastic extensibility of the berry dermal tissue (Matthews *et al.*, 1987).

* Present address: Washington State University Tri-Cities, 2710 University Drive, Richland, WA 99354, USA.

† To whom correspondence should be addressed. Fax: +1 530 752 8502. E-mail: kashackel@ucdavis.edu

Coombe and McCarthy (2000) presented a generalized model of vascular function during growth and maturation of grape berries. According to this model, both xylem and phloem are functional during Stages I and II, and both contribute to the water requirements of the fruit. However, upon the transition to Stage III (called veraison), the xylem becomes non-functional as the result of a decline in its hydraulic conductance. Post-veraison growth and ripening are described as entirely dependent upon resources transported from vine to berry via the phloem. The evidence of xylem dysfunction comes primarily from microscopic examination and dye uptake studies with a number of grape varieties, namely; Muscat Gordo Blanco (Findlay *et al.*, 1987), Riesling (Düring *et al.*, 1987), Pinot noir (Creasy *et al.*, 1993), and Merlot (Creasy *et al.*, 1993). When the cut pedicel of excised berries was dipped in dye, dye uptake extended into the peripheral and axial xylem system of the entire berry before veraison, but was limited to the basal few millimetres after veraison (Findlay *et al.*, 1987; Creasy *et al.*, 1993; Rogiers *et al.*, 2001). Düring *et al.* (1987) also visually evaluated dye uptake by excised Riesling berries that were fed with dye through the rachis and found a marked reduction around the time of veraison. These observations, and evidence from photomicrographs of stretching or gaps in the helical thickenings of berry tracheids (Düring *et al.*, 1987; Findlay *et al.*, 1987) have been interpreted to indicate that the lignified xylem cells are unable to increase in length during the second phase of berry growth, and hence that growth itself causes a disruption in xylem continuity (Lang and Düring, 1991; Coombe and McCarthy, 2000). This implies that if post-veraison berry growth could be physically restricted while allowing other aspects of berry development to continue (softening, colour change, etc), the disruption in xylem continuity should also be prevented. Düring *et al.* (1987), however, also reported that the appearance of gaps preceded the reduction in dye uptake by about 12 d, and hence suggested that an intervening wound response may actually be the cause of the apparent xylem disruption.

Estimates for net flows of water entering through xylem and phloem tissues have been made based on measurements of the diurnal volume changes in intact, phloem-girdled, and excised berries, and these estimates indicate a rapid change from predominantly xylem to predominantly phloem water influx near veraison (Greenspan *et al.*, 1994, 1996) and a predominantly phloem flow after veraison (Lang and Thorpe, 1989; Rogiers *et al.*, 2001). This evidence indicates that the use of the xylem pathway is substantially reduced, but does not necessarily imply that the reduction is imposed by a physical blockage or other direct reduction in xylem hydraulic conductivity. Tyerman *et al.* (2004) have recently reported direct measurements of hydraulic conductivity along the pedicel-to-berry pathway in Chardonnay and Shiraz varieties, and for both of these varieties, a progressive decline in whole berry conductivity

was found between 65 and 95 d post-flowering (from about 10–20 °Brix). The hydraulic method used in this study was able to determine that the loss in conductivity did not occur in the fruit pedicel or in the pedicel/fruit junction, but was not able to determine whether the loss was associated with a change in the xylem or apoplast pathway per se, or in the apoplast to symplast pathway within the fruit flesh. Tyerman *et al.* (2004) referred to the overall pathway as a ‘xylem/composite membrane’ pathway, and suggested that the observed reduction in conductance may be a combination of xylem restriction and reduced aquaporin presence or activity. Hence, the hypothesis of a physical disruption in xylem continuity at veraison (Lang and Düring, 1991; Coombe and McCarthy, 2000) remains untested. Presumably water moves in the xylem in response to a gradient in hydrostatic pressure, which, because it is caused by tension (negative hydrostatic pressure) in the apoplast, is often referred to as a ‘matric’ gradient (but see Passioura, 1980, for a thorough discussion of this term). Hence, as an alternative to the physical disruption hypothesis, it is possible that the reduction in xylem water flow at veraison is associated with a reduction in the hydrostatic gradient, either between the fruit and the shoot, or more locally between the xylem in the pedicel and the xylem in the fruit flesh. The objective of this study was to evaluate the functionality of xylem in post-veraison berries by observing patterns of apoplastic dye movement that were specifically driven by hydrostatic gradients applied to the fruit apoplast.

Materials and methods

Plant material

Fruit was obtained for dye uptake experiments before and after the onset of fruit ripening (veraison) from mature, field-grown vines of six varieties of grape (*Vitis vinifera* L.) (Chardonnay, Cabernet Sauvignon, Pinot noir, Shiraz, Muscat Gordo, and Grey Riesling) in the Davis vineyard of UC Davis, CA. Entire, representative clusters of each variety were collected before 09.00 h, placed in a plastic bag, and transported to the laboratory within 15 min. Experimental berries were chosen with uniform shape and colour and free of blemishes. For berry hydration experiments and some dye uptake studies, greenhouse-grown Chardonnay berries were used, and for the growth restriction experiment, field-grown Zinfandel was used. The developmental age of berries was determined as the °Brix of the whole berry juice, measured with a refractometer. The transition to ripening (i.e., veraison) occurs at about 7 °Brix.

Dye uptake studies

The ability of the berry xylem tissue to conduct water was evaluated using three dye uptake methods: (i) passive dye infusion, (ii) a pressure membrane method, and (iii) a simple wicking system. For passive dye infusion, the berries were excised from the rachis, and the pedicel of an intact berry was trimmed and immediately dipped in a small reservoir of apoplastic dye solution (basic fuchsin 0.1% aqueous; Talbot, 1955). In most cases, infusion through the cut pedicel was allowed to occur for 5–6 h under laboratory conditions, but for some studies longer times were used, and in some cases the infusion was performed in a humidified chamber maintained at 100%

RH. Except for the environmental conditions of 100% RH, the infusion method was essentially the same as that used by other authors (Findlay *et al.*, 1987; Creasy *et al.*, 1993; Rogiers *et al.*, 2001). Berries were then either cross-sectioned at the proximal (pedicel) and distal (stylar) end, or were sectioned longitudinally through the centre, and the pattern and extent of xylem staining was observed with bright field-optics in a Zeiss microscope (model-SV11).

In the pressure membrane method, a negative hydrostatic pressure potential was used to extract water from the apoplast of grape mesocarp tissue (Fig. 1). The apparatus was constructed with high-pressure brass pipe fittings, and a hydrophilic cellulose ester membrane (0.05 μm pore size, air entry pressure 2.4 MPa, MWP02500, Millipore, Billerica, MA) supported by a screen made of stainless steel and a custom-fabricated support and outlet. The internal dimensions of the cylindrical pressure vessel were 25 mm in diameter and 20 mm in height, with wet filter paper inside the vessel to maintain it at 100% RH. Based on the support screen size and the length and inner diameter of the outlet, the estimated total volume on the atmospheric pressure side of the membrane ('dead volume') was 50 μl . The theory of operation of the pressure membrane apparatus (also known as a 'pressure plate' apparatus) is described in many soil physics texts (e.g. Or and Wraith, 2002). Briefly, it is assumed that there is a single continuous liquid phase (water or solution) throughout the pores of the membrane (solid phase) and the pores of the sample (tissue or soil) solid phase that are in contact with the membrane (Fig. 1). In the case of plant tissue, the solid phase corresponds to the apoplastic space. The surface tension of the water filling the pores of the membrane is sufficient to hold a pressure differential across the membrane of any value less than or equal to

the air entry pressure (also known as the 'bubble pressure'). When a pressure differential (ΔP , MPa) within this range is established across the membrane, water withdraws from the upper surface of the membrane, establishing a negative hydrostatic potential, typically called a 'matric potential' (but see Passioura, 1980) on the membrane surface, with a radius of curvature R (μm) as described in equation 1:

$$R = 0.146 \Delta P \quad (1)$$

The pressure used in this study was 0.8 MPa. Because both the upper surface of the membrane and the entire tissue are under the same applied pressure, it is only the hydrostatic gradient between the membrane upper surface and the tissue apoplast in contact with it, which will cause water movement from the tissue through the membrane to the outlet. Hence, when pressure is applied to the system, the only change that should occur within the system is that the membrane surface will become physically drier, with no other stresses or strains occurring on the berry tissue. Of course, water will only move if the negative hydrostatic potential established by the applied pressure is lower than the initial negative hydrostatic potential (matric potential) of the tissue apoplast. In the absence of an external supply of water, the apoplastic water that is removed from the tissue will be replaced by symplastic water, until the tissue is dehydrated and hydrostatic equilibrium has been reached between the tissue apoplast and the membrane. At this point, the total potential of the symplast will be equivalent to the sum of the hydrostatic and the solute potential (if solutes are present) of the apoplast. If the tissue apoplast is in contact with a supply of water, then the water will flow from the supply through the apoplast to the membrane along the

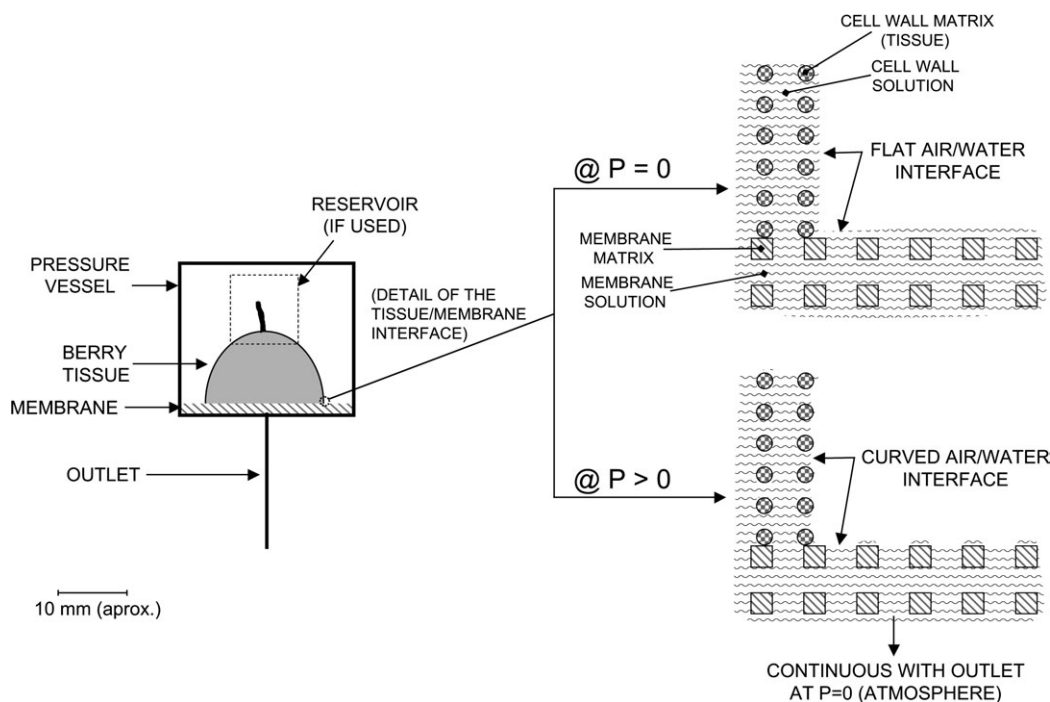


Fig. 1. Diagram of the pressure membrane apparatus (approximate scale), and a schematic depiction of the interface between the solid phases of the tissue (cell wall matrix) and of the membrane. Both the tissue cell wall matrix and membrane matrix are assumed to be hydrated, but in the absence of a supply of water to the tissue. When the pressure in the vessel is at atmospheric ($P=0$) the air/water interface on both sides of the membrane has no curvature (equal pressure on both sides of the interface). When the pressure in the vessel is raised ($P>0$) a curved air/water interface is established on the upper surface of the membrane, and water will move from the tissue to the membrane, and ultimately to the outlet, under the influence of the hydrostatic potential difference (also known as a matric potential difference). At equilibrium, the hydrostatic (matric) potential of the tissue and the membrane will be equivalent. The relative humidity within the vessel was maintained at 100% by lining the sides of the vessel with wet filter paper (not shown).

hydrostatic gradient. In this respect, the pulling of water from the supply through the tissue apoplast by the membrane surface is equivalent to the pulling of water through the tissue by a dry wick or blotter. One advantage of the pressure membrane approach over the use of a dry wick is that a known value of hydrostatic potential can be established and maintained at the membrane surface, compared with the surface of a wick, which would progressively wet and hence increase (become less negative) in hydrostatic potential. For the purpose of following dye movement, the pressure membrane approach also has the advantage that when the dye solution leaves the tissue it flows away from the membrane surface toward the outlet, rather than diffusing laterally, as would occur within a wick material. A potential disadvantage of the pressure membrane approach is that the plant tissue is exposed to a relatively high pressure for a prolonged period.

To prepare the pressure membrane apparatus, the membrane was saturated with water, assembled in the device, and exposed to 0.8 MPa pressure until water flow from the outlet had ceased (typically about 20–25 min). Berries were prepared by sealing a small piece of tubing (inner diameter of 1 mm), using lanolin, to the surface of the berry at the proximal (pedicel) end. The tubing served as a reservoir for the dye solution, and while filling the tube with the dye, it was made certain that the cut end of the pedicel was fully immersed. A cross-section of the berry was made, removing approximately 2 mm of the pericarp at the stylar (distal) end of the berry, and the berry was placed with this flat cut surface directly on the membrane. The berry, reservoir, and membrane were assembled into the pressure membrane apparatus (Fig. 1), and a pressure of 0.8 MPa was applied with nitrogen gas.

A simple wicking system was used to establish a matric potential gradient across a grape berry without the need for berry exposure to high pressure. The berry was prepared in the same manner as for the pressure membrane method, but in this case the berry cut surface was placed in direct contact with a single layer of lint free paper (Kimwipe, Kimberly-Clark, Irving, TX), on a feminine hygiene pad ('always' thin ultra regular, Proctor and Gamble, Toronto, Canada) with the external netting removed, as a wick material. Berries were incubated in a 100% relative humidity environment for 10–14 h. After incubation, cross- or longitudinal sections were observed microscopically. For both the pressure membrane and the simple wick method, about 0.5–0.7 mm of the distal end, which had been in contact with the wick or the membrane surface was removed before examination to eliminate any staining which may have been due to lateral diffusion of the dye at the surface of the membrane or the wick.

Growth restriction studies

In order to inhibit expansive growth after veraison, Zinfandel berries that were about 1 cm in diameter were individually enclosed in small Perspex boxes (1×1×1 cm inner dimensions) at 30 d post anthesis, which in this year was 38 d before 60% veraison. A typical final diameter for these berries would be 1.8–2.0 cm (Mannini *et al.*, 1981). Therefore, this treatment essentially eliminated berry expansion in the second stage of berry growth. Post-veraison berries were collected, carefully removed from the box using a fine wire and silicon lubricant to avoid damage to the berry skin, and evaluated for dye uptake as described above.

Berry hydration

The weight gain of berries which only had their pedicel in water (as for the dye infusion studies) or which were completely submerged in water was measured periodically to follow the kinetics of berry hydration. The berries with only the pedicel in water were in an environment maintained at 100% RH. Weights were recorded after the berries were quickly surface blotted dry with a Kimwipe tissue paper, and the berries replaced into the hydration environment. Experiments were performed on small groups of berries (10–12) on

different dates, and the data were analysed by pooling all of the berries of a similar age, as measured by the days from bloom. The berries for this experiment came from greenhouse-grown plants.

Results

Using the dye infusion method with excised Chardonnay berries under laboratory conditions, the dye was clearly visible throughout the pre-veraison berry vasculature within 30 min of dipping the pedicel in the dye (Fig. 2A). The same rapid dye uptake was observed for berries that were held at 100% RH. However, no dye was visible in post-veraison berries even after the pedicel had been in the dye for several hours (Fig. 2A). Dye movement into the ventral and peripheral vasculature of post-veraison berries was not visible in longitudinal section, even though the xylem traces appeared to be continuous (Fig. 2B), nor was it visible in cross-sections made near the pedicel (Fig. 2C). The same results were obtained when the dye infusion method was used on post-veraison berries that had been restricted from post-veraison growth (Fig. 2D).

In contrast to the results obtained using the dye infusion method, staining was apparent throughout the peripheral vasculature of post-veraison berries when the pressure membrane method was used (Fig. 2F, G). Dye movement into the peripheral xylem was visible in cross-sections at the proximal (pedicel) end of the berry (Fig. 2F), as well as at the distal (stylar) end (Fig. 2G). The pattern of staining that was observed on the membrane also coincided with the position of the vascular bundles in the berry (Fig. 2E), and hence was consistent with the hypothesis that dye was flowing through the berry vascular system under the influence of a gradient in negative hydrostatic pressure (tension), caused by the matric potential of the membrane.

The dye infusion and pressure membrane methods were used as above to test xylem transport in pre- and post-veraison berries of several varieties. The range across varieties in °Brix values was 4.2–5.7 for pre-veraison berries and 18.4–23.4 in post-veraison berries (Table 1), and for all varieties, dye moved into the peripheral xylem of pre-veraison, but not post-veraison berries using the dye infusion method (Table 1). In addition, for all varieties, dye moved into the peripheral xylem (as in Fig. 2G) of post-veraison berries using the pressure membrane method (Table 1).

Use of the pressure membrane method resulted in the appearance of gas bubbles in most samples (Fig. 2F, G). In order to test whether the dye movement that was observed under these conditions represented an artefact associated with elevated pressure, dye was drawn through the berry using a wick under normal atmospheric pressure. Under these conditions, dye also moved into the peripheral xylem of post-veraison berries (Fig. 2H).

Both pre- and post-veraison berries took up water when either the entire berry or just the pedicel was submerged,

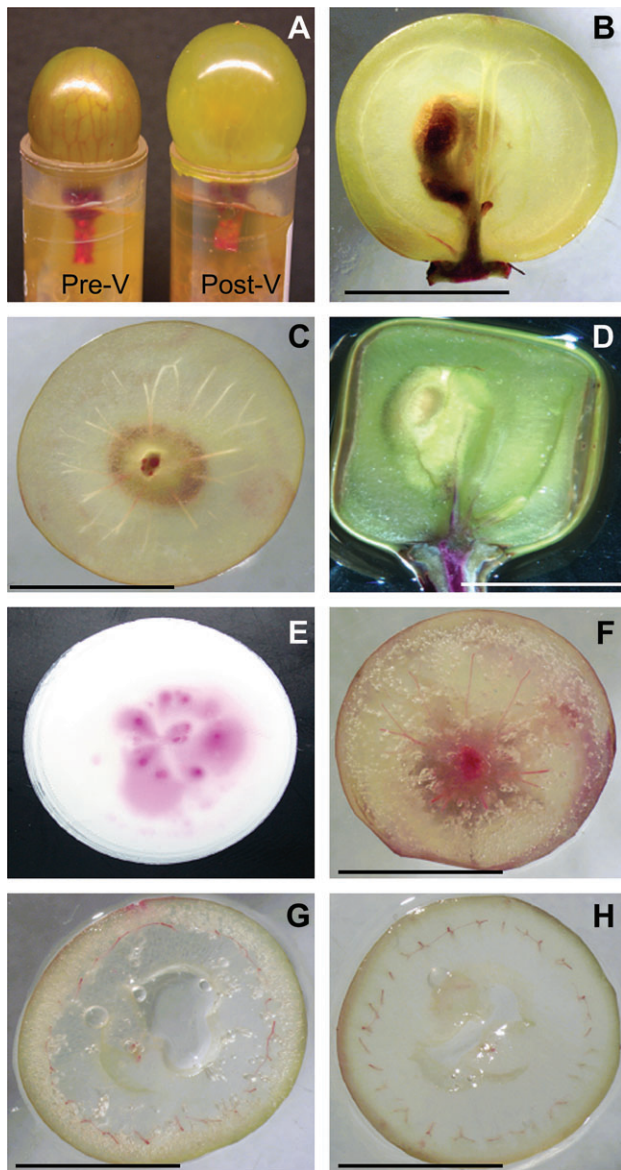


Fig. 2. (A) Whole pre- and post-veraison Chardonnay berries in the infusion assay (pedicel in 0.1% basic fuchsin). The dye is clearly present throughout the peripheral veins of the pre-veraison berry, but not the post-veraison berry. (B) Longitudinal section of a post-veraison berry of Chardonnay following the infusion assay. The axial and peripheral vasculature are both apparently intact, but the dye in the peripheral xylem moved only to a distance that was within the vicinity of the brush area. (C) Cross-section of the proximal (pedicel) end of a post-veraison berry of Chardonnay following infusion, showing dye presence in the peripheral xylem surrounding the brush area. (D) Longitudinal section of a post-veraison growth-restricted Zinfandel berry after infusion, showing dye only in the brush area. (E) Dye stains on the membrane in positions that corresponded to the positions of peripheral vascular bundles on the cut surface of the fruit, following incubation in the pressure membrane apparatus. (F) Cross-section of the proximal (pedicel) end of a post-veraison Chardonnay berry following incubation in the pressure membrane apparatus, showing dye throughout the peripheral xylem. (G) Cross-section of the distal (stylar) end of a post-veraison Chardonnay berry as in (F), showing dye throughout the peripheral xylem. (H) Cross-section of the distal (stylar) end of a post-veraison Chardonnay berry following the wicking method, showing dye throughout the peripheral xylem as in (G).

Table 1. Juice °Brix and basic fuchsin uptake into the peripheral xylem strands by pre- and post-veraison berries using the dye infusion method (DIM) or the pressure membrane method (PMM)

Variety	Pre-veraison ^a		Post-veraison ^a		
	Brix	DIM	Brix	DIM	PMM
Chardonnay	4.2	+	22.2	–	+
Cabernet Sauvignon	4.3	+	23.4	–	+
Pinot noir	4.9	+	22.2	–	+
Shiraz	5.7	+	19.5	–	+
Muscat Gordo	4.4	+	18.4	–	+
Grey Riesling	4.4	+	20.3	–	+

^a The presence (+) or absence (–) of basic fuchsin, respectively, in the peripheral xylem strands.

and water uptake continued over a 24 h period (Fig. 3). Water uptake was essentially the same for both fully submerged and pedicel submerged fruit, suggesting that essentially all of the water taken up was entering through the pedicel. For pre-veraison berries, the pattern of water uptake appeared to be biphasic, with an initial rapid phase of about 9.6 mg h^{-1} decaying to a linear phase (constant rate of uptake) of about 2.5 mg h^{-1} by about 3 h (estimated from the slopes of the lines in Fig. 3). In post-veraison berries, uptake appeared to be linear with time throughout the entire 24 h period, with a rate similar to that of the linear phase of the pre-veraison berries (Fig. 3). Because of initial differences in size, the water uptake after 24 h represented a larger fraction of the initial fresh weight in pre-veraison berries (7%) than in post-veraison berries (3%).

Discussion

These results indicate that the conducting elements (tracheids) of both the axial and the peripheral xylem remain intact and apparently functional throughout grape berry development. The pattern of tissue staining that has been reported in this study shows that an apoplastic dye, and hence presumably water, can flow from the pedicel throughout the axial and peripheral xylem conduits to the stylar end of the post-veraison grape berry under the influence of an applied hydrostatic gradient. The gradient could be established either with an applied pressure or with a wick in the absence of any applied pressure. Previous results using dye infusion (Findlay *et al.*, 1987; Creasy *et al.*, 1993; Dichio *et al.*, 2003), have led to a general consensus that the xylem of post-veraison berries is non-functional (Coombe and McCarthy, 2000) as a direct or indirect consequence of post-veraison berry growth. However, when post-veraison growth was prevented, dye uptake was not influenced, and hence it is proposed that the changes in dye uptake at veraison, which we and many others have observed, are not mechanistically related to post-veraison berry growth and a consequent disruption of xylem continuity.

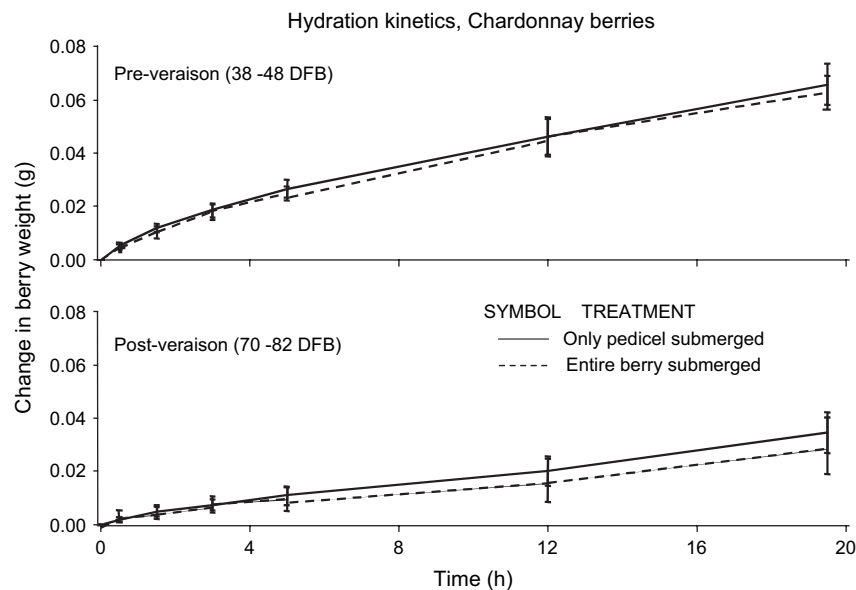


Fig. 3. Kinetics of water uptake for pre- and post-veraison Chardonnay berries. Berries either had only the pedicel submerged (as for the dye infusion technique), or were completely submerged under water. Each point represents the mean ± 2 SE for a pooled sample of fruit (8–10 berries, depending on treatment) having the indicated age (days from bloom, DFB).

Greenspan *et al.* (1994) questioned the conclusion that lack of dye uptake in post-veraison berries implies a blockage in apoplastic transport, and both Greenspan *et al.* (1994) and Rogiers *et al.* (2001) have speculated that water might move through alternative apoplastic paths. In this study, however, with both the pressure membrane and the wick method, dye movement was essentially restricted to the vascular bundles, indicating that this pathway represents the pathway of least resistance. Other explanations for diminished infusion after veraison might be the development of tyloses or embolisms that would impede xylem transport. Findlay *et al.* (1987) looked for, but did not observe tyloses in post-veraison tracheids. The presence of embolisms is consistent with our hypothesis that the berry xylem remains physically intact throughout grape berry development, although the observed change from xylem to phloem dominated flow during fruit development has not generally been attributed to xylem embolism. If the xylem of post-veraison berries were largely embolized, then exposure to 0.8 MPa pressure, as used in this study, might be expected to eliminate gas emboli, but the application of a dry wicking material to pull dye through the berry would not. Hence the similarity of the results that were obtained using these two contrasting methods would argue against a xylem embolism explanation for these results. Further, if embolism is responsible for the change from xylem to phloem dominated flow at veraison in grape berries, then a reduction in xylem contribution might be expected, even in pre-veraison berries, as the degree of water stress increases. This expectation is not consistent with the observation of Greenspan *et al.* (1994) that the amplitude of diurnal berry contraction (1–15%) was well correlated to midday

leaf water potential in pre-veraison, but not post-veraison berries, even though the range in leaf water potential caused by withholding irrigation was about the same for both groups (–0.9 to –1.5 MPa). Hence, to attribute the change from xylem- to phloem-dominated flow at veraison to embolism, it would also be necessary to propose that xylem vulnerability to embolism dramatically increases at this time. Further research will be required to test this hypothesis.

Tyerman *et al.* (2004) have shown that a clear decline occurs in whole-berry hydraulic conductivity at veraison, and suggest that it may be a result of both apoplast (xylem disruption) and symplast (aquaporin/membrane hydraulic conductivity) effects. The evidence of dye movement through the length of the peripheral xylem system in post-veraison berries (Fig. 2), demonstrates that this system remains physically conductive, and because the dye specifically follows the path of the xylem, further suggests that this continues to be the low resistance pathway through the berry. Further support for the conclusion that there may be little or no change in xylem conductivity per se at veraison is that a clear difference between pre- and post-veraison berries was only found in the initial rate of water uptake of about 9.6 and 5.0 mg h^{–1}, respectively, as calculated from the slopes in Fig. 3. After about 3 h, the rate of steady-state water uptake in pre- and post-veraison berries was similar, at about 2.5 and 1.5 mg h^{–1}, respectively. Overall, these rates are of a similar magnitude to those reported by Greenspan *et al.* (1996) for xylem flows in field-grown Cabernet Sauvignon, with average rates over a 24 h period of 7.5 and 4.1 mg h^{–1} for pre- and post-veraison berries, respectively, although short-term rates as high as 30 mg h^{–1}

were also reported. The relatively high initial rates and the overall similarity of steady-state rates of water uptake in pre- and post-veraison berries in this study, were interpreted to indicate either that the conductance of the xylem pathway is similar in both instances, or at least that it is not a limiting factor in either case.

Water transport through any pathway can be described as the product of the pathway conductance and the magnitude of the driving force for water flow across that pathway. For most plant organs, the xylem is considered as a low resistance axial path, for instance from the stem to the stylar end of the fruit, compared with a higher resistance radial path, namely from the xylem into the inner or outer mesocarp tissue. For post-veraison fruit, Tyerman *et al.* (2004) found that the resistance to flow in the pedicel and receptacle portions of the pathway were relatively low, compared with the proximal (brush) and distal (entire berry) portions. The distal portion had the highest resistance, but for non-transpiring berries, this represents the overall pathway from the pedicel to the mesocarp. Hence the relative importance of the axial (xylem) resistance, from the brush to the stylar end of the fruit, to the overall resistance of water flow from the brush into the fruit cells, remains unknown.

If the xylem pathway through the pedicel, into the fruit, and throughout the flesh remains available for the transport of water after veraison but is not used, and if the reason for this change is not due to a decrease in xylem conductance, then the change must be due to a reduction in the driving force for water flow. In this case the driving force is the hydrostatic (tension) gradient within the xylem between the vessels/tracheids of the pedicel and the tracheids of the peripheral bundles in the outer pericarp. In the dye infusion technique, the xylem of the pedicel is exposed to a solution at atmospheric pressure, and hence, in order for solution to flow from the reservoir into the berry, a tension in the xylem of the berry is required. In pre-veraison berries, the uptake of dye (Fig. 2A) is very rapid, and there is also a relatively high initial rate of water uptake (Fig. 3). Since both can occur under conditions of 100% RH, it is concluded that both dye uptake and at least the initial water uptake in pre-veraison berries is due to the presence of significant tension in the pre-veraison berry xylem. The biphasic pattern of water uptake in the pre-veraison berry (Fig. 3) is interpreted to indicate that the tension was progressively reduced as water was taken up, and that a steady-state was reached. Presumably, the steady-state is due to a continued osmotic water uptake by cells of the fruit mesocarp. In view of the relatively large distance between the vascular bundles and the bulk of the fruit (Coombe, 1987; Harris *et al.*, 1968), equilibrium may be approached slowly or never. If, as is generally assumed, the conductance for water transport in xylem is much higher than the conductance for symplastic water transport, then there would essentially be no tension in the xylem of the pre-veraison berry, once the steady-state conditions had been reached. Hence, the lack of dye uptake

and slow initial hydration of post-veraison berries may simply indicate that there is no tension initially present in the post-veraison berry xylem. This explanation is consistent with the comparable steady-state water uptake observed for pre- and post-veraison berries and with the finding of Tyerman *et al.* (2004) of an increase in the equilibrium hydrostatic pressure (from tension to slightly positive) in the berry pedicel near veraison. This explanation does not require that there be any substantial structural or functional changes in the berry xylem at veraison.

The hypothesis that post-veraison berries have substantially reduced tension in the xylem compared with pre-veraison berries carries with it a number of important physiological consequences for grape berries, and perhaps other similar fruits. Where it has been directly measured, the turgor in tomato fruit cells has been found to be less than 0.2 MPa (Shackel *et al.*, 1991; Mingo *et al.*, 2003), which is relatively low, considering that the solute potential of fruit tissue is typically on the order of -1 to -2 MPa. Recently, grape cell turgor was reported to decrease from *c.* 0.4 MPa before veraison to less than 0.1 MPa after veraison (Matthews and Shackel, 2005). An absence of tension in the xylem, and presumably also the rest of the fruit apoplast, would imply that low total water potentials must be due to the presence of solutes in the apoplast. Accordingly, a number of authors have found evidence for the presence of apoplastic solutes in fruits (Shackel *et al.*, 1991; Pomper and Breen, 1995) and other organs (Cosgrove and Cleland, 1983; Welbaum and Meinzer, 1990). For grapes in particular, Delrot *et al.* (2001) have proposed a model of fruit development in which apoplastic solutes are the result of a breakdown in apoplast/symplast compartmentation. Whether or not the hypothesized occurrence of apoplastic solutes in the case of grape is due to a passive loss of compartmentation or to an active partitioning, however, one important issue is how water and apoplastic solutes are prevented from being removed from the fruit by bulk flow due to tension in the xylem of the rest of the plant. For a similar question related to seed development, Bradford (1994) suggested that a semi-permeable barrier might occur in the apoplast between the seed and fruit, acting essentially as a membrane, to retain apoplastic solutes. In the model proposed by Delrot *et al.* (2001), presumably the loss of xylem function would prevent a substantial water or solute backflow from the fruit to the plant, but the evidence from this study is not consistent with any change in the xylem pathway of the post-veraison grape. This study's evidence is also difficult to reconcile with the hypothesis of a semi-permeable apoplastic barrier between the pedicel and fruit, because any barrier that would retain sugars and other solutes within the fruit should also have prevented the movement of dye from the pedicel to the fruit under the influence of a hydrostatic gradient. Direct measurement of xylem tensions at different positions along the berry and pedicel using the pressure probe (Wei *et al.*, 2001), would

be useful in determining whether or not the tensions that normally occur in the plant are also experienced in the berry xylem, and this should improve an understanding of the biophysical basis for the change from xylem- to phloem-dominated water flow in the post-veraison grape berry.

Despite the presence of intact and apparently functional xylem conduits in the berry, it appears clear that, at veraison, there is a substantial reduction in the proportion of water that is transported to the berry through the xylem compared with the phloem (Greenspan *et al.*, 1994; Rogiers *et al.*, 2001). The xylem and phloem pathways operate in parallel, and the interaction between conduits is not well studied (Cernusak *et al.*, 2003; Lampinen and Nojonen, 2003). A reduction in the contribution of one pathway could result from changes to that pathway (i.e. a reduction in pathway conductivity or in the magnitude of the driving force) or from contrary changes in the parallel pathway (increases in conductivity or driving force). Hence, the possibility that an increase in phloem transport could contribute to an increased (less negative) hydrostatic potential in the apoplast, and thereby a reduction in xylem inflow, remains. The girdling methods that have been used to evaluate phloem and xylem flows into fruit (Lang and Thorpe, 1989) must make the assumption that disrupting phloem transport by girdling does not alter xylem transport. This has not been tested directly, although an analysis of the errors attributable to that assumption showed that, except at low flow rates close to nil, the errors were small (Fishman *et al.*, 2001). Moreover, several independent methods have given rise to data indicating that a transition from xylem to phloem water transport occurs during the development of a number of other fleshy fruits (Ho *et al.*, 1987; Lang and Ryan, 1994; Dichio *et al.*, 2003). Thus, this phenomenon may be of very general occurrence, and probably importance, in fleshy fruit. The grape berry represents a particularly appropriate model system in which to study this process, in part because of its agricultural importance, and in part because of the relatively complete transition from xylem to phloem water transport over a very short and well-defined period of fruit development.

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