**Ripening grape berries remain hydraulically connected to the shoot**

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**Abstract**

Berry diameter was monitored during dry-down and rewatering cycles and pressurization of the root system of *Vitis vinifera* (cv. Merlot) and *Vitis labruscana* (cv. Concord) to test changes in xylem functionality during grape ripening. Prior to veraison (onset of ripening), berries maintained their size under declining soil moisture until the plants had used 80% of the transpirable soil water, began to shrink thereafter, and recovered rapidly after rewatering. By contrast, berry diameter declined slowly but steadily during post-veraison water stress and did not recover after rewatering; irrigation merely prevented further shrinking. Preconditioning vines with a period of water stress after flowering did not influence the berries’ reaction to subsequent changes in transpirable soil water. Pressurizing the root system led to concomitant changes in berry diameter only prior to veraison, although some post-veraison Concord, but not Merlot, berries cracked under root pressurization. The xylem-mobile dye basic fuchsin, infused via the shoot base, moved throughout the berry vasculature before veraison, but became gradually confined to the brush area during ripening. When the dye was infused through the stylar end of attached berries, it readily moved back to the plant both before and after veraison. Our work demonstrated that berry-xylem conduits retain their capacity for water and solute transport during ripening. It is proposed here that apoplastic phloem unloading coupled with solute accumulation in the berry apoplast may be responsible for the decline in xylem water influx into ripening grape berries. Instead, the xylem may serve to recycle excess phloem water back to the shoot.

Key words: Apoplastic dye, deficit irrigation, grape berry, phloem, root pressure, vascular connection, water stress, xylem.

**Introduction**

In premium wine production, fruit quality is considered to be far more important than crop yield, and small berry size is deemed desirable. There is a widespread belief in the wine industry that rain or irrigation close to harvest may increase berry size and cause a ‘dilution’ of solutes (sugars, acids, anthocyanins, tannins, etc.) or even cracking (splitting) of berries. In Europe, this belief is often written into the law, and irrigation is prohibited or strictly regulated. Even in the New World, wineries may encourage growers to withhold irrigation water at this critical time to avoid any perceived adverse effects. Surprisingly, there is little scientific evidence that late-season water uptake by the roots and transport to the fruit is detrimental to grape quality. In the absence of rain, which may lead to direct water uptake through the skin (Considine and Kriedemann, 1972; Lang and Thorpe, 1989), water influx into fruits occurs via both the xylem and the phloem. Conversely, efflux may be due to fruit transpiration and xylem ‘backflow’
from the fruit to the vine. Many studies found that water flow via the xylem into grape berries decreases markedly during ripening (reviewed by Ollat et al., 2002). Whereas xylem sap is the main source of water for the berries before veraison (colour change, beginning of ripening, and resumption of cell expansion after a brief lag phase), phloem sap becomes the primary water source after veraison. However, the causes of the observed decrease in xylem water influx remain unclear. Physical disruption of the xylem conduits inside the berry is usually cited as the main reason for impeded xylem flow (Düring et al., 1987; Findlay et al., 1987), but this has been disputed (Creasy et al., 1993). Fruit transpiration declines during development (Rogiers et al., 2004), which implies that evaporative water loss ceases to function as the driving force for water influx. Moreover, the cessation of xylem inflow into grape berries may not be complete (Greenspan et al., 1994; Rogiers et al., 2001).

Evidence for a breakdown in xylem functionality comes mainly from studies of apoplastic dye perfusion through the berry pedicel (Düring et al., 1987; Findlay et al., 1987). Other experimental findings were sometimes at odds with this dogma, but while doubts have been raised about the validity of the interpretation of dye uptake studies (Lang and Thorpe, 1989; Rogiers et al., 2001; Tyerman et al., 2004), the basic view has remained largely unchallenged. Indeed, the development of a xylem discontinuity in the pedicel or inside the fruit (i.e. hydraulic isolation of the fruit) is now often regarded as a prerequisite to prevent loss of solutes via the xylem in fruits that employ apoplastic phloem unloading, such as grape (Patrick, 1997; Sarry et al., 2004), tomato (Ho et al., 1987; Patrick and Offler, 1996), or apple (Dražeta et al., 2004; Zhang et al., 2004). It has been argued that high hydraulic resistance ($r_h$) inside such fruits and restricted xylem influx may be required to promote phloem unloading and fruit softening and to protect the fruit from excessive backflow, for example, during periods of high evaporative demand (Malone and Andrews, 2001; Tyerman et al., 2004). However, it is not clear how such hydraulic isolation should come about. Even if fruit tracheids were indeed disrupted, the cell wall and intercellular space apoplast of the fruit would still be contiguous with the rest of the plant. Moreover, the xylem and phloem are interconnected along their entire length and can readily exchange water and solutes (Esau, 1977; Zwieniecki et al., 2004). Thus, while physical xylem disruption would certainly increase $r_h$ within the fruit pericarp, it would not entirely prevent apoplastic exchange of water and solutes between the fruit and the plant. On the other hand, although Lang and Thorpe (1989) reported xylem flow from ripe grape berries back to the vine, direct evidence for xylem backflow is thus far lacking.

The present study examined if water stress during the early development of grape berries would affect their response to late-season water stress, presumably by altering the hydraulic connection of the berries to the shoot, as reported for tomato (Davies et al., 2000). Berry diameter was monitored during repeated dry-down/rewetting cycles both before and after veraison. In addition, water was forced through the xylem using a root pressure chamber to determine if soil water uptake would contribute to berry volume changes (Davies et al., 2000). Changes in both soil moisture and air pressure applied to the root system are useful to manipulate xylem pressure ($P_x$) (Wei et al., 1999). Finally, an apoplastic dye was used to trace xylem connections between the berries and the rest of the plant. Two genetically distinct grape cultivars, Merlot (Vitis vinifera L.) and Concord (Vitis labruscana Bailey), were used for the three sets of experiments.

Materials and methods

Plant growth

Three-year-old, own-rooted grapevines V. vinifera cv. Merlot and V. labruscana cv. Concord were grown in white 20.0 l PVC pots containing a mixture of 50% sandy loam, 25% peat moss, 25% pumice, and 30 g l$^{-1}$ dolomite, with a volumetric water content of 30% at field capacity. The vines were grown outside at the Irrigated Agriculture Research and Extension Center in Prosser, Washington, USA (46°17’ N; 119°44’ W; elevation 270 m), before being transferred to a whitewashed, air-conditioned greenhouse (photosynthetic photon flux $\sim$860 $\mu$mol m$^{-2}$ s$^{-1}$ under clear sky at midday) for the series of experiments described below. Meteorological conditions in the greenhouse were recorded with a HMP45C temperature and relative humidity sensor (Vaisala, Helsinki, Finland). Before starting the experiments, water deficit was imposed during the post-anthesis cell division phase of Merlot berries in an attempt to enhance the xylem connection between the berries and the rest of the plant (Davies et al., 2000). After watering the pots to field capacity, they were dried down twice for two consecutive 14 d periods (Merlot DI) before being returned to the same daily (standard) irrigation regime used for the remaining Merlot (Merlot STD) and Concord vines (Concord STD). Plant leaf area was estimated by measuring the length of the main vein of each leaf and regressing area against length as determined on surplus plants, using a Li-Cor 3100 leaf area meter (Lincoln, NE, USA). Upon completion of the experiments, vines were removed from the pots to determine root distribution and dry weight following drying at 60 °C to constant weight.

Dry-down experiments

Three separate experiments were conducted to determine the response of berry size to dry-down and rewetting cycles applied before and after veraison. At the start of each experiment, the pots were watered to field capacity and then allowed to dry down. The pot surface was sealed to prevent water loss by means other than transpiration. During the second (pre-veraison) and third (post-veraison) dry-down cycles, daily plant water use for transpiration was determined gravimetrically, and all pots were watered back to field capacity when daily water use had decreased to 10% of the maximum rate. Daily water use was calculated as a fraction of the total transpired soil water (FTSW), which gives an indication of the actual plant-available water in the soil (Lebon et al., 2003). This water depends on root distribution and, therefore, differs from the commonly used (potential) plant-available soil water, which is...
calculated based on soil properties alone and thus ignores the fact that roots may not access a portion of the total available soil volume because they do not grow there. Although the calculation of FTSW ignores plant water capacitance, the error due to this omission is likely to be small, considering that 3-year-old, pot-grown grapevines were found to contain <250 ml of water in the roots, trunk, and shoots (Keller and Koblet, 1995; M Keller, unpublished results).

During each dry-down and rewatering cycle, changes in berry diameter were recorded on three berries per vine, using FI-XSM linear variable displacement transducers (Phytech, Rehovot, Israel). Transducers were connected via an AM416 relay multiplexer (Campbell Scientific, Logan, UT, USA) to a CR10X data logger (Campbell Scientific), and their output was calibrated against berry starting diameter measured with electronic calipers (0.01 mm resolution). Testing of the FI-XSM sensors found negligible temperature difference between 10 °C and 50 °C and <2.1% deviation from the specified displacement of 200 mV mm⁻¹. It was also tested whether the force exerted on the berries by the spring-loaded transducers (0.40 N at the beginning of stroke to 0.75 N at the end of stroke) would lead to fruit deformation. Two sensors were placed simultaneously at a 90° angle on mature, detached Concord berries: one sensor with high force (as indicated by higher voltage, greater sensor compression) and one with low force. There clearly was a directional decrease in diameter in the direction of the stronger sensor. Over a 21 d period, the high-force sensors indicated a diameter loss of 17.4±1.6% (n=4) and the low-force sensors a loss of only 2.0±1.2%. In no case did the weaker sensor register an increase in diameter, implying that the berries were not pressed into an oval shape by the stronger sensor. It was therefore assumed that the sensor output was a reasonable reflection of fruit volume changes. At the end of each experiment, the selected berries were removed and the solute concentration (%Brix) recorded by refractometry.

Root pressurization experiments

When pressure is applied to a plant’s root system, the entire xylem becomes pressurized until the pressure at the surface reaches $P_s \geq 0$ (atmospheric pressure, see Wei et al., 1999). If the pressure is increased to a point where xylem flow exceeds transpiration, water floods the apoplast and drips from the hydathodes. A custom-built 26.0 l metal root pressure chamber, based on the design by Yong et al. (2000), was used to determine the effect of rapid changes in plant water status on berry size. The chamber was designed with a split lid such that the entire pot (i.e. root system) could be placed inside the chamber and the lid sealed around either side of the stem. Pneumatic pressure was applied to the chamber under manual control with nitrogen. The appearance of xylem sap at the surface of a cut petiole above the cluster was used to indicate full hydration at that position on the shoot. Prior to veraison, the pressure was increased in 0.2 MPa increments up to 1.0 MPa. Berries at the apical, middle, and basal part of the fruit cluster were monitored using the transducers described above, with chamber pressure logged automatically at the same time interval. Additional FI-XSM sensors were used to monitor the trunk diameter and shoot node diameter at the point of cluster attachment. The plants were not watered during the 2 d prior to root pressurization, and the leaf above the cluster did not reach full hydration ($\Psi_{leaf} \approx 0$ MPa) until the chamber pressure reached 1.0 MPa.

For the post-veraison measurements, the procedure was simplified to a rapid one-step increase in root pressure to bring the plant to full hydration within a period of 6 min. This pressure was held for ~4 h with adjustments made manually to maintain sap flow from a cut petiole above the cluster. The diameter of two berries on each of two clusters per plant was recorded using FI-XSM sensors. Berry sugar concentration (%Brix) was subsequently determined by refractometry.

Dye movement experiments

Shots with four mature leaves and one cluster were cut from field-grown grapevines in a nearby vineyard before (all berries green), during (berries ranging from green to blue), or after veraison (all berries blue). The cut end was immediately placed in a centrifuge tube containing 30 ml of a 0.1% aqueous solution of the xylem-mobile dye basic fuchsin (C$_{20}$H$_{19}$N$_3$HCl; mol. wt 338 Da), and the tube was sealed around the shoot to prevent evaporation (‘forward’ dye infusion). The diameter of 10 marked berries per cluster was measured with digital calipers, and the stylar (distal) end of selected berries was sliced off with a razor blade to stimulate water evaporation from the exposed surface. The pedicel of additional berries on the same cluster was girdled with the blunt end of a razor to inhibit phloem influx. Berries were sampled at regular intervals to monitor the pattern of dye movement through their xylem conduits (tracheids with spiral thickenings; Pratt, 1971; Findlay et al., 1987). The berries were visually rated for the progression of skin pigmentation (green, blush, pink, red, purple, blue) before being sectioned longitudinally through the centre for light microscopy. The extent of dye movement into the berry from the shoot was rated visually and assigned a number from 1 (in brush only) through 6 (continuous throughout the entire vascular network). The berry juice was then expressed to determine sugar concentration.

To test whether water can move back from grape berries to the parent vine (‘xylem backflow’), dye was also fed to berries on intact, well-watered Merlot and Concord vines before and after veraison. The root pressure chamber was used to bring the plant to full hydration. While the chamber was under pressure, the stylar end of selected berries was removed with a razor blade to expose the peripheral (dorsal) and axial (ventral) vascular bundles, and the cut end of each berry was placed in 0.1% basic fuchsin (‘reverse’ dye infusion). The pressure, which served to avoid cavitation in the berry vasculature due to the cut, was then released and the dye was allowed to move back through the berry. The experiment was repeated with a different set of Merlot and Concord vines without pressurizing the root system, similar to an experiment with immature (green) tomato (Malone and Andrews, 2001). After ~3 h, the clusters were removed, and cross-sections of the pedicel (proximal) end of the treated berry, the pedicel, the rachis, other berries on the same cluster, and the shoot above and below the cluster were examined for dye movement by light microscopy.

Results

Dry-down experiments

The average solute concentration at the end of each experiment was 5.1 °Brix (early pre-veraison Merlot only), 7.2 °Brix (pre-veraison Merlot), 8.5 °Brix (pre-veraison Concord), 21.1 °Brix (post-veraison Merlot), and 20.0 °Brix (post-veraison Concord). During the second interval (34 d), Merlot berries expanded ~1.6-fold, gaining ~16 µl d⁻¹ in volume and ~0.4 °Brix d⁻¹ in solutes (~22 mM hexoses d⁻¹). Similarly, Concord berries grew ~1.4-fold and gained ~15 µl d⁻¹ and ~0.3 °Brix d⁻¹. Grape berries of both cultivars reacted similarly to changes in soil moisture and plant water status. However, the response of pre- and post-veraison berries differed in two important ways. First, pre-veraison berries were able to maintain their diameter (and thus their volume) for several days into the dry-down period, after which they shrank rapidly (Fig. 1A).
After veraison, the berry diameter appeared to decrease slightly but gradually from the first day (Fig. 1B). When normalized against FTSW, pre-veraison berries suddenly began to shrink after 80% of the transpirable soil water had been used, regardless of cultivar and irrigation history (Fig. 2A). After veraison, the decrease in diameter as a function of FTSW was more gradual, but occurred over the entire range of transpirable soil water (Fig. 2B). Nevertheless, the volume loss also intensified beyond 80% FTSW, although the extent of shrinkage was much less severe than before veraison. During the earliest dry-down experiment, conducted well before veraison, there was a pronounced diurnal pattern of diameter decrease during the day, when evaporative demand of the air was high (high VPD), and partial recovery overnight (data not shown). However, during the second dry-down experiment, the berries were close to veraison (in the lag-phase of growth) and did not show the same diurnal fluctuations in size as water stress became more severe (Fig. 1A). In particular, berry diameter failed to recover overnight, but still decreased during the day. After veraison, the berries were even less responsive to plant water stress, with only a small decrease in diameter during the day and no overnight recovery (Fig. 1B).

A second major difference between pre- and post-veraison fruit was the berry response to rewatering at the end of the dry-down period. In the early pre-veraison experiment, berry diameter recovered entirely within 12 h of rewatering (data not shown). In the second experiment, where the fruit was closer to veraison at the start of the dry-down period, the recovery after rewatering was less rapid, especially for Merlot STD (Fig. 1). Nevertheless, recovery continued throughout the day, regardless of the diurnal fluctuation in vapour pressure deficit (VPD). One of the berries on the Merlot STD vine began to change colour from green to red (i.e. it underwent veraison) 5 d into the dry-down period and simultaneously began to increase in size, even before the vine was rewatered (Fig. 1A). All berries had shrunk at this point, and the size increase was due to the berry returning to full turgor as it began to ripen. In the post-veraison experiment, the daily decrease in berry diameter was stopped by rewatering, but the berries did not return to their initial size (Fig. 1B).

Overall, the response of the Merlot STD, Merlot DI, and Concord STD berries to the dry-down and rewatering cycles was very similar. Merlot DI plants used more water
and took longer than Merlot STD to respond during the dry-down period. This was not related to differences in plant leaf area (means, n=3: Merlot STD 0.69 m², Merlot DI 0.68 m², Concord STD 0.76 m²), but instead appeared to be due to the DI vines having a deeper and 25% larger root system (Merlot STD 151 g, Merlot DI 188 g, Concord STD 182 g) which enabled greater water extraction from the pots. Plant water status was not monitored during the dry-down cycles to avoid increasing defoliation over time. However, midday stomatal conductance to water vapour (gs, measured using a PLC-6 broadleaf chamber connected to a CIRAS-2 from PP Systems, Amesbury, MA, USA) of the leaf opposite the cluster fell from ~300 to <50 mmol m⁻² s⁻¹ during the course of a typical dry-down cycle. Similar cycles with potted pre-veraison Concord plants led to Ψleaf ≈−1.6 MPa, which was associated with stomatal closure and berry shrinkage, and complete recovery overnight upon rewatering (Liu et al., 1978). In addition, whole-plant transpiration decreased progressively with increasing duration of water stress (data not shown). Transpiration is closely coupled with gs which, in turn, is tightly linked to leaf water status.

Root pressurization experiments

Pre-veraison Concord berries responded readily to changes in plant water status imposed by changing the pressure applied to the root system. In the absence of pressure, berry diameter decreased during the day, while plants were transpiring rapidly. Trunk and shoot node diameter reacted almost instantaneously to step-wise increases in root pressure, and then dropped rapidly as the pressure was released to zero (Fig. 3). The trunk diameter was particularly responsive, indicating rapid transmission of the chamber pressure to a change in trunk water capacitance. The berry response was initially less obvious, with the first increase in diameter seen 1 h after pressure was first applied to the roots (Fig. 3). The subsequent increase in berry diameter appeared more buffered from changes in plant water status than the trunk, and increased gradually as the chamber pressure was stepped up. The position of the berry on the cluster also appeared to influence its response to the increase in root pressure; the diameter of the apical, middle, and basal berries increased by 0.8, 1.0, and 1.4%, respectively, during the pressurization period. Assuming a spherical berry shape, this was equivalent to a volume increase of 27, 34, and 82 µl, respectively. When the chamber pressure was released, berry diameter began decreasing almost immediately, but the rate was less rapid than for the trunk and node at the point of cluster attachment. The volume loss amounted to 26, 29, and 70 µl for the apical, middle, and basal berry, respectively, by dusk, after which there was no further decrease in berry volume. These observations suggest that the change in berry diameter is a consequence of changes in cellular water uptake and turgor, rather than the more direct changes in xylem diameter that may have been responsible for the rapid response of trunk diameter to changes in root pressurization.

For the post-veraison pressure runs, vines were watered prior to being sealed in the chamber. Consequently, the pressure required to bring the plants to Ψleaf ≈0 MPa was lower than before veraison. The response of berries to root pressurization was somewhat variable; the diameter of some berries did not change, while other berries decreased in diameter by ≤0.2 mm during the measurement period (Fig. 4). This reaction was not related to the solute concentration of these berries, which ranged from 15.5 to 24.2 °Brix, nor to berry diameter, which varied from 11.8 to 18.5 mm (data not shown). However, in no case did root pressurization result in a measurable increase in berry diameter. For some berries, the rate of decline in diameter was slowed by bringing the plant to full hydration and then accelerated again as the pressure was released. Earlier work in our laboratory (unpublished) had shown that it was possible to crack mature (22–32 °Brix) Concord berries on intact vines, using the root pressure chamber, and this was verified in the present study (Fig. 4C). There was no measurable increase in berry diameter leading up to the berry cracking, and cracking did not appear to be due to the force exerted by the sensor as several other berries on the vine also cracked. In addition, spontaneous cracking occurred during humid nights following irrigation.

Dye movement experiments

Basic fuchsin clearly behaved as a xylem lumen-mobile dye, but not as a general apoplastic tracer. The dye always remained confined to xylem vessels and tracheids, regardless of whether it was fed via the shoot base (forward infusion) or via the berries (reverse infusion). On shoots with clusters bearing only green (i.e. pre-veraison) berries, the dye was consistently found to be continuous throughout the entire vascular network, including the ovular,
ventral (axial), and dorsal (peripheral) vascular bundles, and the dorsal network, giving the berries a characteristic ‘chicken-wire’ appearance. In clusters undergoing veraison, dye movement into the berries did not stop suddenly at a certain developmental stage. Instead, the extent of dye penetration through a berry’s vascular system decreased gradually as the berry changed colour from green to blue and began to accumulate sugar (Fig. 5). As soon as berries began to blush, the dye became discontinuous in the stylar end, while still moving into the axial and peripheral vascular bundles. With increasing pigmentation and sugar concentration, the dye was found to penetrate progressively less far into the berry until, in purple and blue berries (>15 °Brix), it was confined to the brush region. This pattern was independent of initial berry size or volume change during dye infusion ($r=-0.03$, $P=0.92$, $n=44$) and was unaffected by the duration (up to 3 d) of dye uptake by the shoot. Neither removing the stylar end of the berry nor pedicel girdling had any effect on the extent of dye penetration into the berry. Feeding basic fuchsin to additional shoots or excised clusters of V. vinifera cvs Chardonnay, Cabernet franc, Cabernet Sauvignon, Muscat, and Sangiovese, and V. labruscana cv. Niagara growing in the same vineyard always gave the same results; the dye moved into the berries and throughout their vascular network prior to veraison, but only into the brush of post-veraison berries. Regardless of cultivar and developmental status, the dye always moved through the pedicel and the vascular traces in the receptacle.

In a reversal of this standard procedure, basic fuchsin was also fed to the cut stylar end of berries on vines subject to root pressurization. Prior to veraison, root pressurization resulted in xylem sap exudation from the severed xylem ends in the berries upon excision of their stylar end. However, after veraison, such sap exudation could never be observed. When the pressure on the root system was released, the dye rapidly moved back through the berry tracheids and out through the pedicel of both pre- and post-veraison Merlot and Concord berries (Fig. 6). Dye movement through berries was identical when roots were not pressurized, indicating that root pressurization to prevent xylem cavitation prior to dye infusion was unnecessary. In both cases, movement of dye could be traced to adjacent and nearby transpiring leaves, but also downward (basiptely, i.e. against the transpiration stream) in the vessels on the cluster side of the shoot, beyond the most basal leaf, towards the trunk of the vine (Fig. 7).
Moreover, although dye also always moved to other berries on the same cluster, it moved into those berries (i.e. beyond the brush) only prior to veraison but not afterwards.

**Discussion**

The response of grape berry diameter to changes in soil moisture was similar for two genetically distinct cultivars and independent of the previous irrigation history. The extent of berry shrinkage during soil drying was much more severe before than after veraison, which is consistent with earlier research showing that developing berries become progressively less sensitive to plant water status (Creasy and Lombard, 1993; Greenspan et al., 1994, 1996). Moreover, pre-veraison berries rapidly rehydrated and resumed growth upon rewatering, whereas no such response was found in post-veraison berries. Application of irrigation water after veraison merely prevented further shrinkage. In real time, the Merlot DI vines responded more slowly than the STD vines to the dry-down episodes before veraison, but they were able to exploit more soil water due to their larger and deeper root system. Thus, plotting berry diameter against FTSW showed that pre-veraison berries did not begin to shrink until the vines had transpired 80% of the available soil water. This is remarkably similar to the rapid drop at FTSW of <20% in grapevine $\Psi_{leaf}$ and $g_s$ (Lebon et al., 2003). Our finding that a shrinking berry undergoing veraison during a dry-down episode suddenly began to re-expand (before rewatering and while the pre-veraison berries on the same cluster continued to shrink) indicates a sudden increase in phloem influx at veraison. This increase must have exceeded the total water loss from the berry, suggesting that phloem water may be sufficient to sustain pericarp cell expansion in ripening berries.

Changes in pneumatic pressure applied to the root system were readily transmitted to the trunk, as shown by the stepwise and almost immediate changes in trunk diameter. Since pressure applied to the roots is rapidly transmitted to $P_x$ (Wei et al., 1999), fluctuations in trunk diameter may be a good indicator of changes in $P_x$. The fact that changes in grape berry diameter before veraison occurred much more gradually compared with the trunk suggests that they were a direct result of water influx and efflux. By contrast, when root pressure was applied after veraison, it was at best able to offset the decrease in berry diameter during the day. This is consistent with the effect of rewatering following a post-veraison dry-down episode and suggests that water may continue to flow through the berry xylem after veraison. However, the general direction of water flow may be from the fruit back to the leaves, especially during periods of high evaporative demand and low $P_x$. Greenspan et al. (1994, 1996) claimed that xylem backflow from ripening grape berries was likely to be insignificant.
although Lang and Thorpe (1989) had reported consistent and substantial backflow.

When the cut end of the peduncle of whole clusters was immersed in basic fuchsin, dye always ended up at each berry within ~30 min, covering a distance of 50–100 mm from the point of infusion. However, dye penetration into the berries declined once they started to accumulate hexose sugars, and was confined to the brush (proximal) area after they had reached \( \geq 15 \text{ Brix} \). This agrees well with the increase in ‘pedicel equilibrium pressure’ during ripening (Tyerman et al., 2004) and with all studies using the standard forward dye infusion (reviewed by Ollat et al., 2002), but contrasts sharply with the dye movement out of the berries during reverse infusion in both cultivars. Intriguingly, the decline in dye penetration coincided with the abrupt reversal of the shrinking trend of berries that began ripening while the plant suffered increasingly severe water stress. Taken together, these results suggest that (slow) transpiration from the pedicel and receptacle, in addition to water transfer to the transport phloem (Thompson and Holbrook, 2003), may provide the driving force for dye (i.e. water) movement towards the berries, while dye movement into the berries may be inhibited by positive \( P_x \) inside the ripening berries. Thus, although the berry tracheids apparently remain intact after veraison, grapevines seem to discontinue (or at least substantially diminish) the use of this pathway for water influx, beginning at the stylar (distal) end as the berries begin to ripen. Our data demonstrate that ripening berries of the genus \( Vitis \) remain hydraulically connected to the shoot, confirming recent results by Bondada et al. (2005).

Both physical disruption and air embolisms (cavitation) can therefore be excluded as possible culprits for the alleged xylem discontinuity and hydraulic isolation of the berries (see Introduction), and also rule out the existence of axial, apoplastic, solute-reflecting barriers to the movement of dye (but not water) through the berry vasculature. Though such semipermeable apoplastic ‘membranes’ have been conjectured (Bradford, 1994), the idea is at odds with our observation that reverse-infused dye readily moved out of the berries.

Grape berries are thought to switch from symplastic to apoplastic phloem unloading at veraison (Xia and Zhang, 2000), a change that they share with developing tomato fruits (Ruan and Patrick, 1995). The deposition of solutes in the fruit apoplast raises the apoplast’s osmotic pressure \( (\pi_{\text{apoplast}}) \), leading to water efflux from the phloem (van Bel, 2003). The resulting decrease in phloem pressure \( (P_p) \), in turn, enhances phloem influx (Patrick, 1997). However, berries require very little water compared with leaves, even during rapid expansion. The demand for growth water per Merlot berry in this study was \( \leq 0.00 \text{ d}^{-1} \) (over a 30 d post-veraison period), and berry transpiration has been estimated at \( \leq 100 \text{ d}^{-1} \) (Greenspan et al., 1996; Rogiers et al., 2004). Assuming that active removal of unloaded sugar from the mesocarp apoplast (Sarry et al., 2004) occurs at a faster rate than water influx into mesocarp cells (as indicated by increasing hexose concentration), an excess of phloem water influx during ripening should be expected (which is supported by the present results). Dissipation of surplus phloem water by berry transpiration may not be a sufficiently robust strategy, because transpiration is highly dependent on VPD and declines during development (Rogiers et al., 2004). Indeed, grape berries seem to ‘design’ to minimize evaporative water loss; compared with leaves, they have very few stomata that become non-functional by veraison (Blanke et al., 1999; Rogiers et al., 2004), and the amount of (epi-)cuticular wax is ~10-fold greater (Radler, 1965; Possingham et al., 1967). Therefore, excess phloem water (that has moved to the apoplast osmotically) may be recycled back to the leaves in the xylem, at least partly without entering the mesocarp symplast. Recirculation of excess phloem water was originally proposed by Münch (1930) and has been suggested for various fruits, including grape (Lang and Thorpe, 1989), apple (Lang, 1990), and tomato (Van Ieperen et al., 2003). Lang and Thorpe (1989) assumed the apoplastic pressure in the fruit to be higher than in the plant, and this was recently confirmed by direct measurements of \( P_x \) in grape berry pedicels (Tyerman et al., 2004). Thus phloem influx may cause (positive) ‘back pressure’ leading to xylem efflux due to the limited extensibility of the skin (Matthews et al., 1987), especially if phloem water influx exceeds berry growth and transpiration. This pressure appears to dissipate in the pedicel, so that xylem-mobile dyes can move to (but not into) the berry. Indeed, some of the water exiting the berry may be reabsorbed in the pedicel and beyond by the incoming phloem to maintain water potential equilibrium between the transport phloem and the apoplast (Thompson and Holbrook, 2003).

The key to the formation of berry-xylem back-pressure could lie in the accumulation of solutes in the berry apoplast. In roots, release of sugar into the apoplast leads to positive hydrostatic \( P_x \) due to osmotic water influx from the soil, which forces water up the plant (Scholander et al., 1955; Sperry et al., 1987). A similar mechanism, in which both apoplastic sugar and water are derived from phloem influx, could not only greatly reduce xylem influx into post-veraison berries, but even reverse the flow direction. The increasing \( \pi_{\text{mc}} (=\text{RTC}_s) \) during ripening may not translate into an increasing \( P_{\text{mc}} \) (Thomas et al., 2004) due to the simultaneous accumulation of solutes in the apoplast \( (P_{\text{mc}} \approx \pi_{\text{mc}} \approx \pi_{\text{apoplast}}) \) and because \( \pi_{\text{mc}} \approx \pi_{\text{apoplast}} > 1 \text{ MPa} \) (Li and Delrot, 1987). Indeed, \( \pi_{\text{x}} \) \( (=\pi_{\text{apoplast}}) \), unless the two ‘compartments’ do not equilibrate inside the berry) of grape berry xylem exudate was found to increase in step with \( \pi_{\text{mc}} \) during ripening (Lang and Düring, 1991). At the same time, the high \( \pi_{\text{apoplast}} \) would also raise \( \pi_{\text{x}} (=\pi_{\text{mc}} + \pi_{\text{x}}) \). The proposed mechanism for the generation of hydrostatic xylem back-pressure in
grape berries would not only account for the observed pattern of dye movement after veraison but would also explain our inability to increase berry diameter (or tomato fruit diameter in the study of Davies et al., 2000) by root pressurization. The inverse relationship between dye penetration into berries and sugar concentration (rather than just the amount of sugar per berry) and the lack of correlation with berry size in this study are consistent with increases in \( \pi_{\text{apoplastic}} \) and \( P_s \) during ripening.

Estimations of net water flow into grape berries have usually been based on the assumption that pedicel girdling eliminates phloem flow without affecting xylem flow (Lang and Thorpe, 1989; Greenspan et al., 1994, 1996). However, interruption of phloem influx would render the direction of xylem flow independent of phloem flow and could effectively remove the main driving force for xylem efflux, which would result in systematic underestimations of both phloem influx and xylem efflux. Alternatively, partial occlusion of xylem conduits in girdled pedicels could increase \( r_h \) (Zwieniecki et al., 2004). However, there is no need to postulate hydraulic isolation of ripening fruit to account for any of our observations. Water recycling via the xylem would make fruits less responsive to phloem influx >xylem efflux+berry transpiration. The cracking of post-veraison Concord (but not Merlot) berries under root pressurization without any perceptible increase in diameter confirms that fruit expansion is limited by the elastic properties of the skin cell walls (Considine and Kriedemann, 1972; Matthews et al., 1987).

One implication of our results is that, in addition to xylem conduits, membrane integrity, and thus cellular compartmentation, also apparently remain intact in ripening grape berries. Although this contradicts the conclusion reached by Lang and Thorpe (1989) and Lang and Dürring (1991), their results are actually consistent with apoplastic phloem unloading, and continued hydraulic connection and cell compartmentation. While our interpretation allows for some diffusional loss of solutes from the berries, the ‘damage’ should be small, since the proportion of the berry volume occupied by the apoplast is very small (Hardie et al., 1996; Diakou and Carde, 2001). Solutes leaking back to the pedicel also could be actively reabsorbed ‘en route’ by the transport phloem (Patrick and Offler, 1996; van Bel, 2003). Moreover, diffusion would be far slower than active removal into the sink symplast by transporters and channels. In fact, the expression in ripening grape berries of membrane proteins, such as sucrose (Davies et al., 1999; Ageorges et al., 2000) and hexose transporters (Fillion et al., 1999), and aquaporins (Picaud et al., 2003), is incompatible with the idea of compartmentation breakdown. Membrane disintegration should result in rapid downregulation of membrane-associated proteins. Thus, solute permeability of the plasma membrane and tonoplast may differ for influx and efflux, since solutes are actively ‘pumped’ into the vacuoles and retained there, while water can diffuse in and out down the \( \Delta \Psi \).

Nevertheless, vacuolar water is likely to be relatively well protected from being pulled back into the berry tracheids and shoot xylem vessels. The rising solute concentration inside the vacuoles decreases \( \Psi_{\text{mc}} (=P_{\text{mc}} - RTC_s) \) and makes it more and more difficult for the leaves to ‘extract’ water from the ripening berries. Moreover, since water moving out of the pericarp has to cross both the tonoplast and plasma membrane, closure of aquaporins upon loss of turgor (Chrispeels et al., 1999) would greatly increase \( r_h \) across the pericarp cell membranes. Nevertheless, prolonged plant water stress coupled with declining phloem influx and continued (albeit very slow) cuticular berry transpiration (Rogiers et al., 2004) may lead to slow water loss from the berries. Therefore, berries may shrink if phloem influx becomes too slow to balance water efflux.

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References


