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Developmental changes in the diurnal water budget of the grape berry exposed to water deficits

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ABSTRACT

The diurnal water budget of developing grape (*Vitis vinifera* L.) berries was evaluated before and after the onset of fruit ripening (veraison). The diameter of individual berries of potted 'Zinfandel' and 'Cabernet Sauvignon' grapevines was measured continuously with electronic displacement transducers over 24 h periods under controlled environmental conditions, and leaf water status was determined by the pressure chamber technique. For well-watered vines, daytime contraction was much less during ripening (after veraison) than before ripening. Daytime contraction was reduced by restricting berry or shoot transpiration, with the larger effect being shoot transpiration pre-veraison and berry transpiration post-veraison. The contributions of the pedicel xylem and phloem as well as berry transpiration to the net diurnal water budget of the fruit were estimated by eliminating phloem or phloem and xylem pathways. Berry transpiration was significant and comprised the bulk of water outflow for the berry both before and after veraison. A nearly exclusive role for the xylem in water transport into the berry was evident during pre-veraison development, but the phloem was clearly dominant in the post-veraison water budget. Daytime contraction was very sensitive to plant water status before veraison but was remarkably insensitive to changes in plant water status after veraison. This transition is attributed to an increased phloem inflow and a partial discontinuity in berry xylem during ripening.

Key-words: Vitis vinifera; berry; grape; growth; leaf water potential; phloem; transpiration; vascular water flow; Vitaceae; xylem.

INTRODUCTION

Diurnal variations in the size of fleshy fruit are common (Elfving & Kaufmann 1972; Tromp 1984; Johnson, Dixon & Lee 1992), superimposed on growth and usually attributed to changes in hydration (Kozlowski 1972). It is important to elucidate the nature of such variations in order to model accurately growth and water transport (Lee 1990; Bussieres 1993), and because fruit size is often an important economic factor. For example, berry size in grapevine is a quality factor of fresh, raisin and wine grapes (Webb 1981), and the timing of harvest could exploit such diurnal cycles if warranted.

Diurnal fruit growth occurs when the nighttime expansion in volume exceeds the daytime contraction. These diurnal fluctuations arise from changes in water flows into and out of the fruit. Water uptake from the parent plant presumably occurs through the low-resistance pathways of the phloem and xylem tissues. Outflow occurs via transpiration and in some cases (e.g. cowpea, Pate 1988; apple, Lang 1990) through the xylem, but the prevalence of water 'recycling' has not been established. This is an important conceptual issue since a water potential gradient generated both by transpiration (Lee, Dixon & Johnson 1989; Lee 1990) and by an osmotic gradient (Lang & Düring 1991) has been implicated in the control of fruit growth and water economy.

The relative contributions of the components of the fruit water budget are experimentally difficult to access. There is indirect evidence that the relative contributions of xylem and phloem to the diurnal water budget of fruit vary with species and development. From measurements of fruit transpiration and mass, Pate and coworkers estimated that water was supplied by xylem and phloem, approximately 60:40 (v/v), to white lupin fruit (Pate, Sharkey & Atkins 1977), and that the phloem supply was greater than 100% of the fruit water gain in cowpea (Peoples *et al.* 1985; Atkins, Pate & Peoples 1986). In cowpea, fruit recycled 23% and 70% of supplied water early and late in fruit development, respectively (Peoples *et al.* 1985). From measurements of fruit mass, transpiration and Ca influx, Ho, Grange & Picken (1987) estimated that the water supply to tomato fruit was predominately via the phloem, increasing from ca. 85% to ca. 95% of the total water uptake during fruit development.

For the grape berry, which also undergoes diurnal expansion and contraction (Coombe & Bishop 1980; Nakano 1989), Lang & Thorpe (1989) produced evidence that water transport to a ripening berry was similar to that in tomato, i.e. predominately via the phloem. However, grape berry development differs from that of tomato. In the long term, increase in berry size is multiphasic, occurring in two rather distinct periods (Stages I and III) separated by a lag phase (Stage II) of little or no growth (Coombe & Bishop 1980; Matthews, Cheng & Weinbaum 1987a).

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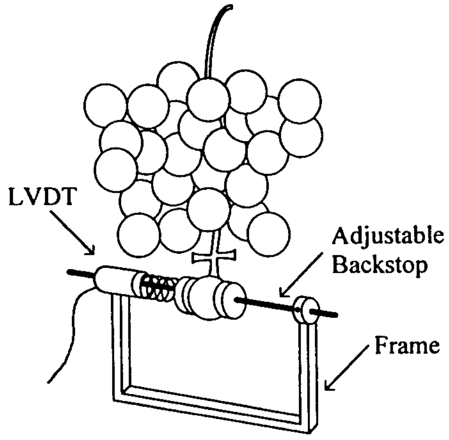

Figure 1. Diagram showing the placement of a linear variable differential transformer (LVDT) on a berry for continuous measurement of berry diameter. A plastic disk LVDT core was appressed to the berry surface with a 9·5 *g* spring, and the berry was immobilized with an adjustable backstop.The frame was mounted to a ring stand with clamps. When necessary, neighbouring berries were removed to provide clearance for the device.

Also, part of the xylem vasculature within the berry may lose integrity upon the transition to Stage III (Düring, Lang & Oggionni 1987; Findlay *et al.* 1987) and, hence, become a much higher resistance pathway.

Water deficits result in reduced size of harvested berries (Williams & Matthews 1990), changes in fruit composition (Matthews & Anderson 1988), and significant differences in wine sensory attributes (Matthews *et al.* 1990). However, little is known about the response of diurnal fruit water relations to water deficits. Changes in berry size, via hydration/dehydration or inhibited growth, may alter fruit composition by simple dilution or concentration of flavour components. This study is based on our earlier work which showed significant differences in growth inhibition and solute accumulation between berries exposed to water deficits during Stage I and Stage III (Matthews, Anderson & Schultz 1987b; Matthews & Anderson 1988, 1989). Experiments were conducted to determine the extent to which reduced berry size could be attributed to contraction during water deficits and to test the hypothesis that phloem is the predominant source of water throughout berry development.

MATERIALS AND METHODS

Plant material and growth conditions

Two- or three-year-old 'Cabernet Sauvignon' and 'Zinfandel' grapevines (*Vitis vinifera* L.) were grown from dormancy in a soil:peat:perlite (1:1:1) mixture in 20 dm^3 pots in a growth chamber [14 h photoperiod, 34/20 ± 2 °C, 50/90 ± 5% relative humidity (RH) and PPFD of 700–900 µmol photon m^{-2}s^{-1}]. The day/night transitions were ramped over a 1 h period. The vines were pruned such that two to four shoots with two clusters per shoot were retained per vine and the shoots were trained horizontally to promote a uniform light environment. The plants were automatically irrigated to soil saturation twice daily, and nutrients were supplied by a complete, slow-release fertilizer (15-15-15 + micronutrients) applied when the shoots were ca. 8 cm in length. The shoots attained a length of ca. 2·5 m at veraison (48–56 d post-anthesis) and grew little thereafter. These are red wine grape varieties for which veraison was identified as the first appearance of red colour.

Measurement of berry diameter and volume

To determine the diurnal pattern of fruit size, the diameters of six berries were continuously and simultaneously measured using Linear Variable Differential Transformers (LVDT, Schaevits Engineering, Pennsauken, NJ) mounted in a custom frame constructed from hollow brass bar stock (Fig. 1). A return spring, applying a force of ca. 9·5 *g*, provided proper contact of the device with the berry. Relative to diurnal fluctuations in berry diameter (typically 0·05–0·2 mm), LVDT sensitivity to environmental changes was insignificant (±0·005 mm) when several diurnal cycles were recorded with the device's adjustable backstop placed against the moveable core. Thermal expansion of berries was also considered to be insignificant because: (i) for a berry diameter of 10 mm (volume = 1570 mm^3), a 15 °C change in temperature resulted in only a ca. 1·5 mm^3 change in volume compared to observed xylem and phloem flows of the order of 80–100 mm^3 (see 'Results'); (ii) thermal changes occurred only at day/night transitions. Experimental berries were selected randomly, and neighbouring berries were removed to allow clearance for the LVDT attachment and movement. The daily growth of berries monitored by transducers did not differ from that of undisturbed fruit as determined with a hand-held micrometer.

A data logger (Campbell Scientific, Logan, UT) recorded the transducer output once per minute, and the mean of each 15 min period was logged to obtain the complete diurnal record of berry diameter. The amplitude of diurnal contraction was calculated as the difference between the maximum diameter attained during the dark period and the minimum diameter reached during the following light period. Berry volume was estimated from a regression of volume on diameter from a random sampling of berries at both pre- and post-veraison stages (Fig. 2). For this calibration, the volume of water displaced by each sampled berry was used to determine berry volume, and a micrometer was used to measure berry diameter at the equator. From the regressions, berry volume could be estimated to within ±5 m^3 before veraison and ±10 mm^3 after veraison at $p \leq 0{\cdot}05$.

Transpiration restriction experiments

In some experiments, transpiration of the shoot or the experimental berry was restricted at the beginning of the

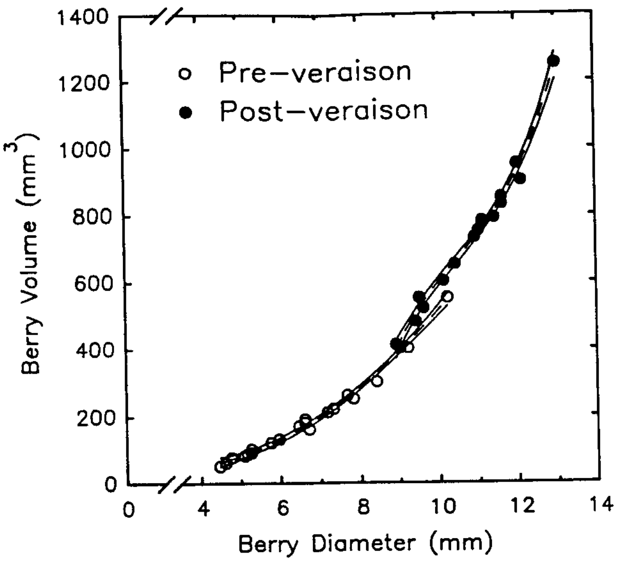

Figure 2. Regression (dashed line) of berry volume on diameter measurements for 'Cabernet Sauvignon' berries pre-veraison (open circles; $n=52$, $r^2=0{\cdot}98$) and post-veraison (filled circles; $n=23$, $r^2=0{\cdot}99$). Second- and third-order polynomials were used for the pre-veraison and post-veraison berries, respectively. The polynomial order was chosen to be the lowest order for which the r^2 was at least 0·95. The 95% confidence interval is indicated by the outer curves.

day while the berry diameter was logged throughout a diurnal cycle. Shoot transpiration was restricted by covering the entire shoot, except for the cluster, with a polyethylene film bag. Berry transpiration was restricted by coating the experimental berry with a thin layer of petrolatum up to the point of pedicel attachment. Treatments were imposed and diurnal data collected on two consecutive days beginning 27 d before veraison (23 and 24 d post-anthesis) and 12 d after veraison (62 and 63 d post-anthesis). Two days with no imposed restriction separated the shoot from the berry experiments, this interval serving as a control. Six berries were measured simultaneously, yielding 12 replications per treatment per growth stage, although slippage of the LVDT required the elimination of two replications each from three of the experiments.

Berry water budget analysis

The xylem, phloem and berry transpiration components of the fruit water balance were estimated pre- and post-veraison using methods similar to those of Lang & Thorpe (1989) and Lang (1990). The diameters of three berries per plant were simultaneously measured using three LVDTs. The berries were subjected to three treatments: (1) the berry was left intact (no manipulation); (2) the pedicel was girdled by heat produced by passing an 11 amp electric current for 5 s through a 26 ga Ni–Cr wire encircling, but not contacting, the pedicel; and (3) the berry was severed at the pedicel, the cut end was sealed with petrolatum and the berry was resuspended in its original position.

For well-irrigated vines, the experiments were performed simultaneously on two plants for two consecutive days starting at 17 d before veraison (33 and 34 d post-anthesis) and 4 d after veraison (54 and 55 d post-anthesis). Treatments were imposed at the beginning of each day and handling of the berry was minimized. Measurements from girdled or cut berries were analysed for one diurnal measurement only, although the observed patterns continued for at least 2 d following treatment. These experiments were repeated on plants subjected to a short-term water deficit before and after veraison.

The three treatments made it possible to separate the three water flow components using a linear combination of the diurnal volume measurements. The phloem component is determined by subtraction of the girdled berry volume (xylem and transpiration intact) from the intact berry volume (phloem, xylem and transpiration intact). The xylem component is determined by subtraction of the detached berry volume (transpiration intact) from the girdled berry volume. Finally, the berry transpiration component is determined directly from the detached berry volume measurement. A scaling factor was applied to normalize the data from each berry to a mean initial volume.

Diurnal contraction during water deficits

To determine the effects of plant water status on berry contraction, water deficits were imposed at both pre- and post-veraison stages by withholding irrigation. Pre-veraison measurements were made 37 to 46 d post-anthesis. The post-veraison water deficit was imposed 2 to 3 d after the first appearance of red skin colour (approximately 58 d post-anthesis). Midday leaf water potential was measured using the pressure chamber technique (Scholander *et al.* 1965; Turner 1981). The sampled mid-shoot leaves were bagged in polyethylene film prior to excision, and the excised leaves were placed immediately into a humidified pressure chamber, where pressure was increased slowly during measurement.

RESULTS

Berries exhibited a diurnal pattern of expansion at night and contraction during all or part of the day throughout development (Figs 3a,b). Berry diameter responded rapidly to the environmental conditions of the growth chamber by exhibiting distinct transitions from expansion to contraction at the beginning of the day and from contraction to expansion at the beginning of the night. There was a striking difference, however, between the amplitudes of the pre- and post-veraison diurnal fluctuations in berry diameter. The well-watered, pre-veraison berry contracted for several hours after the day began. The daytime contraction of representative 'Zinfandel' fruit was ca. 0·18 mm (1·5% of diameter) 15 d prior to veraison (Fig. 3a) and ca. 0·05 mm (0·3% of diameter) on the same day as fruit colouration (Fig. 3b). Similar patterns were observed

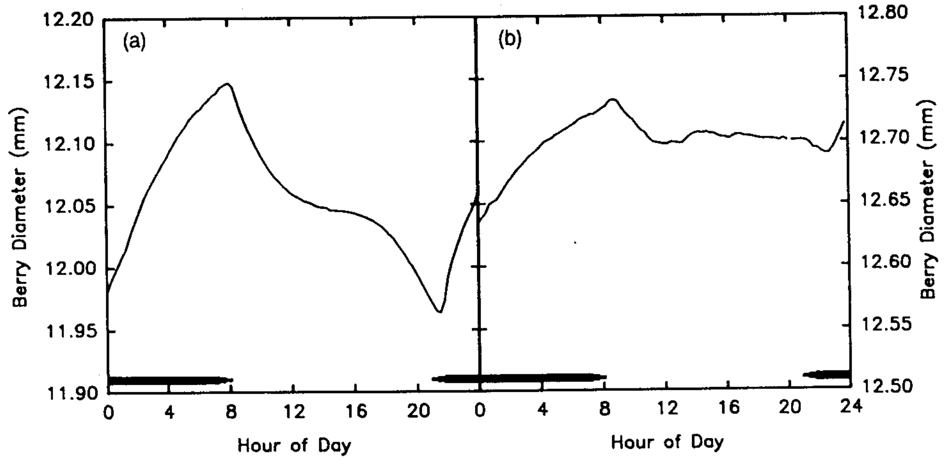

Figure 3. Representative diurnal patterns of diameter for 'Zinfandel' berries (a) before the onset of veraison (37 d post-anthesis) and (b) at the onset of veraison (61 d post-anthesis). Horizontal bars indicate the dark period; changes in environment were ramped over a 1 h period.

in 'Cabernet Sauvignon', although the pre-veraison contraction was less. The transition from the pre- to post-veraison pattern was rapid, being completed in 2 or 3 d. Reduced contraction was frequently observed 1 or 2 d prior to initial berry colouration.

Restriction of shoot and berry transpiration

While control berries exhibited contractions of 0·56% of diameter, restriction of shoot transpiration 25 d prior to veraison reduced the berry contraction to 0·06% of diameter (Fig. 4). The reduction in diurnal contraction from restriction of berry transpiration was only about half as great, resulting in contraction that was ca. 0·32% of diameter. This suggested a large role for shoot transpiration in the pre-veraison water budget of the berry. Similar experiments conducted ca. 12 d post-veraison revealed a diminished influence of shoot transpiration on berry contraction. At that stage, berry contraction was 0·21 to 0·14% of diameter for control and shoot-bagged vines, respectively, but the difference was not statistically significant. In contrast, post-veraison restriction of berry transpiration again significantly reduced diurnal contraction, although the contraction was less than in pre-veraison experiments.

Pre- and post-veraison analysis of berry water budget

Representative diurnal patterns of the cumulative contribution of xylem, phloem and transpiration components to the net volume change of the well-irrigated berry are shown in Fig. 5. At both stages of development large gains via vascular connections and large losses via berry transpiration resulted in relatively small net volume changes (Figs 5a,b). Diurnal changes in net volume were largely paralleled by changes in transpiration, but xylem and phloem contributions also exhibited distinct diurnal patterns with most flow occurring during the day. For the examples shown, the berry water inflow at both the pre- and post-veraison stages was comprised primarily of a single vascular component: xylem during pre-veraison and phloem during post-veraison.

During most of the contraction of pre-veraison berries, vascular inflow was nil, while water was lost via transpiration (Fig. 5a). Xylem inflow then increased to just balance transpiration outflow when contraction ceased. Both transpiration and xylem inflow rates decreased at night. The well-watered, post-veraison berry exhibited much less contraction, as the phloem inflow increased simultaneously with increased transpiration outflow (when light

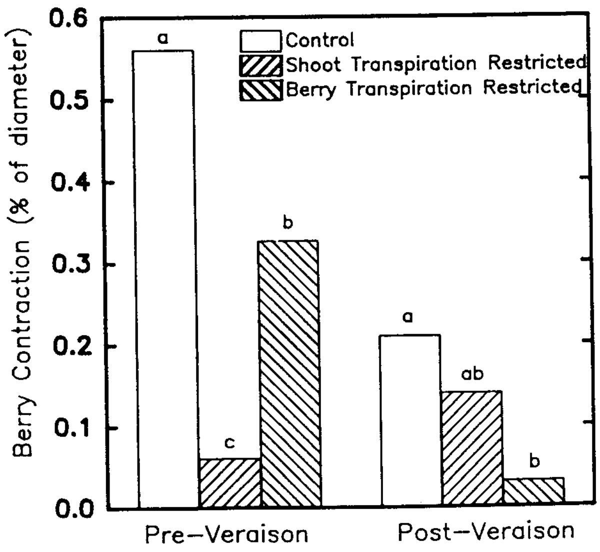

Figure 4. Diurnal contraction of 'Cabernet Sauvignon' berries when treatments were imposed to restrict shoot or berry transpiration. Means with the same letter within each stage of development are not significantly different (Fishers LSD) for $p<0·05$; $n=10$ for pre-veraison shoot and post-veraison berry and shoot transpiration treatments, and $n=12$ for all other treatments.

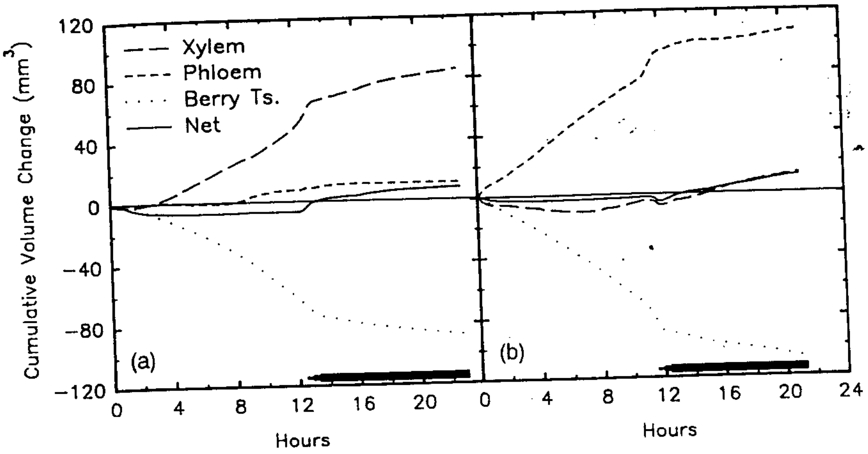

Figure 5. Typical plot of the cumulative water flow for each component of the diurnal water budget for 'Cabernet Sauvignon' berries during the (a) pre-veraison and (b) post-veraison stages of development. Water flow was estimated from a linear combination of diurnal volume changes in intact, excised and girdled berries (for details, see 'Materials and Methods'). Plants were well irrigated. The solid line represents the net water flow, the long-dashed line the xylem water flow, the short-dashed line the phloem water flow, and the dotted line the berry transpiration.

intensity increased) (Fig. 5b). Phloem inflow balanced transpiration outflow throughout the day, while very little water flowed via xylem.

The change in the primary water source for berry expansion that was suggested by the individual berry data of Figs 5a and b was confirmed when repetitions ($n=4$) with irrigated vines were pooled. Cumulative water flows for each component at the end of the measurement day showed that the xylem functioned as the source before veraison, providing ca. 75 mm^3 in 24 h; the phloem played a substantial but secondary role (Fig. 6). This was reversed after veraison, when the xylem supply diminished greatly, and the phloem became the major vascular pathway for inflow, supplying more than 80 mm^3. For well-irrigated vines, berry transpiration provided the primary pathway for berry water loss during both pre- and post-veraison stages. Under well-watered conditions, we observed net backflow over a 24 h period from the xylem only once after veraison and at no time before veraison.

When water was withheld, diurnal berry contraction increased by the second day, and there was a transient loss of water (approximately 60 mm^3) via xylem backflow at midday, eliciting a sharp contraction in berry volume (Fig. 7a). This was in contrast to well-watered conditions where there was essentially no water loss attributable to xylem backflow (Fig. 5a). Note that the contraction began hours before the onset of xylem backflow, indicating that the vascular inflow rate was insufficient to balance the transpirational outflow during that time interval. The water-stressed, pre-veraison berry continued to contract longer into the day than the well-watered berry because the small phloem and xylem inflows were insufficient to balance transpiration. Xylem inflow resumed during the night, allowing for the recovery of berry volume. A decreasing phloem inflow accompanied the recovery in volume.

When a similar experiment was conducted post-veraison, berry contraction was not increased (Fig. 7b). The phloem inflow was markedly reduced, providing a net diurnal inflow of only ca. 35 mm^3, compared to a typical inflow of nearly 90 mm^3 for an irrigated vine (Fig. 6). The reduced phloem inflow was compensated by additional xylem inflow, which comprised a substantial proportion of the post-veraison water flow only in stressed vines. Consequently, contraction was slight.

Estimates of the daytime flow rate for each component in the berry water budget indicated that, at both stages of

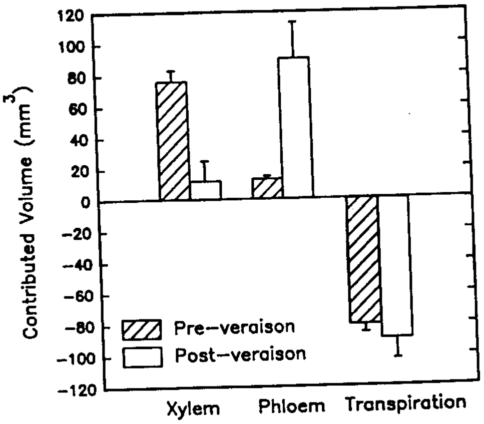

Figure 6. Pooled data for pre- (open bars) and post- (hatched bars) veraison measurements of daily water flow for each component of the water budget of well-irrigated 'Cabernet Sauvignon' berries. Water flow was estimated from a linear combination of diurnal volume changes in intact, excised and girdled berries (for details, see 'Materials and Methods'). Error bars represent one standard error, $n=4$.

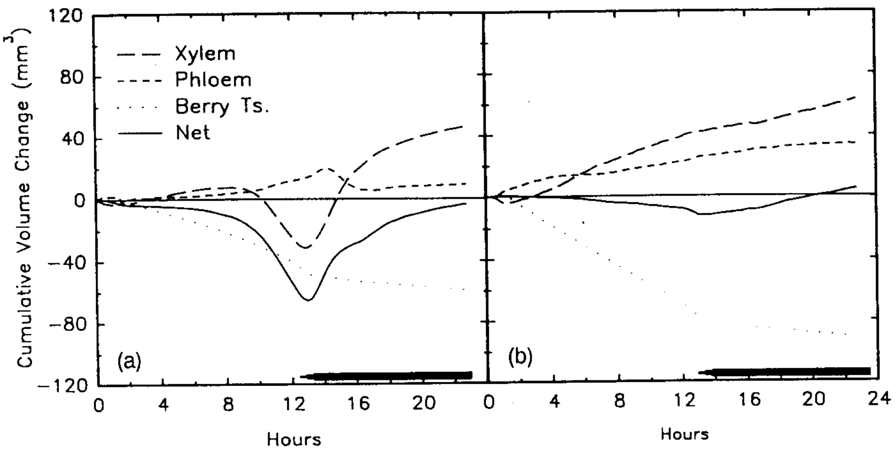

Figure 7. Cumulative water flow for each component of the diurnal water budget of a berry from a 'Cabernet Sauvignon' vine when irrigation was withheld for 1 d prior to treatments and measurements: (a) pre-veraison; (b) post-veraison. Water flow was estimated from a linear combination of diurnal volume changes in intact, excised and girdled berries (for details, see 'Materials and Methods'). Lines are as in Fig. 5.

development, the water inflow rate via xylem plus phloem was only slightly less than the rate of berry transpiration (Table 1). Xylem inflow rate in the light was nearly twice the rate in the dark pre-veraison, but was negligible after veraison. After veraison, the daytime phloem flow rate was more than 3 times the rate in the dark and 10 times the pre-veraison rate.

Effect of water deficit on diurnal contraction

The difference in net volume change observed during the water deficit experiments described in Fig. 7 suggested a developmental change in the sensitivity of berry growth and contraction to low water availability. A series of experiments was therefore conducted to test this. During well-irrigated periods, midday leaf water potentials were within the range of −0·80 to −1·00 MPa before veraison and −0·90 to −1·10 MPa after veraison. The leaf water potential of well-irrigated plants decreased slightly throughout ontogeny, a phenomenon that has been observed in field-grown grapevine (Matthews *et al.* 1987a) and other crops well supplied with water. In pre-veraison berries, diurnal contraction was quite sensitive to vine water status and exhibited a linear relationship ($r^2 = 0·80$) with decreases in leaf water potential (Fig. 8). Daily contraction increased by approximately 2% of diameter for each 0·10 MPa decrease in midday leaf water potential. At severe water deficits, contractions of up to 15% of berry diameter (approximately 39% of volume) were observed. At this water status ($\Psi_l = -1·40$ MPa), the berries were visibly shrivelled during the day, but regained much of their volume during the night.

In contrast, there was a sharply diminished effect of water status on the diurnal contraction of post-veraison berries. Water deficits creating midday leaf water potentials ranging from −1·0 to −1·7 MPa had very little effect on the amplitude of the contraction (Fig. 8). Indeed, post-veraison contraction was never observed to be greater than the well-irrigated pre-veraison berry contraction, although the leaves displayed considerable wilting during the more severe water deficits. The patterns of and contrasts between pre- and post-veraison contractions on a per cent of diameter basis were virtually identical to comparisons on a volume basis.

	Flow rate			
	Pre-veraison		Post-veraison	
Component	light	dark	light	dark
Xylem (mm^3 h^{-1})	4·63 ± 0·30	2·57 ± 0·14	−0·18 ± 0·91	1·37 ± 0·38
Phloem (mm^3 h^{-1})	0·52 ± 0·28	0·32 ± 0·15	5·79 ± 1·99	1·62 ± 0·32
Transpiration (mm^3 h^{-1})	−5·41 ± 0·28	−1·79 ± 0·04	−5·95 ± 1·19	−1·39 ± 0·38
Transpiration (mmol cm^{-2} h^{-1})	−0·118 ± 0·006	−0·039 ± 0·001	−0·099 ± 0·020	−0·023 ± 0·006

Table 1. Flow rates during light and dark periods for each component of the grape berry water budget, estimated from a linear combination of diurnal volume changes in intact, excised and girdled berries (for details, see 'Materials and Methods') in experiments conducted pre- and post-veraison. Data are means ± standard errors for four replications with well-irrigated vines

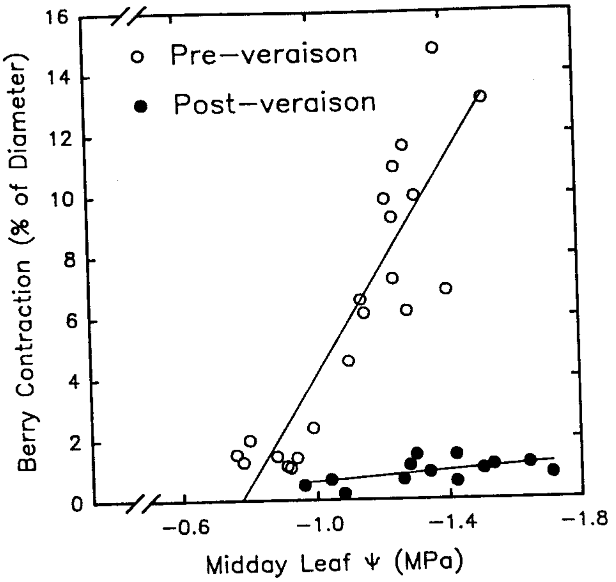

Figure 8. Effect of plant water status on the amplitude of diurnal berry contraction for 'Zinfandel' berries at pre-veraison (open circles, $r^2 = 0{\cdot}80$) and post-veraison (filled circles, $r^2 = 0{\cdot}26$) stages of development. Water stress was induced by withholding irrigation for several days during each developmental stage.

DISCUSSION

These results demonstrate that the grape berry exhibits significant diurnal expansion and contraction even when well watered. Under growth chamber conditions of 34 °C and 50% RH days, virtually all berry growth occurred during the night; day periods were characterized by either contraction or absence of expansion. This pattern was greatly modified after veraison such that daily contraction was less than 50% of that observed before veraison and the sensitivity of daily contraction to water deficits was virtually eliminated. There are earlier reports showing repetitive shrinking and swelling in grapes (Shimomura 1967; Coombe & Bishop 1980; Nakano 1989). However, the developmental transition shown here evidently has not been documented previously. The reduced sensitivity of berry hydration to plant water status that occurred after veraison may partially explain the greater role of pre-veraison than post-veraison water deficits in determining fruit size and composition in the field (Hardie & Considine 1976; Matthews *et al.* 1987b; Van Zyl 1984).

Vascular flows in the developing berry

Prior to veraison and under well-watered conditions, the bulk of vascular water flow from parent plant to the berry occurred via the xylem (Figs 5 & 6). During this developmental stage, the phloem contributed only ca. 12% of the total inflow. When shoot transpiration was restricted, the marked reduction of diurnal contraction (Fig. 4) indicated that there was a strong hydraulic connection between the fruit and shoot at this developmental stage. Despite this strong connection there was no evidence of vascular water flow from transpiring berries to the shoot for well-watered vines under our growth conditions. Hence, the decreased berry contraction that occurred when the shoot was bagged was probably the result of increased xylem inflow almost matching berry transpiration. Our observation that contraction was not eliminated when berry transpiration was eliminated (Fig. 4) would appear to be in contradiction with our hypothesis of no backflow under well-watered conditions. However, it simply indicates that backflow can occur when evaporative water loss is prevented artificially, whereas under control conditions (Fig. 5) backflow did not occur.

Several observations imply a diminished hydraulic conductance in the xylem from berry to parent plant after veraison. Greatly reduced xylem flow was measured after veraison. The decrease in berry water potential after veraison (Matthews *et al.* 1987a) without a corresponding increase in inflow suggests an increased resistance to berry water inflow. Compared to pre-veraison experiments, both the reduced effect on contraction of restricting shoot transpiration and the reduced contraction observed when restricting berry transpiration are also circumstantial evidence of impaired xylem water exchange with the parent plant.

Düring *et al.* (1987) and Findlay *et al.* (1987) suggested that xylem function becomes disrupted in grape berries at veraison. In both studies, (i) staining of lignin in the berry revealed evidence of stretching or gaps in the xylem appearing at or after veraison, and (ii) dye uptake from cut pedicels and transport into the berry were diminished after veraison. The direct implication of these observations within berries on water exchange between berry and parent plant is not clear. Disrupted xylem within the berry probably contributes to a reduced conductance to the parent plant. However, it is unlikely that the berry becomes completely hydraulically isolated since symplastic and apoplastic pathways occur in parallel in the plant. Also, in the study of Düring *et al.* (1987), dye movement declined gradually over a 2-week period and anatomical evidence indicated disruption of the dorsal vasculature only; the ventral vessels of the berry remained largely intact. Thus, some water movement may also occur via remaining intact xylem conduits and the non-vascular apoplast.

In experiments with post-veraison 'Italia' berries, Lang & Thorpe (1989) reported that significant xylem backflow occurred and that phloem inflow was responsible for all water inflow, but the measurements may not have been replicated. We occasionally observed backflow during the day, but positive flow during the night caused the daily net xylem flow to be slightly positive (uptake) in all but one replication. The xylem (or apoplast) appears to contribute to the recovery of berry volume during the night, as indicated by the positive nighttime xylem flow in the irrigated, post-veraison vine.

We suggest that xylem flow is reduced but not eliminated at veraison, and that its post-veraison reduction under irrigated conditions arises in part from an increase in phloem inflow. After veraison, phloem became the primary source of berry water, contributing more than 80% of the total inflow under well-watered conditions. The diurnal behaviour of berry size when the transpiration of leaves or fruit was restricted, when xylem and phloem transport was interrupted, and when water deficits were imposed was consistent with an abrupt shift from xylem to phloem at veraison. It is clear that there is an increased rate of sucrose translocation to the fruit at this time (Brown & Coombe 1985; Coombe, Bovio & Schneider 1987), and this must be associated with an increased transport of the solvent water through the phloem. The estimated inflow rate through the phloem increased ca. 10 times after veraison.

Partial maintenance of the xylem path could serve as a means to recycle excess water delivered via the phloem back to the plant, as shown by Pate *et al.* (1985) and Peoples *et al.* (1985) for cowpea fruit. This capability may be particularly important in fleshy fruits such as grape berries that crack. Coincidently, the partial disruption of xylem function, thus limiting water export, combined with increased phloem inflow may contribute significantly to the resumption of growth in the ripening berry.

Berry transpiration

Water budget experiments (Figs 5, 6 & 7) and transpiration restriction experiments (Fig. 4) showed that, despite high diffusive resistance (Nobel 1975; Blanke & Leyhe 1988), berry transpiration remains an important pathway for berry water outflow throughout fruit development and appears to be the primary path for fruit water loss, at least in well-watered plants. Indeed, gas exchange measurements of berry transpiration also indicate that it is sufficient to comprise a substantial portion of the diurnal berry water budget (Düring & Oggionni 1986; Frieden, Lenz & Becker 1987). Inhibition of berry transpiration markedly reduced the diurnal contraction before and after veraison (by ca. 45 and 80%, respectively).

Blanke & Leyhe (1987) found significant cultivar variability as well as developmental changes in berry transpiration rate. Our measurements of berry water budget components gave estimates of the berry transpiration rate (Table 1) that are in good agreement with their measurements. Errors in our estimates due to excision or desiccation are unlikely to be great because in most experiments berries lost small fractions of the total volume and the rate of transpiration was steady throughout the day period. Both studies indicate that when expressed on a surface area basis the rate of transpiration declines during fruit development, presumably as a result of continued deposition of surface waxes (Rosenquist & Morrison 1988), but the volume of water lost per berry changes little.

Low water status is expected to have little effect on berry transpiration because of the scarcity of stomata on the berry and a diminishing stomatal function throughout fruit development. Blanke & Leyhe (1987) observed a decreasing response of berry transpiration rate to light throughout development. However, there are no data available regarding the response of berry transpiration to decreasing plant water status.

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Berry contraction during plant water deficits

Prior to veraison, fruit contraction increased dramatically as plant water status declined. The amplitude of the pre-veraison diurnal contraction was well correlated with decreasing leaf water potential, as was the case in citrus (Elfving & Kaufmann 1972; Maotani, Machida & Yamatsu 1977) and apple (Tromp 1984). We consistently observed reversed xylem flow when water was withheld pre-veraison, and this was apparently the source of the increased contraction in excess of that observed in well-watered controls. Our estimates indicate that transpiration alone can account for only about one-half of the 39% contraction in volume (15% of diameter) observed at leaf water potentials of ca. −1·4 MPa. Contraction in excess of transpirational water loss must have been due to backflow. Thus, the high sensitivity of berry enlargement to low water status pre-veraison (Williams & Matthews 1990) is in part a result of xylem conduction in both directions during that period.

The diurnal excursions in diameter of the post-veraison berry were insensitive to low water status. We attribute this transition both to diminished hydraulic continuity of the berry xylem through the pedicel xylem and to greatly increased water inflow through the phloem. Increased hydraulic resistance of the pathway from pericarp tissue to xylem vessels in the pedicel would render negative $\Delta\Psi_{\text{pedicel–berry}}$ gradients less effective in removing water from the berry. In addition, the rate of water inflow through the phloem will be determined in part by metabolically active sugar transport, which increases at veraison (Coombe *et al.* 1987). Hence, the transition to phloem-sourced water can be considered as imparting dehydration avoidance to the berry because translocation is less sensitive to low water status in the phloem than in the xylem. The transition from large to small diurnal contractions and the reduction in sensitivity of berry contraction to plant water status were observed only 2 d after veraison. Although hexose accumulation during ripening constitutes osmotic adjustment, it is unlikely that the observed change in water flow patterns is primarily due to increasing sugar concentrations because berry osmotic and water potentials decrease only ca. 0·067 MPa d^{-1} (Matthews *et al.* 1987a). Furthermore, the water budget data indicate a decrease in xylem inflow after veraison, whereas an effective increased gradient should increase flow. Consequently, the post-veraison desiccation resistance of the berry may be a manifestation of both a strong phloem component budget at veraison and a loss of xylem conductance. Whereas the loss of xylem function and conductance, for example from cavitation, is potentially disastrous for most higher plant tissues (Tyree & Ewers 1991), when the water requirements can be met by phloem flow, as in grapes and perhaps other strong sinks, it may constitute an adaptive mechanism that prevents dehydration and, in fruits, disruption of the ripening process. The apparent post-veraison resistance of the berry to water loss indicates that moderate vine water deficits during the ripening period are unlikely to affect fruit composition directly via berry hydration. Changes in the concentration of amino acids, phenolics and anthocyanins in response to late-season vine water deficits reported earlier (Matthews & Anderson 1988) are more probably a result of berry responses to low vine water status than a result of a concentration of berry solutes via loss of water.

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