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Direct *in situ* measurement of cell turgor in grape (*Vitis vinifera* L.) berries during development and in response to plant water deficits

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ABSTRACT

***Vitis vinifera* L. berries are non-climacteric fruits that exhibit a double-sigmoid growth pattern, and at the point known as 'veraison', which is just before the beginning of the second period of rapid fruit growth, these berries undergo several abrupt physiological changes. Cell pressure probe was used to examine the *in situ* turgor (P) of cells in the mesocarp during berry development and in response to plant water deficits. Initial tests comparing attached and detached berries demonstrated that cell P was stable for up to 48 h after detachment from the vine, provided that water loss from the berry was prevented. Cell P at pre-dawn was on the order of 0.25 MPa pre-veraison (PreV) and was reduced by an order of magnitude to 0.02 MPa post veraison (PostV). Cell P declined slightly but significantly with depth from the berry surface PreV, but not PostV. When water was withheld from potted vines, cell P declined about 0.2 Mpa, as pre-dawn vine water potential declined about 0.6 MPa over 12 d, whereas cell P was completely insensitive to a 1.10 MPa decrease in pre-dawn vine water potential after veraison. Rewatering of stressed plants also resulted in a 24 h recovery of cell P before, but not after veraison. The substantial decline in cell P around veraison is consistent with the decline in berry firmness that is known to occur at this time, and the PostV insensitivity of P to changes in vine water status is consistent with current hypotheses that the PostV berry is hydraulically isolated from the vine. The fact that a measurable P of about 0.02 MPa and typical cell hydraulic/osmotic behaviour were exhibited in PostV berries, however, indicates that cell membranes remain intact after veraison, contrary to many current hypotheses that veraison is associated with a general loss of membrane function and cellular compartmentation in the grape berry. We hypothesize that cell P is low in the PostV berry, and possibly other fleshy fruits, because of the presence of regulated quantities of apoplastic solutes.**

Key-words: apoplast; cell pressure probe; compartmentation; membrane integrity; symplast; veraison; water deficit.

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INTRODUCTION

Many fleshy fruits exhibit a double-sigmoid growth pattern and accumulate substantial quantities of solutes (sugars) as part of normal development (Coombe 1976). Grape (*Vitis vinifera* L.) berries are typical of this developmental pattern, with initial (stage I) growth consisting of cell division and cell enlargement, followed by reduced growth during seed maturation (stage II), and final rapid berry growth and solute accumulation (stage III). The accumulation of solutes, predominately in the form of glucose and fructose, causes the tissue osmotic potentials of grape berries to reach –3 to –4 MPa (Matthews, Cheng & Weinbaum 1987b). The low osmotic potentials of fleshy fruits are typically assumed to be important for fruit growth and water relations because they allow the development of low total water potentials, and hence the maintenance of a favourable water potential gradient from the plant to the fruit, for continued fruit growth and water uptake (e.g. Grange & Andrews 1994). Of course, it must be recognized that low fruit osmotic potentials will only result in low fruit total water potentials if the fruit is essentially separated from the plant by an intact, semipermeable membrane. At the cell level, low osmotic potentials will also only result in low total water potentials if turgor (P) is low, and hence the level of cell P is central to an understanding of the consequences of solute accumulation to fleshy fruit growth and water relations. For instance, low osmotic potentials at the stylar end of *Prunus domestica* fruits were associated with irrigation-induced cracking (skin mechanical failure and fruit flesh expansion) at the stylar end of the fruit, presumably the result of higher cell P in these cells (Milad & Shackel 1992). Many other fleshy fruits, including grape berries, can also exhibit cracking under environmental conditions that are associated with a sudden increase in plant or soil water availability (Considine & Brown 1981).

Cell P plays an important general role in plant growth, translocation, tissue firmness and the responses of plants to water-limited conditions, and has a central role in hypotheses regarding the increase in solute accumulation that occurs at the beginning of stage III (also known as 'veraison') in grape berries (Lang 1983; Coombe 1992). Grape berries may provide a useful model system to study the physiology of solute accumulation and growth of fleshy

fruit because the transition from a low to a high growth and solute accumulation rate occurs relatively rapidly (1–2 d, Matthews & Shackel 2005), presumably reflecting physiological rather than anatomical changes. Before veraison, reductions in berry growth are associated with reductions in vine water status, whereas after veraison, berry growth is insensitive to vine water deficits, and at veraison, the water budget of the berry shifts from a combination of xylem and phloem water supply to predominantly phloem (Greenspan, Shackel & Matthews 1994). Water stress is an important aspect of viticulture and has been found to influence berry growth, composition and wine quality; managing vine water stress is widely practised and is thought to improve wine quality (Matthews *et al*. 1990). Changes in cell P are reported in response to plant water deficits in tomatoes and citrus fruits (Huang & Gao 2000; Mingo, Bacon & Davies 2003). For grapes, veraison is characterized by accumulation of pigments, changes in cell wall composition, gene expression and berry water budget, all of which have been studied extensively (Coombe 1992; Robinson & Davies 2000) because of the economic importance of these traits. Specifically, low cell P after veraison is thought to promote solute import (phloem unloading) into the berry. Lang & Thorpe (1989) have hypothesized that veraison is associated with a 'collapse of membrane function', resulting in isoosmotic solutions in both the apoplast and the symplast, and hence, zero cell P. This hypothesis has been explicitly incorporated into a model of grape berry development (Robinson & Davies 2000).

Given the recognized importance of cell P in grape berry physiology, it is not surprising that several attempts have been made to assay P (Bernstein & Lustig 1985; Matthews *et al*. 1987b; Lang & Düring 1991; Huang & Huang 2001). However, all of the methods used to date have been indirect, and many of these previous results are in conflict. Using isopiestic psychrometers, Matthews *et al*. (1987b) concluded that berry cell P progressively increased during the course of development, reached about 0.4 MPa at the end of development with a transient decrease around veraison and never dropped below 0.1 MPa. Despite the sound thermodynamic basis for psychrometric methods, a number of concerns have been expressed about the potential for physiological artefacts, particularly for reproductive tissues in plants, during the long equilibration times that are typically required for this method (Bradford 1994). Indeed, a relatively high cell P of 0.4 MPa is inconsistent with the current hypothesis that translocation efficiency into grape berries is only high when high solutes are found in the apoplast and there is no cell P (Lang & Düring 1991; Coombe 1992; Davies & Robinson 1996). Huang & Huang (2001) found low relative P before and after veraison separated by a transient but significant increase in P at veraison, but their method of evaluating P was highly indirect and was based on the rate of juice exudation after berry puncture. Bernstein & Lustig 1985) reported very low (0–0.04 MPa) post-veraison (PostV) P, based on the pressure required to inject water into the berry through a hypodermic needle. The authors postulate that water would enter the berry only after the external pressure applied to the needle exceeded berry P; thus, the amount of P necessary to obtain water flow into the berry was assumed to be equal to berry internal P. Because the needle tip dimensions were much larger than cell dimensions, however, the P measured in this way would only reflect an internal P of the berry as a whole (if it existed), and not the P of the berry cells.

The cell pressure probe is the only available method for the direct measurement of P in plant cells (Tomos 2000). Investigators have used it to map P spatially and temporally in several plant organs and tissues (Rygol *et al*. 1993). Additionally, the cell pressure probe has been used to determine the effects of water stress on leaf and fruit P and its relation to the growth of these organs (Shackel, Matthews & Morrison 1987; Mingo *et al*. 2003). P has generally been shown to decline with increasing water stress and to respond as expected to changes in transpiration in roots (Rygol *et al*. 1993) and leaves (Shackel & Brinckmann 1985). In the only study to date of fruit cell P *in situ*, Mingo *et al*. (2003) found that a decline in P was associated with reductions in soil water content when roots were in a uniformly drying soil, but not when part of the root system was maintained in wet soil. With the exception of this study, previous use of the pressure probe in fruit tissue has been restricted to post-harvest studies, and has only been used in grape to determine cell P in expanding leaves. Therefore, given the importance of cellular P and uncertainties about its indirect determination in grapes, the cell pressure probe was used to assay grape berry P before and after veraison, and to test the hypothesis that membrane degeneration results in 0 P after veraison. Additionally, changes in P induced by withholding water and by normal diurnal changes in plant water status was evaluated.

MATERIALS AND METHODS

Plant materials

Two-year-old *V. vinifera* L. cv. Chardonnay, Pinot Noir, Merlot and Cabernet Sauvignon were grown from dormancy in 5 L pots of GrowCoir (Greenfire, Chico, CA, USA) potting mix in a greenhouse (30/20 ± 3C; 40/70 ± 10% RH; and natural light with a maximum of 1200 μmol s^{-1} m^{-1} PAR). The vines were pruned such that two shoots with 1–2 clusters per shoot were retained per vine, and the shoots were vertically trained to approximately 1.5 m. Vines were watered daily with 280 mL of nutrient solution (0.11 gL^{-1} of GrowMore Gaidera, CA, USA, 4-18-38, boron removed). Additionally, some berries were sampled from field-grown Cabernet Sauvignon located in the Variety Collection Block of the Department of Viticulture and Enology facility at the University of California, Davis (38°32′N latitude and 121°46′W longitude, elevation is 18 m above sea level).

The anthesis date was noted as the day in which 50% of the cluster was flowering. All flowering within a cluster occurred within approximately 2–3 d. Veraison date was noted as the days after anthesis (DAA) when berries were

soft. Berries were selected at different parts of the cluster, and in cases where the berries were excised at the pedicel, they were immediately stored in sealed, aluminized mylar bags.

Assaying P

The cell pressure probe technique (Hüsken, Steudle & Zimmerman 1978) modified as described previously (Shackel *et al*. 1987) was used to measure the P of individual cells in the mesocarp of grape berries at various depths (100–2500 μ) from the epidermis. Microcapillary tips were prepared with a Kopf Mod. 750 micropipette puller (Model 750, David Koph Instruments, Tujunga, CA, USA) and were bevelled (widened and sharpened) in a jet stream of bevelling solution (Ogden, Citron & Pierantoni 1978) modified as described previously (Shackel *et al*. 1987). The orientation of the berry, probe and microscope was similar to that described by Shackel & Turner (2000), with the microcapillary parallel to the microscope plane of focus, penetrating normal to the skin surface of the berry. A small apparatus was constructed to hold the berry between two small, adjustable silicone rubber cups, which allowed the berry to be precisely positioned as well as rotated about one axis. In contrast to epidermal cells (Shackel *et al*. 1987), penetration of the micropipette into the subepidermal cells could not be directly observed. However, penetration could be inferred by the behaviour of the meniscus that divided the silicon oil of the probe from the cell sap. As reported by Cosgrove & Cleland (1983), after a slight manual reduction in oil pressure, a rapid backward movement of the meniscus during a forward advance of the probe into the mesocarp tissue indicated that the tip of the probe had penetrated into a turgid cell. A motorized piezo-electric manipulator (Stoelting, IL, USA) was used to carry the pressure probe and to impale the cells as described previously (Tong *et al*. 1999). Using the piezo-electric manipulator and inferring cell penetration by meniscus behaviour allowed for multiple P measurements along a single axis within the berry, noting the cell P and depth as stable values were obtained. On occasion, a rapid increase or decrease in volume was also applied to an individual cell, and the resulting pressure relaxation (Steudle 1993) was used as a qualitative indicator of individual cell membrane integrity. Penetration was always along the equatorial plane of the berries. Cell P of several berries across a wide range of depths (200–2000+ μ), and DAA between 23 and 100 (5–20°Brix) were used for each variety. All measurements were performed under laboratory conditions (diffuse fluorescent light and 25–28 °C air temperature) and were completed within 1–5 h of detachment from the cluster unless otherwise noted. Additionally, the berries were assayed to determine P changes after excision using potted vines that were transported to the laboratory. Merlot and Chardonnay berries were assayed while still attached to the vine and were then excised and placed in sealed, aluminized mylar bags (unless otherwise noted), and held at room temperature for 24 or 48 h before determining P again on the same berries.

Statistical analysis was performed using Statistical Analysis System (SAS System 8.1, SAS Institute, Inc., Cary, NC, USA).

Vine water status

All treatments were irrigated similarly until 28 or 71 DAA for pre-veraison (PreV) and PostV experiments, respectively. Water was then withheld from non-irrigated (NI) PreV vines for 14 d ($n = 3$), and NI PostV vines for 8 d ($n = 2$). Irrigated (I) vines ($n = 2$) were watered throughout the course of the experiment on a daily basis with enough water to run out the bottom of the pot. After measurement, NI vines were watered as I vines and were assayed the following day for recovery. Vine water status, evaluated as both midday leaf water potential (LWP) and stem water potential (SWP, pre-dawn and midday), was determined using the pressure chamber technique (Scholander *et al*. 1964; Matthews, Anderson & Schultz 1987a), taking care to avoid leaf water loss. Pre-dawn and midday SWP were determined as described previously (Choné *et al*. 2001). Intact leaves were placed into aluminized mylar bags for at least 10 min to equilibrate LWP and SWP, after which the leaf was removed and SWP was measured in the pressure chamber. There was good agreement between LWP and SWP measurements made on I and NI vines at midday ($r^2 = 0.9461$, $P < 0.005$).

RESULTS

P changes as a result of excision were small

Across a range of P between 0.10 and 0.35 MPa, the P of Chardonnay and Merlot grapes was essentially the same up to 48 h after excision as it was while still attached to the vine, provided that berry water loss was prevented (Fig. 1). After 24 h, the slope and intercept were not significantly different from 1 ($r^2 = 0.9993$, $P < 0.0001$) and 0 ($P = 0.095$), respectively. However, after 48 h, the slope was not significantly different from 1 ($r^2 = 0.9985$, $P < 0.0001$), but the intercept had a small value (0.01 MPa) which was significantly different from 0 ($P = 0.017$). Interestingly, the difference occurred at low P and reflected a slight increase in P during storage. When transpiration was not prevented, P was almost completely lost after 48 h (Fig. 1).

P progressively decreased during development, reaching low but measurable values PostV, and decreased slightly with increasing depth from the berry surface PreV

All of the berries that were measured in this study were divided into three developmental stages, PreV, PostV or uncertain (transitional), and analysis of variance (ANOVA) for the effects of stage and individual berry, with depth considered as a within-berry repeated measure, indicated highly significant effects of stage ($P = 0.003$) and individual berry ($P = 0.0001$, ANOVA not shown). To evaluate whether

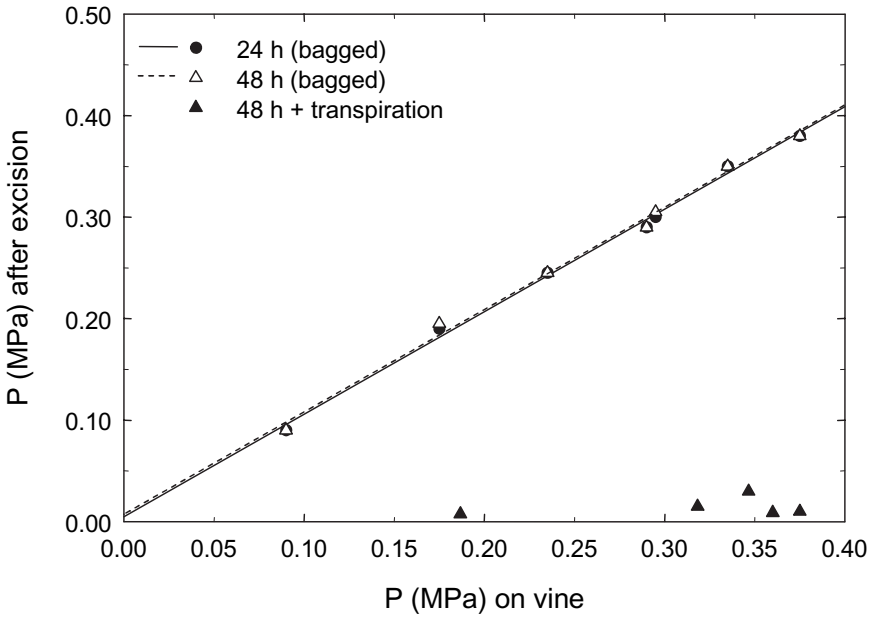

Figure 1. Relation of the cell turgor (P) measured on Chardonnay berries while still attached to the vine (*x*-axis) to the cell P in the same berries 24 or 48 h after excision from the vine. The berries were held at room temperature and were either protected from water loss (bagged) or not protected (+ transpiration). Straight lines are regressions for 24 and 48 h (bagged) values ($r^2 = 0.9993$ and 0.9985, respectively, $P < 0.0001$).

any changes in cell P were associated with depth from the berry surface, a mean P for each berry was calculated, and the residuals from this mean were pooled into 100 μ depth categories for each stage. For the purposes of plotting, the pooled residuals were adjusted for the respective means of each developmental stage (Fig. 2). Regression analysis of the residuals showed that only the PreV group had a significant relation of depth to P; this was highly significant, but the r^2 was only 0.12, and the slope was 0.04 MPa mm^{-1}. Hence, there is some evidence for a depth effect PreV, but for the most typically sampled depths (300–500 μ), it was small. Before veraison, the mean P was as high as 0.27 MPa, near the hypodermis, and as low as 0.20 MPa at 2000 μ (Fig. 2). However, P was low (0.03–0.05 MPa) in PostV, and there was not a significant effect of depth from the berry surface on P (Fig. 2, $P = 0.12$). For both PreV and PostV fruit, pressure relaxations could be induced by rapidly injecting or withdrawing solution from the cells (Fig. 3). These relaxations exhibited approximately exponential behaviour, and the steady-state P after relaxation was higher than the initial value for injections and lower than the initial value for withdrawals (Fig. 3), both indications of apparently intact and functional cell membranes. During the slow forward advance of the probe into the mesocarp tissue, rapid backward movement of the cell solution/oil meniscus within the probe tip occurred at relatively regular intervals of *c.* 50–200 μ, similar to the anticipated radial dimension of mesocarp cells in PreV and PostV berries.

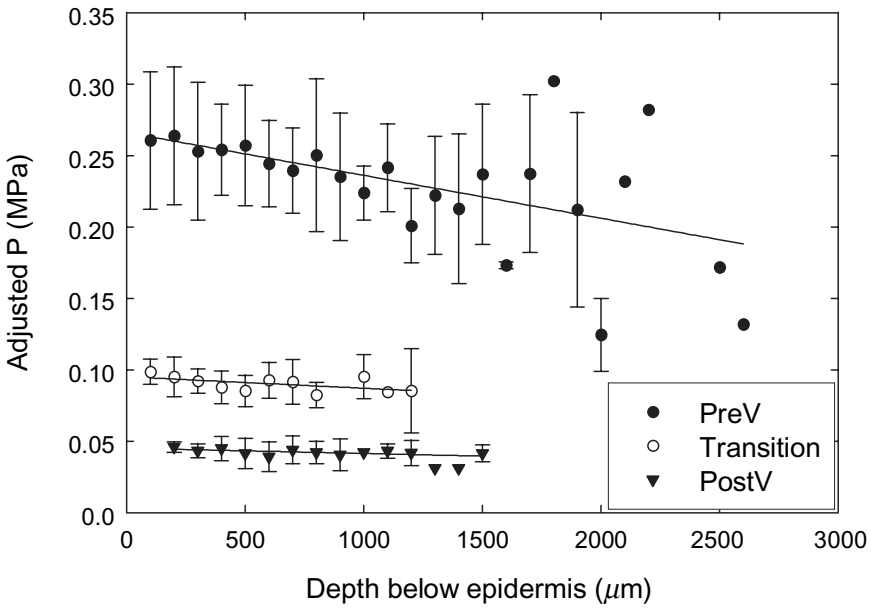

Figure 2. Relation of cell P to depth below epidermis in pre-veraison (PreV), post-veraison (PostV) and uncertain (transitional) Chardonnay berries. Points represent the mean of all measurements pooled into 100 μ depth categories, with error bars of 1 SD, and lines shown are regressions to the raw data. Only the regression for the PreV berries was significant ($P < 0.01$), with $r^2 = 0.12$ and slope = 0.035 MPa mm^{-1}.

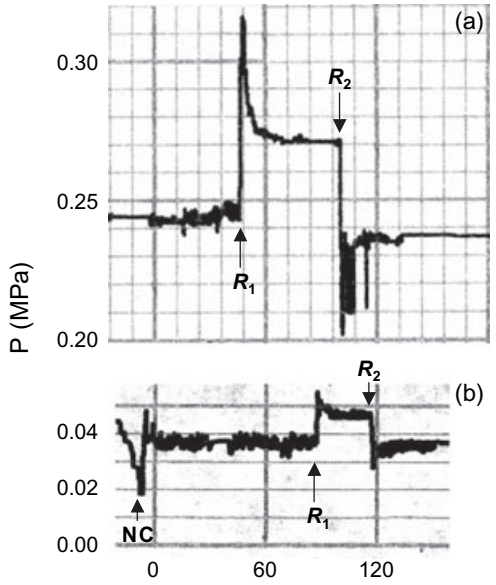

Figure 3. Relaxation behaviour of pre-veraison (PreV) (a) and post-veraison (PostV) (b) cells in grape berries. R_1 indicates the beginning of a volume-injection relaxation cycle, when pressure was increased to move the meniscus to a new location, closer to the cell. After a new stable pressure was observed, the meniscus was replaced to its original position (R_2). NC indicates the entry into a new cell before relaxation behaviour was assayed. Before this point, turgor (P) was slowly decreased as the probe was advanced, to keep the meniscus in the same absolute spatial position, until the meniscus rapidly moved away from the cell. The P increase is the P required to return the meniscus to its initial location.

Pre-dawn water potential decreased in NI vines both before and after veraison

Before veraison, withholding water caused pre-dawn water potential of the NI vines to decrease over several days to a minimum of –0.85 MPa on day 15 (Fig. 4a). A similar pattern was observed when water was withheld after veraison, although the decrease in pre-dawn water potential of NI vines developed more quickly and reached more negative values (Fig. 4b). Before veraison, water use was slowed by significant cloud cover between 4 and 9 d after water was withheld. After veraison, the weather was more consistently sunny. For both treatments, the pre-dawn water potential of NI vines increased to close to that of I vines by 24 h after rewatering (Fig. 4).

P decreased diurnally and with decreasing SWP before, but not after veraison

SWP decreased as a result of withholding water (Fig. 4), and also decreased from pre-dawn to midday (data not shown). PreV P was about 0.25 MPa at an SWP of –0.20 MPa, and declined to about 0.02 MPa for SWP's less than or equal to –0.80 MPa (Fig. 5). In contrast, PostV P was about 0.02 MPa across the entire range of SWP exhibited (from near 0 to –1.60 MPa, Fig. 5).

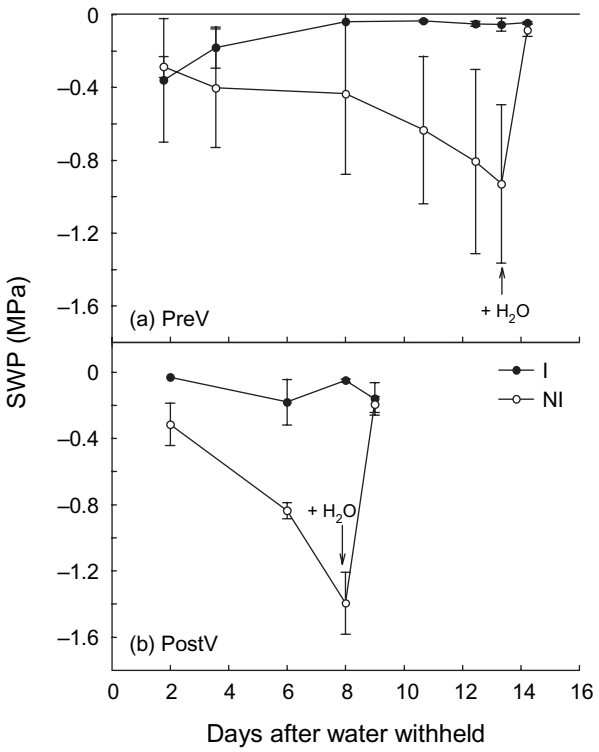

Figure 4. Pre-dawn stem water potential (SWP) for irrigated (I, $n = 2$) and non-irrigated (NI, $n = 3$) vines at various times after withholding water (H_20) from the NI vines during pre-veraison (PreV) (a)and post-veraison (PostV) (b) periods. Irrigation of NI vines at the end of the drying period is indicated by '+ H_2O', and error bars represent approximate 95% confidence intervals.

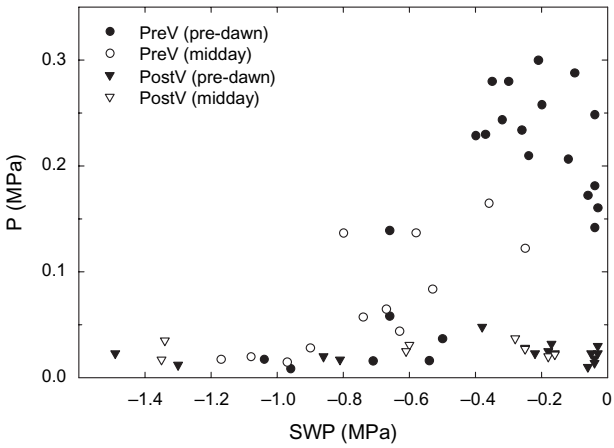

Figure 5. Mean turgor (P) of pre-veraison (PreV) and post-veraison (PostV) berries at various levels of stem water potential (SWP). Variation in SWP was associated with changes from pre-dawn to midday conditions, and with differences in irrigation. Each point represents the mean P of three to eight cells from each of one to two berries.

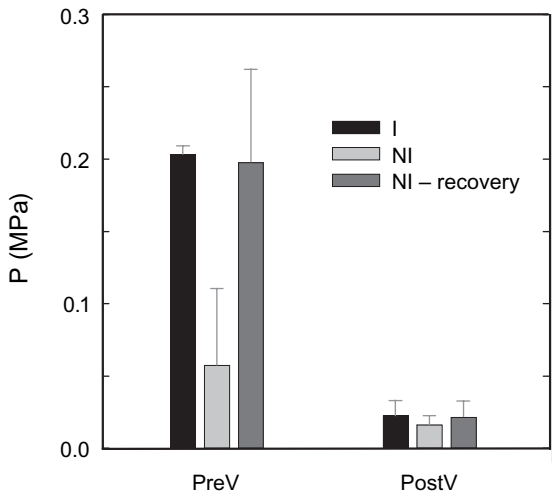

Figure 6. Mean turgor (P) (± 1 SD) of three to five cells of a single berry from irrigated (I) and non-irrigated (NI) vines just before irrigation, and of berries from NI vines 24 h after watering (NI – recovery), at pre-veraison (PreV) and post-veraison (PostV) periods. For NI vines, SWP increased 0.85 MPa after watering PreV, and 1.20 MPa PostV.

NI vines were watered on day 15 before veraison and on day 8 after veraison. P was measured on berries from I and NI vines before watering, and on NI vines 24 h afterward. Before veraison, the P of NI berries increased from 0.05 to 0.20 MPa as pre-dawn SWP increased by 0.85 MPa (cf. Figs 4 & 6) . However, after veraison, increases in berry P of NI vines were negligible, when the pre-dawn SWP increased by 1.20 MPa (cf. Figs 4 & 6). PostV berry P was significantly lower, but positive, in I and NI vines when compared with PreV P (Fig. 6).

DISCUSSION

This report of the first direct and *in situ* measurement of cell P in grape berries indicates that P declines from 0.25 MPa just before veraison to 0.02 MPa at or near veraison, and that berry cell P is responsive to plant water deficits before veraison, but not after veraison. At veraison, a number of important physiological processes, such as berry softening, sugar accumulation, resumption of growth and color changes, all occur (Coombe 1992). Because the low PostV P that we observed occurred sometime within 2 weeks of veraison, our observations may have important implications for the understanding of the role of cell P in these processes in grapes and perhaps, in other fleshy fruits. The current understanding of softening and increased sugar transport into grape berries at veraison is that cell membrane degeneration causes solute leakage to the apoplast, resulting in a loss of compartmentation and essentially no cell P (Lang & Düring 1991; Coombe 1992; Robinson & Davies 2000). We have found that there is low, but measurable, PostV P, and that mesocarp cells exhibit pressure relaxations (Fig. 3) that are typical of cells with intact and functional membranes. Both of these observations are inconsistent with a general loss of membrane function in these cells. Cell pressure relaxations can be used to estimate a number of important cell water relations parameters (Steudle 1993), if cell volume and/or cell osmotic potential is known, but neither of these were determined as part of this study. Further, estimates of overall cell size based on freehand sections indicate that grape mesocarp cells are between 20 and 500 μs in diameter, and hence give a very large predicted range (about four orders of magnitude) in cell volume, precluding any meaningful estimates based on average values from the literature. For any particular pressure relaxation, the quantitative difference in steady-state pressure before and after the relaxation primarily depends on the ratio of cell volume to the volume of fluid that was injected or withdrawn. The fact that higher pressures were observed for added volumes, and lower pressures for volumes withdrawn (Fig. 3), is clear qualitative evidence that at least some of the solutes added or subtracted with injection/withdrawal were retained by the membrane of the cell being measured.

Because the osmotic potential of PostV berry tissue, and presumably the mesocarp cell symplasm, is low (Matthews *et al*. 1987b), a low cell P indicates that a low total water potential exists in both symplastic and apoplastic compartments. Low apoplastic water potentials may be due to the presence of apoplastic solutes and/or a significant apoplastic matric potential (negative hydrostatic pressure), but there is a growing body of evidence that significant concentrations of apoplastic solutes occur in PostV berries, as well as in a number of fruits and other tissues which accumulate high amounts of sugars (Matthews & Shackel 2005). Hence, we suggest that the occurrence of apoplastic solutes are the result of a regulated compartmentation of solutes rather than a general loss of membrane function in these cells. Compartmentation may be regulated by active processes or by selective changes in specific membrane properties, such as the reflection coefficient for a particular solute species. A decrease in P at veraison is consistent with the hypothesis that low P, both in the phloem and sink tissue, is mechanistically important for sugar translocation into sink tissues (Lang 1983), but the specific role that low cell P may play in sugar influx is unclear. The flux of sugar through hexose and sucrose symporters in sugar beets is P sensitive, increasing in efficacy with decreasing P (Wyse, Zamski & Tomos 1986). There is evidence that hexose and sucrose transporters are expressed at veraison (Robinson & Davies 2000) and hence, additional research into the relation of low P to expression and activity of sugar transporters in grape is warranted.

Berry softening and the resumption of expansive growth at veraison (Coombe & Bishop 1980) have often been attributed to changes in cell wall metabolism and composition (Davies & Robinson 2000). An alternative explanation, however, is that much of the softening may be primarily a result of the substantial decline in cell P that we have reported. Tong *et al*. (1999) reported that post-harvest softening in apple was associated with a loss in cell P, and post-harvest losses of P in cucumber (Sajnín *et al*. 2003) and

carrot (Greve *et al*. 1994) have also been correlated with a loss in tissue firmness. Further studies by Thomas, Shackel & Matthews (unpublished results) have shown that changes in firmness throughout grape berry development are coincident and positively correlated with changes in P, consistent with reports by others (Bernstein & Lustig 1985; Lang & Thorpe 1989). Hence it is clear that a loss in P should not be ignored when considering the mechanism of softening in grape berries and perhaps in most other plant organs.

Cell P is widely recognized as mechanistically important for the expansive growth of plant cells (Cosgrove 2000), but the relation between P and the rate of growth in plant organs remain unclear, in part because of the possibility for serious methodological artefacts. Excision and removal from a water source results in wall relaxation and P loss in some growing systems (Cosgrove 1993) and not in others (Nonami & Boyer 1993). In growing grape leaves, excision under high humidity conditions caused no change in P (Schultz & Matthews 1988). Similarly, in this first report of direct measurement of P in intact and excised fruit, there was no change in P upon excision, provided that the grape berry was stored to prevent water loss. Because the berries used in this study represented a range of developmental stages, including stages during which growth occurs, these results indicate that P loss due to wall relaxation is of minimal importance in grape berries. With this assumption, our results indicate that the substantial expansive growth rates that occur in PostV berries occur at very low values of cell P. Work in other plant systems has demonstrated that P can vary significantly across small distances, and because it is possible that the growth of particular tissues (e.g. the epidermis) may determine overall organ growth, spatial difference in P was examined as part of this study. Differences in P as a function of depth from the berry surface were observed only before veraison: P was slightly higher closer to the surface of the berry and gradually declined towards the centre of the mesocarp. Turgid inner cells may cause additional stresses in epidermis regardless of gradients in P (Dale 1988; Kutschera 1989), although there is no current theory for the distribution of stresses in multicellular tissues. In any case, the distribution of forces in tissues will be complicated by turgor gradients. Pressure gradients associated with transpiration have been observed in leaves (Shackel & Brinckmann 1985) and roots (Rygol *et al*. 1993). In the latter case, a gradient of 0.2–0.3 MPa developed with the low P occurring near the epidermis, and this was correlated with corresponding gradients in cell solute potential. In the berry, the P gradient was in the opposite direction to that reported for the root and to that which would be expected from the direction of transpirational water loss from the berry. The P gradient, cell-to-cell variability in P and the wide distribution of cell sizes (Hardie, O'Brien & Jaudzems 1996) complicate understanding of the distribution of wall stress in the growing berry (Serpe & Matthews 1994), and hence preclude speculation about the mechanistic relation of cell P to berry growth. As discussed by Mingo *et al*. (2003), however, there is also a growing body of evidence against a direct, 'Lockhartian' connection of growth rate to P, and hence a detailed model of wall stress distributions in multicellular tissues may not be critical to our understanding of growth in these tissues.

Imposition of water deficits on grapevines is an important way in which viticulturists may impact berry composition and wine quality (Matthews *et al*. 1990). Because changes in cell P may directly or indirectly influence a number of important physiological processes, understanding the P behaviour of the developing grape berry in response to water deficits has important implications for understanding fruit growth and development as well as for practical aspects related to manipulation of crop quality. Loss of P is an expected response at the cell level to water deficits experienced at the plant level, although organs and tissues may differ in sensitivity. Our results show that the sensitivity of fruit P to water deficits was dramatically different PreV and PostV. PreV P was moderate (up to 0.4 MPa, Fig. 1) and responded to changes in SWP both diurnally and after withholding water, whereas PostV P was low (on the order of 0.02 MPa) and did not respond to changes in SWP. The PreV changes in P were only about half of that of the corresponding changes in SWP, but the change in P sensitivity to SWP at veraison is consistent with previous observations that both diurnal and water stress-induced fruit contractions are essentially eliminated at veraison (Greenspan *et al*. 1994; Greenspan, Schultz & Matthews 1996). Presumably, short-term changes in cell P are associated with changes in cell, and hence organ, volume.

During or after veraison, xylem water transport to the berry is almost eliminated; phloem becomes the dominant water source (Lang & Thorpe 1989; Greenspan *et al*. 1994) and, based on this study, cell P no longer responds to diurnal or stress-induced changes in SWP. In the only other pressure probe investigation of fruit cell P during water deficits, a similar behaviour was observed by Mingo *et al*. (2003), in which P reached a minimum (0.08 MPa) in tomato exposed to soil drying. However, the behaviour was not similar to grape in that P only decreased to 50% of the well-watered value, and the change in P occurred during a single day, as volumetric soil water content decreased from 30 to 25% (plant and soil water potential were not reported).

From an economic perspective, berry size is widely recognized as an important factor in wine grape quality (Roby & Matthews 2004). In this study, the sensitivity of P to SWP changed from PreV to PostV, but berry growth occurs in both stages (Coombe 1992). Water stress PreV and PostV reduces berry size (Matthews *et al*. 1987a; Wang *et al*. 2003). Therefore, changes in berry size as a result of water stress after veraison are not due to changes in the P of the mesocarp, and reduction in berry size due to water stress PreV versus PostV may be the result of different mechanisms. PreV, berry interaction with vine water status may be largely through the xylem and perhaps may be more directly subject to changes in vine water status, as observed by berry expansion and contraction, and changes in P. The PreV P behaviour reported here suggests that vine water deficits could impact growth directly by water import

through the xylem, and possibly by a decrease in P. This is consistent with the hypothesis that berry growth is more sensitive to water deficits that are imposed PreV compared with PostV (van Zyl 1984; Matthews & Anderson 1989). However, the difference in sensitivity is not great and has not always been observed (Hardie & Considine 1976; McCarthy 1997). PostV, the berry's connectivity to the vine is through the phloem, and thus may be only indirectly subject to changes in vine water status, for example, through changes in photosynthetic rates that occur because of water stress (Wang *et al*. 2003). Because the rapid changes in whole-berry water relations that occur at veraison appear to be based on physiological changes which occur at the cell level, we suggest that grape may be a useful model system to study the cellular processes important in solute accumulation and the regulation of source-sink relations in fleshy fruits.

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