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ORIGINAL ARTICLE

Mesocarp cell turgor in *Vitis vinifera* L. berries throughout development and its relation to firmness, growth, and the onset of ripening

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Abstract *Vitis vinifera* L. berries are non-climacteric fruit that exhibit a double sigmoid growth pattern and dynamic changes in gene expression, cell metabolism, and water relations at the onset of ripening. The cell-pressure probe was utilized to examine the relationships of turgor pressure (*P*) in mesocarp cells to growth, sugar accumulation, and fruit softening during development. In replications utilizing three different varieties, mesocarp cell *P* demonstrated a consistent pattern of a relative mid-range *P* early in development, followed by an increase to a maximum of about 0.35 MPa, and a subsequent rapid decline before ripening to less than 0.1 MPa. Fruit "apparent elastic modulus" (*E*, units of MPa), was introduced as a standard measure to describe ripening-related softening. *E* changed dynamically and synchronously with *P* during development and in response to water deficits for fruit grown in greenhouse and field conditions. Thus, *E* and *P* were positively and linearly related. Sugar accumulation did not increase significantly until *P* had declined to less than 0.1 MPa. The results suggest that *P* is an important determinant of fruit softening and that *P* decreases in conjunction with many of the physiological and gene expression changes that are known to occur at the onset of ripening.

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Introduction

Fleshy fruit development typically involves expansive growth, sugar accumulation, and softening. Turgor pressure (*P*), a central feature in plant water relations, has been implicated in growth (Welbaum and Bradford 1988) and sugar accumulation (Lang and During 1991; Pomper and Breen 1996) of fruit, but *P* was either estimated indirectly or not measured in these studies. Although fruit softening is intensively investigated, and *P* has been long recognized as a fundamental aspect of tissue mechanical properties (Bruce 2003; Preston 1901), its possible role in softening during fruit development has received little attention. Indeed, in contrast to extensive developmental studies of fruit softening, reliable data on *P* during cell and tissue development are scarce for any system.

The grape (*Vitis vinifera* L.) berry has emerged as a potential model system for the study of fruit development and water relations. The developmental patterns of growth, sugar accumulation, and water and solute potentials are established (Coombe 1992; Matthews et al. 1987), the organ is a massive hexose accumulator and is large and easily manipulated for source-sink study including the separation of exocarp and mesocarp, and several developmental aspects of gene expression are being elucidated, including sugar transport, color change, and fruit softening (Goes da Silva et al. 2005). Grape berries are representative of nonclimacteric fruit that exhibit a dynamic, double sigmoid growth habit. The two periods of growth, Stage I and Stage III, are separated by a period of little or no fruit expansion, Stage II (Coombe 1976; Matthews et al. 1987).

At approximately the onset of Stage III, several genetic, metabolic, and physiological changes occur beginning with fruit softening (Coombe 1992) and including upregulation of hexose and sucrose transporters (Davies et al. 1999) and a large increase in phloem influx (Greenspan et al. 1994). Greenspan et al. (1994) and Greenspan et al. (1996) have shown that the increased phloem influx is coordinated with decreased xylem water influx. Earlier evidence of xylem dysfunction (Düring et al. 1987; Findlay et al. 1987) was recently countered by evidence that the xylem conduits remain functional (Bondada et al. 2005; Keller et al. 2006; Chatelet et al. 2008). Reduced P of sink cells is fundamental to some models of phloem transport (Patrick 1997), and reduced mesocarp cell P due to loss of mesocarp cell viability is proposed as the mechanism leading to increased phloem influx during fruit ripening (Lang and During 1991). However, Thomas et al. (2006) have recently reported that mesocarp cells exhibit hydraulic and osmotic properties that are typical of cells with functional (semi-permeable) membranes, and hence it is not necessary to attribute a reduced cell P to a loss of cell viability or a loss of cell membrane integrity (Krasnow et al. 2008). The hydraulic conductivity at the tissue level decreases (Tyerman et al. 2004), and this may contribute to cell water deficits and lower P, but P has not been measured at this time.

Much previous research on fruit development in grape has utilized the skin fold caliper to non-destructively estimate the elastic/rheological properties of a berry, where berry deformation (D, expressed in mm) (Coombe and Bishop 1980; Creasy et al. 1993; Franke et al. 1995; Davies et al. 1997; Tattersall et al. 1997; Tyerman et al. 2004), or deformability (D as a percent of berry diameter) (Lang and During 1991) resulting from an applied load (force) has been used as a measure of fruit softening. While it is clear that for a constant applied load, D will increase as fruits soften, the basis for using D rather than the standard material property of bulk elastic modulus (E, expressed in MPa, ASAE 2003) has not been presented. For the compression of a spherical object between two parallel plates, D is inversely proportional to both the two-thirds power of E, as well as to the cube root of the sphere radius, as shown in the Hertz equation (e.g., Ravi et al. 2006.). Thus, E and D are inversely related, and a linear decrease in E over time, which would indicate a steady and progressive softening, may correspond to a strongly curvilinear increase in D over time, depending on the numerical range exhibited by E and D. Since Coombe and Bishop (1980) reported that the earliest evidence of ripening in grape was an increase in the rate of change in D, it is important to verify whether this is also exhibited in E. This is particularly important for studies of berry development because an increase in D has been used to identify the onset of ripening (termed 'veraison' in viticulture), and this time point has been used as the developmental basis for investigations of ripening-related changes in gene expression (Tattersall et al. 1997; Davies and Robinson 2000b). Increased D has also been attributed to an increase in cell wall elasticity (Coombe and Bishop 1980), but a reduction in P could also have the same effect. Low P in mesocarp cells was observed in grape berries during ripening (Thomas et al. 2006), when there is rapid phloem influx (Greenspan et al. 1994, 1996) and fruit softening (Coombe 1992). Therefore changes in mesocarp cell P, sugar accumulation, and E throughout grape berry development were examined using the cell-pressure probe and the skin fold caliper.

Materials and methods

Plant material

Two-year-old *Vitis vinifera* L. cvs. Chardonnay (CH), Pinot noir (PN), and Cabernet Sauvignon (CS) were grown from dormancy in 5 L pots of GrowCoir^{TM} (Houston TX) potting mix in a greenhouse (30°/20° ± 3°; 40/70% ± 10% RH; natural light with a maximum of 1,200 μmol photons m^{-2} s^{-1} PAR). The vines were pruned to two shoots with one or two clusters per shoot, and the shoots were vertically trained to approximately 1.5 m. Vines were watered daily with 280 mL of nutrient solution [0.11 g/L of GrowMore (Gardena, CA, USA 90248) 4-18-38, boron removed]. Additionally, some berries were sampled from field-grown CS located in the Variety Collection Block of the Department of Viticulture and Enology facility at the University of California, Davis, CA, USA (38°32′N latitude and 121°46′W longitude, elevation 18 m above sea level). The anthesis date was noted as the day in which 50% of the cluster was flowering. All flowering on a cluster occurred within 3 days. Except where otherwise noted, berries were sampled between 0700 and 0900 hours. For some berries, diameter was measured repeatedly during development with hand-held calipers, returning to the same berry and same caliper orientation on each sample date. The accumulation of soluble solids was measured on small aliquots of juice from individual berries with a hand held refractometer (Reichert A2R200, Reichert GmbH, Seefeld Germany) and reported as °Brix (Amerine and Ough 1980).

Assaying turgor

The cell-pressure probe technique (Hüsken et al. 1978) modified as described previously (Shackel et al. 1987) was used to measure the turgor of individual cells in the mesocarp of grape berries at depths between 90 and

2,500 μm from the epidermis (Matthews and Shackel 2005). 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 behavior of the meniscus that divided the silicon oil of the probe from the cell sap. Micropipettes were inserted into the berry along the equatorial plane of the berries and perpendicular to the berry surface. All measurements were performed under laboratory conditions (diffuse fluorescent light and 25–28°C air temperature) and were completed within 5 h of detachment from the cluster, and generally within 2 h depending on the sample size. For most measurements berries were excised at the pedicel and immediately placed into aluminum foil-coated bags that excluded light and prevented transpiration (McCutchan and Shackel 1992). When stored in this way, mesocarp cell turgor is unchanged for 2 days (Thomas et al. 2006). In one experiment with CH, the entire potted vine was transported to the laboratory at 0700 hours on sample dates. A single berry on the end of one cluster was selected and the turgor of mesocarp cells monitored repeatedly throughout the development. Previous work, using sampling capillaries much larger than the microcapillary tubes used to assay turgor, showed that repeatedly puncturing the berry during sampling did not significantly change its development or composition (Coombe and Phillips 1980).

Berrry rheological measurements

Berry deformation (D) was measured as described previously (Lang and During 1991). A Harpendon skinfold caliper (British Indicators Ltd, St Albans, United Kingdom) was placed along the equator of the berry, recording the initial diameter (d_i), releasing the calipers to apply the force of the spring to the berry, and recording a second reading (d_f):

$$D(\text{mm}) = \left(d_i - d_f\right)$$

An alternative method of expressing the response of the berry to an applied load was Elasticity (E), calculated from the Hertz equation:

$$E(\text{MPa}) = 3F\left(1 - v^2\right)/\left(2RD^3\right)^{1/2}$$

where F is force (N), v is Poission's ratio (dimensionless, assumed = 0.5), and R is the undeformed sphere radius (mm). The Hertz equation corrects for the geometrical effects of both D and R on the area of contact between a flat plate and an elastic sphere, but neither D nor E correct for viscous effects. For these berry measurements, the initial value of D was followed by very small changes (viscous flow under an applied load), and so the initial value of D was consistently used, as in Lang and During (1991). The force applied by the skin fold caliper was 4.4 N (450 g), similar to that of 3.4 N (350 g) used by Coombe and Bishop (1980); however, large deformations occasionally resulted with mature fruit. A comparison was made between values of E obtained using a 450 g load and a 110 g load on the same berry for berries whose E values covered the entire range observed in this study (0.5–14.0 MPa). The two values never differed by more than 1 MPa, indicating that this method was reasonably robust.

Results

Mesocarp cell P of greenhouse-grown CS berries increased from a relatively mid-range value early in berry development (Stage I) to a maximum at about 50 DAA, then declined during Stage II to a low value that was maintained throughout ripening in Stage III (Fig. 1a). Thus, P had a consistent mean value of ~0.16 MPa during early Stage I. At 36 DAA, P began to increase and reached a maximum of 0.30 MPa at 48 DAA. P began to decline about 12 days prior to the transition to Stage III and reached about 0.05 MPa at that time (Fig. 1a). Apparent elasticity (E) exhibited a similar pattern, increasing from about 6–9 MPa, and then decreasing to about 2 MPa at the onset of Stage III (Fig. 1b), and remaining low thereafter. When P in the berry mesocarp declined from 0.30–0.16 MPa (between 49 and 56 DAA), there was a negligible increase in soluble solids (Fig. 1c). After a further drop in P to less than 0.1 MPa, soluble solids increased 3.6 °Brix in 3 days (between 56 and 59 DAA) and continued increasing thereafter. Thus, the data suggest that rapid sugar accumulation (Fig. 1c) and growth (Fig. 1d) did not begin until P was approximately 0.1 MPa, but the resolution of growth and sugar accumulation was not sufficient for a definitive interpretation.

The relationships of P and solute accumulation to fruit age in field-grown CS berries were similar to greenhouse-grown fruit. The maximum P was 0.31 MPa at the initial sample date of 55 DAA (Fig. 2). Thereafter, P declined more than 0.2 MPa, reaching 0.035 MPa at about 70 DAA. Turgor remained ≤0.05 MPa throughout the ripening period. When P declined 0.14 MPa during the first 10 days from 0.31 to 0.17 MPa at 64 DAA, soluble solids increased from about 5–7 °Brix. After a further drop in P to less than 0.1 MPa in the 10 days between 66 and 76 DAA, soluble solids increased 10 °Brix and continued increasing thereafter. The apparent coincidence of the decrease in mesocarp cell P and rapid increase in soluble solids of field-grown berries is consistent with the observations made in greenhouse-grown fruit. The patterns of E and mesocarp cell P in greenhouse-grown PN berries and in an individual CH berry during development were similar to

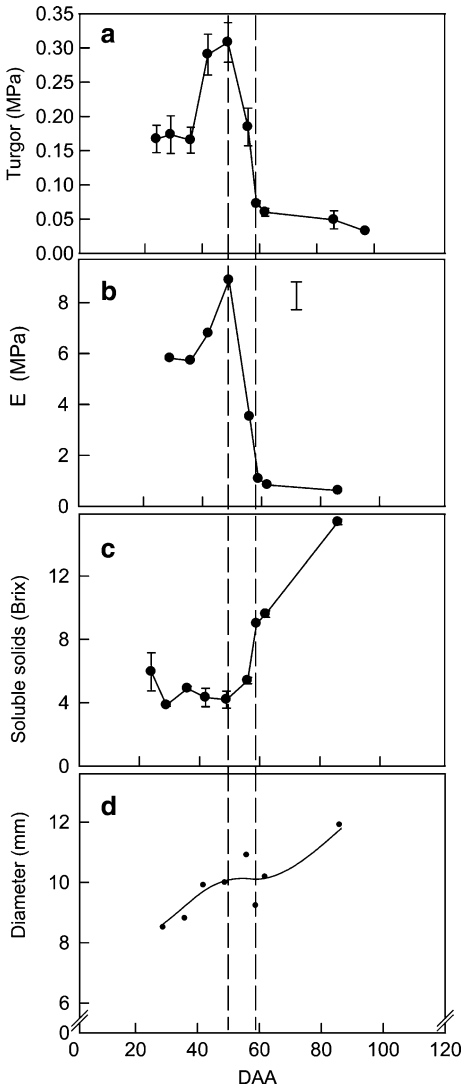

Fig. 1 **a** Mesocarp cell turgor, **b** apparent elastic modulus (E), **c** soluble solids reported as °Brix, and **d** diameter of greenhouse-grown CS berries at various days after anthesis (DAA). *Error bars* in **a** and **c** represent 95% confidence intervals, and are hidden when smaller than the symbols. *Error bar* in **b** is a pooled estimate of 1 SD (root MSE) from a regression analysis of independent pairs of Pinot noir and Chardonnay berries collected over development from clusters with the same DAA. Data represent the mean turgor of 6–22 cells in each of two berries up to 62 DAA and in only one berry after that, corresponding E, °Brix, and diameter. *Vertical dashed lines* indicate arbitrary transitions from Stages I to II and Stages II to III. *Curve* in **d** is a 65% smoothed cubic spline (SAS Institute Inc., Cary, NC, USA) drawn through the mean points

those observed in CS (data not shown). Both E and P increased during Stage I and decreased to low values prior to Stage III, although the decrease in E and P started earlier than in CS.

The relationship of P and E throughout fruit development was evaluated in CS berries, in an individual berry of CH, and in a population of berries from all three varieties. Mesocarp cell P and E were well correlated when

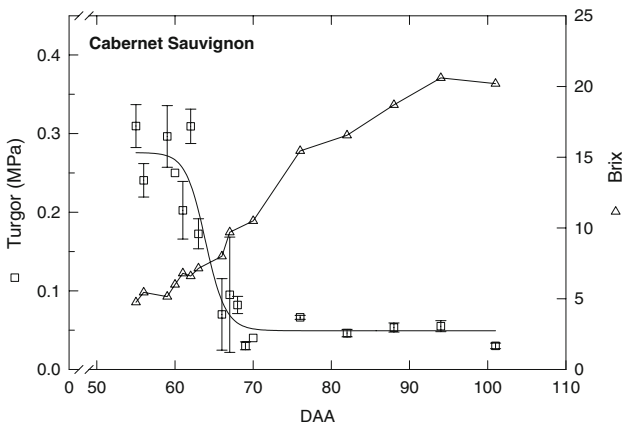

Fig. 2 Soluble solids (Brix) and mesocarp cell turgor of individual, field-grown CS berries. Data are mean turgor from 5 to 8 cells per berry in 2–4 berries and corresponding °Brix

evaluated in field-grown CS (Fig. 3a; $r^2 = 0.89$, significant at $P < 0.001$). When the same CH berry was repeatedly sampled in situ, P and E followed a similar pattern as that previously observed in CS and PN: mesocarp cell P increased to a maximum of 0.39 MPa and then declined to 0.045 MPa before Stage III (data not shown). E changes were coincident with changes in P. For this single berry, the correlation of P and E was positive and linear (Fig. 3b; $r^2 = 0.83$, $P < 0.01$). When data were pooled from all three varieties, E was again significantly and positively correlated with P (Fig. 3c, $r^2 = 0.84$, $P < 0.001$). To further test the relationship between E and P, several CH berries were excised, assayed for E and mesocarp cell P, and then allowed to transpire for 48 h before determining E and P again. In all cases, P was reduced to <0.015 MPa and E was reduced to approximately 0.1 MPa, although both parameters still had positive values (Table 1).

Discussion

Mesocarp cell P was assayed directly on intact excised berries and on intact berries in situ during fruit development. In all experimental berries from three varieties, P exhibited a similar, complex pattern in which P increased as growth slowed in Stage I and then decreased during Stage II to quite low values where it remained during ripening. The same complex pattern was observed in fruit E, indicating a strong role of P in fruit softening during berry development. The specific timing of the decrease in P from a maximum of about 0.35 MPa to less than 0.1 MPa is interesting because it began 10 days before the transition from Stage II to Stage III when resumption of growth and rapid sugar accumulation occur. The control and coordination of these changes in grape berries are poorly understood, but for each of these processes individually

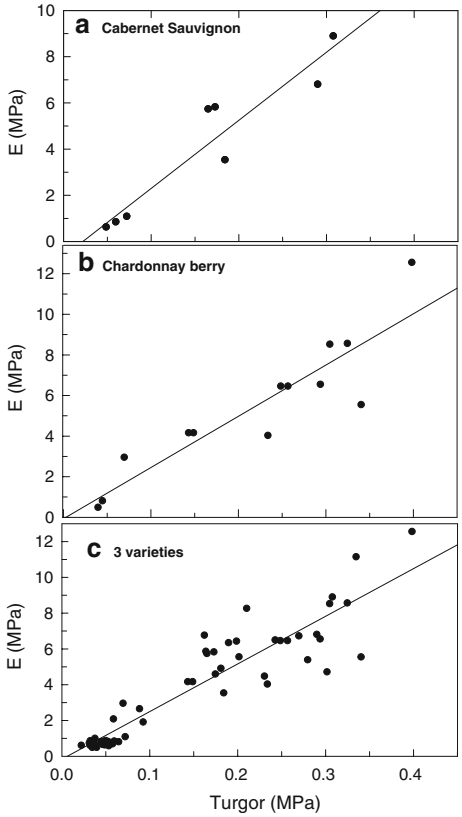

Fig. 3 Relation of apparent elastic modulus (E) to mesocarp cell turgor in **a** 16 greenhouse-grown CS berries ($r^2 = 0.890$), **b** an individual CH berry in which each point represents the E and turgor of the same berry throughout development ($r^2 = 0.827$), and **c** 52 greenhouse-grown CS, CH, and PN berries ($r^2 = 0.814$). *Lines* are linear regressions; in **a** and **c** E data are means of two berries and turgor data are means of 5–12 cells per berry from the same two berries

(growth, sugar transport, and softening), a central role of P has been hypothesized.

Fruit softening

Biological materials are typically viscoelastic, and hence any simple force/deformation approach, such as used in this and other previous studies of berry development, will only provide a relative index of fruit softening. From this standpoint it may be argued that berry deformation (D) and apparent elasticity (E), while inversely related, may be equally reliable as a softening index, and in fact when the D, berry diameter, and force data of Coombe and Bishop (1980) are recalculated as E, a developmental pattern very similar to that found in this study is observed, with large decreases in E occurring relatively early in berry development (Fig. 4). However, because the interpretation of these data was based on the patterns exhibited by D, and because D and E are inversely related, it is clear that most of the change in E was complete by the time that D had an apparent sudden increase on day 0, as proposed by Coombe and Bishop (1980) (broken dashed line in Fig. 4). Hence, we propose that the apparently 'sudden' softening described by Coombe and Bishop (1980), was in part an artifact of the use of D as a measure of berry material properties, and in part the arbitrary selection of segments of data over time. The approximately fourfold higher E for Muscat Gordo may reflect a larger and firmer table grape berry than the winegrape berries used in this study, but may also be due in part to the caliper method used by Coombe and Bishop (1980), which involved the use of two different spring forces.

The close correspondence between mesocarp cell P and fruit E during development and under a wide range of treatments and conditions that cause E or P to vary indicates that a fundamental relationship exists between these two measurements. The complex developmental pattern (increase followed by dramatic decrease) was present in both P and E. There was a linear relationship between P and E for all varieties tested, and the same relationship was observed in an individual berry during its development. The linear relationship between E and P during berry development suggests that cell P may be the primary component that determines berry firmness. When excised and stored in nontranspiring conditions, P and fruit firmness remained unchanged for at least 2 days (Thomas et al. 2006). In this study, when excised and transpiring, the berry coincidentally reached a P of near zero and very low E values. When E was calculated from the diurnal D and diameter measurements reported in Coombe and Bishop (1980), E was high in the morning, decreased by midday, then returned to a high value by the next morning. This is the same pattern as the diurnal changes in P that we reported recently (Thomas et al. 2006). When cell P declined in postharvest studies in tomato (Shackel et al.

Table 1 Mean turgor and E of Cabernet Sauvignon berries at 0 and 48 h after excision ($n = 5$)

Time	Turgor (MPa)[a]	Turgor 95% confidence level	E (MPa)[b]	E 95% confidence level
0	0.32	±0.06	9.3	±2.7
48	0.014	±0.007	1.0	±1.1

[a] Mean P of 2–5 cells for each berry

[b] Same berries used to determine turgor

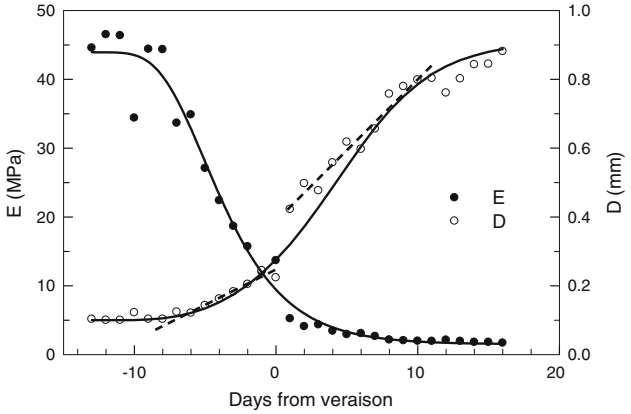

Fig. 4 Deformation (D) and apparent elastic modulus (E) at various days from veraison (onset of ripening) for Muscat Gordo. Data for deformation and for calculation of E appeared in Coombe (1992), and the two linear segments (*dashed lines*) are presented as in Coombe 1992. *Solid line* through E data is a Gompertz sigmoid function: $f = y^0 + a \times \exp(-\exp(-(x - x^0)/b))$, where $a = -42.4$, $b = 3.21$, $x^0 = -5.07$, and $y^0 = 43.93$; $r^2 = 0.981$. *Solid line* through D data is that predicted from the function fit to E data

1991), cucumber (Sajnin et al. 2003), and apple (Tong et al. 1999), a loss in tissue firmness was observed. Additionally, P played a predominate role in the rheological properties of potato and carrot when it was reduced by freeze-thaw or chloroform treatment (Ohnishi et al. 2003) or by heat (Greve et al. 1994).

This is the first report to demonstrate that cell P has an important role in fruit softening. In a recent review concerning the mechanical characteristics of plant tissues, Bruce (2003) states that "All mechanics of primary plant cells is essentially a problem of interplay between turgor and wall properties," and in view of this recognition of the importance of turgor (P), it is surprising that there are very few direct measurements of P available in the literature, particularly in fruits. Recognizing the dependence of fruit firmness on cell P is important because in the voluminous literature on fruit softening, the softening and the genes responsible for softening are discussed almost exclusively in terms of cell wall disassembly (see reviews by Rose and Bennett 1999; Brummell and Harpster 2001). In grape, Coombe and Phillips (1980) hypothesized that softening arises from a decrease in the elastic modulus of pericarp cells, and it is often assumed that berry softening is primarily influenced by cell wall modification (Davies and Robinson 2000b; Ollat et al. 2002). It is clear that structural members (e.g., cellulose microfibrils) of the cell wall are responsible for supporting the tensile stress imposed by cell P, and hence it may be intuitive that cell wall modification may independently influence both P and the structural rigidity of a plant tissue. However, it must also be recognized that at equilibrium, which is approximately the case during Stage II and in excised berries, cell P is only a function of the difference between apoplast total water potential and cell osmotic potential (assuming no meaningful matric potential in the cytoplasm), and hence there is no explicit dependence of P on wall structure per se. We recently showed that P of fruit cells is substantially reduced by the presence of apoplastic solutes (Wada et al. 2008), and we further suggest that softening in grapes and possibly other fruits may be a direct physical consequence of reduced P, rather than or in addition to structural changes in the cell walls.

Could products of cell wall disassembly provide apoplastic solutes to decrease P? The decrease in P and E appear to precede the changes in wall plasticity (Matthews et al. 1987) and wall metabolism (Davies and Robinson 2000a), but this requires further validation with improved rheological methods. Also, Coombe (1992) noted asynchrony of as much as a week among berries in his analysis of softening, and suggested that this may be due to asynchrony in bloom dates of individual berries. In the present study, the decreases of P and E 10–12 days before Stage III are unlikely to be an artifact of asynchrony in fruit development. Bloom dates of individual flowers did not vary more than ± 1 day. And, similar observations of early decreases and close correlation between P and E were made when an individual berry was repeatedly sample in situ, in which case there is no opportunity for asynchrony. The most significant changes discovered in the grape berry cell wall at ripening are a decrease in galactose/galactan (Nunan et al. 1998), and an increase in water-soluble polysaccharides (Silacci and Morrison 1990) and hydroxyproline-rich proteins including expansins (Nunan et al. 1998; Nunan et al. 2001). Several of these changes in cell wall composition could be a result of enzyme activity, which has been proposed to have a primary role in softening (Davies and Robinson 2000a; Nunan et al. 2001; Ollat et al. 2002), but could also have a role in Stage III growth. There are several cell wall related genes and enzymes whose expression and activity are altered at the time berries begin to both ripen and expand (Davies and Robinson 2000b). The current literature on these wall-related gene products does not distinguish their roles between the events of softening and expansion in grape berries.

There are too few studies of P in fruit or during development to ascertain the novelty of the complex pattern of mesocarp cell P in developing grape berries. In most studies where P has been assayed directly (usually to explore the regulatory mechanisms of cell elongation), tissue and cell age was constant. For tissues with localized growing regions, e.g., young stems and roots, the axial (age) profile of cell P has been investigated in several systems. There is usually little difference in P among cells in those studies (e.g., soybean stems, Nonami and Boyer

(1993)), although cortical cell *P* may decrease about 0.1–0.2 MPa as cells mature and growth ceases in rye coleoptiles (Kutschera 2004) and maize roots (Spollen and Sharp 1991). In contrast, epidermal cell *P* in dicot *Begonia* leaves, which do not have localized growing growth regions, increased 0.1 MPa or more as leaves matured and growth ceased (Serpe and Matthews 1994). This behavior, increasing *P* with decreasing rate of growth, is similar to our observations in the grape berry during Stage I. There are three developmental studies that estimated fruit *P* from measurements of tissue water potential and solute potential (Matthews et al. 1987; Welbaum and Bradford 1988; Yakushiji et al. 1996). In muskmelon, which does not exhibit multiphasic growth, estimated *P* of fruit flesh and seed appeared to increase slightly before decreasing along with growth to approximately the low values measured directly in ripening grapes in this study (Welbaum and Bradford 1988). In berries of *Vitis vinifera* cv. Cardinal, estimated *P* increased during Stage I similar to this study, but thereafter tissue *P* was more than 0.2 MPa higher than the low values obtained by direct measurement with the cell-pressure probe in this study (Matthews et al. 1987). Only excised inner pericarp tissue was used in the muskmelon study (Welbaum and Bradford 1988), but the grape berry assay incorporated mesocarp and exocarp tissue. Because the tissue averaged *P* is measured by isopiestic thermocouple psychrometry (Boyer 1995), the grape results suggest a difference between cell and tissue level measurements of *P* as was shown by Nonami et al. (1987) and Nonami and Schulze (1989).

Growth at low turgor

In addition to *P* increasing as growth decelerated in Stage I, the entire second period of growth took place at very low *P*, less than 0.05 MPa. This is the lowest *P* at which expansive growth has been reported, and it occurred under well-watered conditions. For comparison, the minimum *P* for leaf growth at low water status in three dicot species was about 0.25 MPa (Matthews et al. 1984; Schultz and Matthews 1993; Serpe and Matthews 2000). Turgor is also about 0.25 MPa in fruit tissue of muskmelon (Welbaum and Bradford 1988) and bell pepper (Aloni et al. 1999). However, in some fleshy fruit, low *P* of 0.1 MPa or less may be common (e.g., tomato, Grange (1995); Satsuma mandarin vesicle cells, Yakushiji et al. (1996)). In grape, the expansion at extremely low *P*, the decrease in mesocarp cell wall per berry during Stage III expansion (Nunan et al. 1998), and evidence of cell wall thinning (Fougere-Rifot et al. 1997) and disassembly (Silacci and Morrison 1990; Yakushiji et al. 2001) raise the question of whether Stage III expansion is "growth" of a fundamentally different nature than is typical of leaves, roots, etc., and presumably of Stage I in the berry and other fruit with multiphasic growth, where addition of new cell wall material is a key component of growth.

Sugar accumulation

Cell *P* declined to very low values in all three varieties prior to the onset of ripening, which is marked by a precipitous increase in solute accumulation. Shackel et al. (1991) suggest that changes in cell *P* may reflect physiological changes at the cell or membrane level that could precede the morphological expression of these changes at the tissue level. The decline in *P* of sink cells may be mechanistically important for the increased phloem influx (Lang 1983; Patrick 1997). At the tissue level, Greenspan et al. (1994) estimated a tenfold increase in the rate of phloem influx at the onset of Stage III. In young *Phaseolus* roots there was a nonlinear response of increased assimilate accumulation rate to changes in estimated tissue *P*; lower *P* elicited a disproportionately strong response in the rate of accumulation (Lang and Thorpe 1986). Similarly in the grape berry, increases in soluble solids were not linearly related to decreasing *P* (Fig. 3). Soluble solids increased slightly as *P* decreased from 0.3 to 0.1 MPa, but increased rapidly after *P* decreased to less than 0.1 MPa.

Although cell *P* decreased markedly, it did not go to zero as predicted by the decomparmentalization hypothesis (Lang and During 1991). Mesocarp cells display normal pressure relaxations (Thomas et al. 2006), retain viability (Krasnow et al. 2008), and express genes for membrane proteins such as aquaporins occurs well into ripening (Baiges et al. 2002). The low *P* observed during solute accumulation is consistent with a symplastic/apoplastic model for solute unloading in the ripening berry. Initial symplastic unloading from sieve elements with an apoplastic step to allocate solutes to storage parenchyma has been proposed (Coombe 1992; Patrick 1997; Lalonde et al. 2003) and recently supported with direct evidence (Zhang et al. 2006). Symplastic unloading by bulk flow may be limited to the phloem/storage parenchyma interface. If plasmodesmata are *P* sensitive, then *P* of transfer cells will set much of the *P* difference between the phloem and storage parenchyma; therefore, the *P* of the mesocarp cells may be indicative of the ability to unload and transport sugars (Patrick 1997) at least during Stages I and II. If sucrose unloading is diffusive postverasion, then retrieval of unloaded sucrose (via active transport) or cell wall invertase is necessary to maintain a sucrose gradient between sieve element and apoplast. There is evidence for the presence of a cell wall invertase in tissue surrounding the vasculature in grape berries (Famiani et al. 2000), and hexose (Fillion et al. 1999), and sucrose (Davies et al. 1999) transporters are upregulated during ripening.

Furthermore, phloem unloading in berries is decreased by enzyme inhibitors, suggesting active transport of solutes (Wang et al. 2003). The low *P* and high concentration of hexoses in the apoplast are consistent with apoplastic unloading.

Turgor in signal transduction?

The early decrease of *P* invites speculation of its role as a signal in the suite of gene expression and metabolic changes that occur at the onset of ripening. Although there is some evidence for *P*-regulated gene expression (Jones and Mullet 1995), there is surprisingly little direct investigation of *P* in most studies (Neill and Burnett 1999). Nevertheless, several lines of investigation are consistent with a role of decreasing *P* in the dramatic changes in gene expression at the onset of fruit ripening (e.g., Deluc et al. 2007), and present interesting parallels with changes in response to water deficit. The concentration of ABA increases at or near veraison (Coombe and Hale 1973) and in response to water deficits. Tattersall et al. (1997) demonstrated the early and large induction of a thaumatin-like protein; proteins known to be induced by the stress hormones ABA and salycilic acid (van de Rhee et al. 1994). Further, the induction of numerous other genes, i.e. *RAP2* and *WRKY*s, at veraison suggests a stress response (Terrier et al. 2005). A grape ASR (ABA-, stress-, and ripening-induced) protein, VvMSA, was characterized and *VvMSA* expression is strongly enhanced by ABA and sucrose (Cakir et al. 2003). VvMSA binds to and promotes expression from the promoter of a hexose transporter (Cakir et al. 2003) suggesting a role in sugar accumulation. Therefore, Stage II and water deficits may be two distinct developmental contexts in which decreases in *P* followed by increases in ABA promote sugar accumulation.

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