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Research article

The role of fruit exposure in the late season decline of grape berry mesocarp cell vitality

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ABSTRACT

Loss of cell vitality in the mesocarp of grape (*Vitis vinifera* L.) berries during late ripening is programmed and under genetic control. Environmental factors such as temperature and vine water status, however, have a strong influence on the onset and extent of cell death. Following preliminary observations made on field experiment fruit, the hypothesis that exposure (increased light interception and wind velocity) at the berry level is important to the progression of cell death was tested. Transpiration, mesocarp cell vitality and total soluble solids concentration were compared in exposed and sheltered berries within single Shiraz vines. At oenological maturity (24 °Brix), exposed berries exhibited the same extent of cell death as sheltered berries, and it was not until four weeks later that cell death was more extensive in the exposed treatment. Therefore, under the conditions of this study, increased exposure over the ripening period was not a strong predictor of the extent of cell vitality at maturity. However, the results are consistent with an increase in the importance of environmental effects (including rain and exposure) on mesocarp cell death over the course of berry development, particularly in overripe fruit.

1. Introduction

Shiraz berries tend to undergo weight loss beginning around 100 days after flowering, leading to shrivelling (McCarthy, 1999), declines in yield (Rogiers et al., 2006) and altered composition and sensory traits (Bonada et al., 2013a; Tilbrook and Tyerman, 2008). Transpiration combined with reduced phloem inflow (Greer and Rogiers, 2009), and increased susceptibility to reverse flow through the xylem (Tilbrook and Tyerman, 2008; Tyerman et al., 2004) all potentially contribute to dehydration of the berry. Longitudinal cross sections of grape berries of many wine cultivars respond positively to vital stains at the start of the ripening period (Clarke et al., 2010; Krasnow et al., 2008; Tilbrook and Tyerman, 2008). However, as the berries approach oenological maturity regions of non-vital cells typically appear in the inner mesocarp, then increase in number, expand and coalesce, ultimately leaving remnant regions of vital cells associated with the septum and peripheral mesocarp/exocarp (Clarke et al., 2010; Krasnow et al., 2008; Tilbrook and Tyerman, 2008). The dehydration of Shiraz berries during the later stages of ripening is distinct from 'berry shrivel' in that the former is observed after the sugar content per berry has accumulated to normal levels, whereas the latter is associated with premature curtailing of sugar accumulation and an atypically low sugar concentration (Krasnow et al., 2009). Our interest is the former process but does not preclude the pertinence of the research to both phenomena.

Temperature influences the onset of pericarp cell death (Bonada et al., 2013a). Open-top chambers increased air temperature and advanced the onset of the late season decline in the cell vitality of vineyard-grown Shiraz berries by about nine days (Bonada et al., 2013a; 2013b). Moreover, when expressed in terms of thermal time (summation of the range between daily maximum and minimum canopy temperature above a base temperature of 10 °C) the onset of the late season decline in cell vitality across the treatments was uniform (approximately 1000 °Cd (Bonada et al., 2013a) to 1080 °Cd (Bonada et al., 2013b) post-anthesis). This is approximately the same thermal time Shiraz berries grown on plants subjected to shade treatments were last observed to exhibit a high degree of cell vitality (> 90%) (Caravia et al., 2016). These results imply a strong role for temperature in regulating the onset of cell death. However, these preceding studies have manipulated and monitored environmental effects by applying treatments to all or part of the canopy. It is uncertain if the onset of berry

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cell death is a consequence of the heat summation that the vegetative components received, or if it is the berry that integrates and responds to environmental effects such as thermal time.

The aim of the present work was to assess the response of grape berry cell vitality to light and wind exposure at the berry level. Exposure was of interest because it was a factor that could potentially explain the developmental trends and variability observed in a preliminary berry cell vitality dataset obtained on field experiment fruit, the results of which are shown here. The objective was to test the hypothesis that increased berry exposure is an important determinant of the extent of cell vitality at oenological maturity, and over the following weeks. A grape berry total soluble solids concentration (TSS) of 24 °Brix was used to define the technical target for harvest because a TSS-alcohol conversion of 0.59 (Jones and Ough, 1985) would produce a wine close to the median alcohol concentration for the Australian wine industry (Godden et al., 2015). Berries with TSS > 24 °Brix are referred to as 'overripe' (Keller and Shrestha, 2014). After ambient temperature, light interception is the primary determinant of berry temperature (Smart and Sinclair, 1976), therefore a positive result would be consistent with an effect of thermal time at the berry level on cell vitality, and would therefore aid in identifying the most appropriate contexts to monitor, model and mediate exposure with the aim of predicting and influencing grape berry cell vitality.

2. Materials and methods

2.1. Preliminary observations

Preliminary observations were obtained by sampling a set of grape berries from the Charles Sturt University, Wagga Wagga, experimental vineyard replicated variety block once every three weeks, beginning before the onset of véraison. The design and management of the variety block is described elsewhere (Coetzee et al., 2017). Capfall was recorded on the day 50% of the calyptras were shed, on a per grapevine basis. Results reported here are for Shiraz, clone BVRC12, trained as bilateral cordons, each with approximately seven spurs of two buds. Each sample consisted of a berry collected from the top, middle and bottom of a randomly selected bunch within a three-grapevine panel, from the same two adjacent rows within the vineyard. Berries were sampled by cutting through the pedicel with scissors and immediately transported to the laboratory. Using a razor blade, the torus was excised from the berry and the fresh weight (FW) was recorded (analytical balance model BP 221S, Sartorius, Goettingen, Germany). The cell vitality of these grape berries was assessed using previously described methods (Krasnow et al., 2008). Briefly, berries were bisected longitudinally and one half was stained with fluorescein diacetate for 15 min, then blotted with tissue paper before being imaged under white and blue light using a stereomicroscope (Leica MZ 16 FA, GFP2 ET filter, excitation 480/40 nm, barrier 510 nm). Image analysis software (ImageJ, Schneider, et al., 2012) was semi-automated (Supplementary Material) to express cell vitality as the percentage of the berry cross-section that fluoresced green following application of the vital stain. Non-stained controls exhibited negligible fluorescence in the green band. Sampling was terminated when grape berry cell vitality was close to zero. The TSS of the unstained berry half was measured with a handheld refractometer (Atago, Tokyo, Japan). Daily minimum and maximum temperature, and rainfall were recorded using a data logger (Intercap HMP50 Vaisala temperature and RH sensors at 30 min intervals, CR200 data logger, Campbell Scientific, Garbutt, Queensland, Australia). The midday (12–1:30 p.m.) balance pressure of three leaves (fully-expanded, placed in a darkened bag for at least 2 h before excision) selected from random locations across the targeted panels, were measured using a Scholander pressure chamber (PMS Instrument Company, Albany, OR, model 1000) once every two weeks.

2.2. Potted vine experiment

2.2.1. Experiment design

The potted vine experiment was conducted in an outdoor bird-proof enclosure located at the National Wine & Grape Industry Centre, Charles Sturt University, Wagga Wagga. Fifteen plants (cultivar Shiraz, clone 1654, entering their fourth vegetative season) were arranged in five east-west rows with an additional buffer plant located on the western end of each row. The plants were grown in 50 L pots and a freely-draining potting mix obtained from a local landscape supply company was used as the potting substrate. Temperature, relative humidity and wind speed were monitored with a weather station (WXT520, Vaisala, Hawthorne, Victoria, Australia, logged every 30 min using a CR1000 data logger, Campbell Scientific, Garbutt, Queensland, Australia) located at bunch height between the third and fourth replicated rows (counting from the northernmost row).

The plants were initially pruned to four shoots on two spurs. The date of capfall for each bunch was recorded, at which time each plant was fertilised with 2 mL of liquid fertiliser (diluted) (Megamix Plus, Rutec, Tamworth, Australia) supplemented with magnesium sulphate (2 g per plant) (Manutec, Adelaide, Australia). One month after capfall the plants were pruned to three shoots each and the bunches were pruned to approximately 100 berries each. The shoots were trained in a bottle shape with the aid of bamboo canes – spreading slightly at the base and positioned almost vertically at the shoot tips. In combination with the effect of the initial strong dose of fertiliser on leaf size, this structure provided bunches with a range of light exposures. Fertiliser (half-strength) was applied again at the first signs of véraison. At this time the shoots were tip-pruned to 20 primary leaves. The lateral shoots were also pruned to three leaves at this point and each week thereafter. During the ripening period the plants were typically irrigated for 25 min four times per day via one 2 L h^{-1} pressure-compensating emitter (Antelco CETA, Toro Australia, Beverley, South Australia, Australia) located adjacent to the grapevine trunk. Across the experiment the first berries to enter véraison (as judged by the initial blush of red pigmentation) were marked with a liquid wax pen (DecoPaint, Uchida of America Corporation, Torrance, CA). Nine days later berries that were yet to enter véraison were marked with another liquid wax pen. All marked berries were later avoided in the sampling regimes. At the first signs of véraison, three exposed and three sheltered sampling sites were chosen on each of the 15 plants. These sampling sites were sub-locations on bunches consisting of about ten berries. The exposed sites were typically on the northern side of the plant and at the proximal end of a bunch, whereas the sheltered sites were typically on the southern side and at the distal end. Approximately 12% of all bunches had both exposed and sheltered sites.

2.2.2. Characterising early ripening

Four days after marking the last berries to enter véraison, one berry from a randomly chosen exposed and sheltered sampling site on each plant was excised to determine FW and TSS. Eight days later this procedure was repeated on another set of randomly selected berries.

2.2.3. Assessing grape berry cell vitality

The first set of berries sampled to determine cell vitality were collected and prepared as described above, 24 days after the last berries to enter véraison were marked. Follow-up samples were collected one, two and four weeks after the initial set. Each batch comprised one berry from two separate exposed and two separate sheltered sites randomly chosen per plant. The albedo was measured using a spectroradiometer (UniSpec, PP Systems, Haverhill, MA). The spectroradiometer reading (400–700 nm, 50% power, 10 averages, 5 ms integration time) was made on each berry at its widest point after gently wiping waxes from the surface using low-lint tissue paper.

2.2.4. Characterising shrivel symptoms

On the final sampling event for cell vitality assessment, berries were classified according to their visible symptoms of shrivel. Berries in the '0' class exhibited no symptoms, those in class '1' exhibited slight dimples (shallow, ovoid depressions), class '2' berries exhibited a moderate degree of shrivel (short, approximately linear wrinkles), and class '3' berries exhibited pronounced shrivel (multiple wrinkles extending over one half of the berry length).

2.2.5. Quantifying berry light exposure

Berry light exposure was quantified by deploying light-sensitive acetylcellulose film (product code Y-1W, i.e. yellow-one week, Taisei E &L, Tokyo, Japan). The film was cut into 1 cm^2 squares and affixed using a malleable adhesive (Terostat VII, Henkel Teroson GmbH, Heidelberg, Germany) onto a representative berry surface at each of the three exposed and sheltered sites per plant. These representative berries were not sampled for any other analysis. The film was oriented so that the inner surface of the original roll faced outwards and tangential to the edges of the bunch at each site. This resulted in film at exposed sites laying almost horizontal and at sheltered sites angled close to vertical. An additional three squares of film that served as positive controls were affixed using malleable adhesive to the quantum sensor mounting plate. At the end of the deployment period the absorbance of each piece of film was read at 468 nm using a spectrophotometer (Unicam 8625, Cambridge, UK). A batch of film was retrieved when the absorbance of the positive controls had approximately halved (checked after three days and every day or so thereafter) and therefore in the range of a linear relationship between intercepted light and the ratio of final:initial absorbance (manufacturer note and unpublished observation). By deploying a fresh batch of film approximately one week after retrieving the last batch, a total of four batches were used in deployments spanning five to nine days. These deployments covered approximately 70% of the observation period.

The radiation intercepted at each site was calculated from the decrease in the absorbance of the film sample relative to the mean decrease in the absorbance of the positive controls, multiplied by the photosynthetic photon flux density (*PPFD*) recorded by the quantum sensor every 30 min, multiplied by a correction factor ($c = 0.51$) to convert the flux density (μmol m^{-2} s^{-1}) into irradiance (W m^{-2}) (Thimijan and Heins, 1983):

$$I_0 = PPFD \cdot c \cdot \frac{1 - {ABS_s}/{ABS_0}}{1 - {ABS_c}/{ABS_0}} \quad (1)$$

Where ABS_s, ABS_0 and ABS_c are the absorbance of the film retrieved from the sample site, at time zero and the positive control, respectively.

Spot readings made with a handheld meter (LI-185 light meter, LI-COR, Lincoln, Nebraska) suggested (1) irradiance levels greater than those inferred using the light sensitive tape and (2) approximately constant low-level irradiance of sheltered berries throughout the day and greater irradiance of exposed bunches in the afternoon than the morning (Fig. S1). The first observation reflects the difficulty of gauging long-term exposure from subjective spot-readings. The latter observations indicate the need for a measurement regime appropriate to the dynamic nature of light interception at sampling sites. The use of light sensitive tape is beneficial because it accumulates such short term fluctuations in exposure while minimising the biases associated with hand measurements, while integrating readings over a timescale that is well-suited to the present interest in cumulative effects over the ripening period.

2.2.6. Quantifying berry wind exposure

Upon terminating the experiment, sample site-specific wind velocity was estimated using a pedestal fan, centered at bunch height, set 50 cm from each sampling site where wind velocity was measured using a handheld sensor (anemometer, product number QM1644, Jaycar Electronics, Rydalmere, New South Wales, Australia). Wind velocity was recorded with the fan directed towards each exposed or sheltered site from four cardinal and four inter-cardinal directions at low and high speed settings (maximum potential of 2.2 and 3.0 m s^{-1}, respectively). To estimate site-specific wind speed, the ratio of the mean observed to potential wind velocity (decimal percentage) at each site was applied to wind speed recorded by the experiment weather station. The handheld sensor had a lower sensitivity of 0.3 m s^{-1}, so the minimum site-specific calibration factor was set at 0.14 (0.3/2.2).

2.2.7. Berry transpiration

On each day prior to sampling the first, second and third batches of berries for cell vitality assessment, a berry from a randomly selected exposed and sheltered site on each plant set of berries was sampled to assess their transpiration rate *in situ*. The pedicel and torus of each berry was wrapped in malleable adhesive which aided in re-affixing them *in situ*. The berries were retrieved after about 7–9 h of daylight: for the first batch this was mid-morning until late afternoon. For the next two batches this was overnight until mid-afternoon. The transpiration rate of each berry was calculated from the weight loss across the measurement period (respiration being negligible - Greer and Rogiers, 2009) and converted into a molar density flux by estimating surface area from berry width and length with digital vernier calipers, assuming berry shape conforms to a prolate spheroid (less the area of a circle with the diameter of the torus). The mean vapour pressure deficit (VPD) during each measurement period was calculated from weather station temperature and humidity assuming berry surface temperature was equal to ambient temperature, and that the effect of solutes on vapour pressure was negligible.

2.2.8. Data processing and statistical analyses

The data were collated and processed in a spreadsheet (Microsoft Office Excel 2013) and visualised using technical graphing software (Igor Pro version 6.37). Statistical analysis software (GenStat 17th edition, VSN International, Hemel Hempstead, UK) was used to examine treatment effects *via* paired *t*-tests or (for skewed and/or heteroscedastic distributions) Wilcoxon matched-pairs tests on mean data per treatment per plant across the experiment (where $\alpha = 0.05$). In addition, linear least-squares regression was used to examine the relationship between variables. To avoid pseudo-replication in the set of preliminary field observations, results were averaged within panels (i.e. the experimental unit), then across rows (i.e. replications), propagating the uncertainty across the levels. Data are reported as means (± one standard error, SE).

3. Results

3.1. Preliminary observations

Mean capfall for the grapevines sampled from the field experiment occurred on November 13, 2013 (± 3 d). Berries sampled 48 d later had a TSS of *ca.* 5 °Brix and berry cell vitality close to 100%. Six weeks later the berries were technically fit for harvest and cell vitality was 60–70% (Fig. 1a). Up to this point, the seasonal conditions during the sampling period included three days ≥ 40 °C (Fig. 1b), and leaf balance pressures ≥ 1 MPa on two occasions (Fig. 1c). Across the remainder of the sampling period (84 d), maximum temperatures declined from *ca.* 30 to 20 °C, rainfall events > 5 mm were frequent, and leaf balance pressure was < 1 MPa (Fig. 1b and c). Isolated symptoms of shrivel on sampled berries were first noted on February 25, 2014, approximately two weeks after technical ripeness. Cell vitality and TSS had changed little three weeks after technical ripeness. Cell vitality and TSS then became highly variable, with the former declining at a rate similar to that observed ahead of technical ripeness (*ca.* 20% per month). Final TSS was 31 ± 7 °Brix (Fig. 1a).

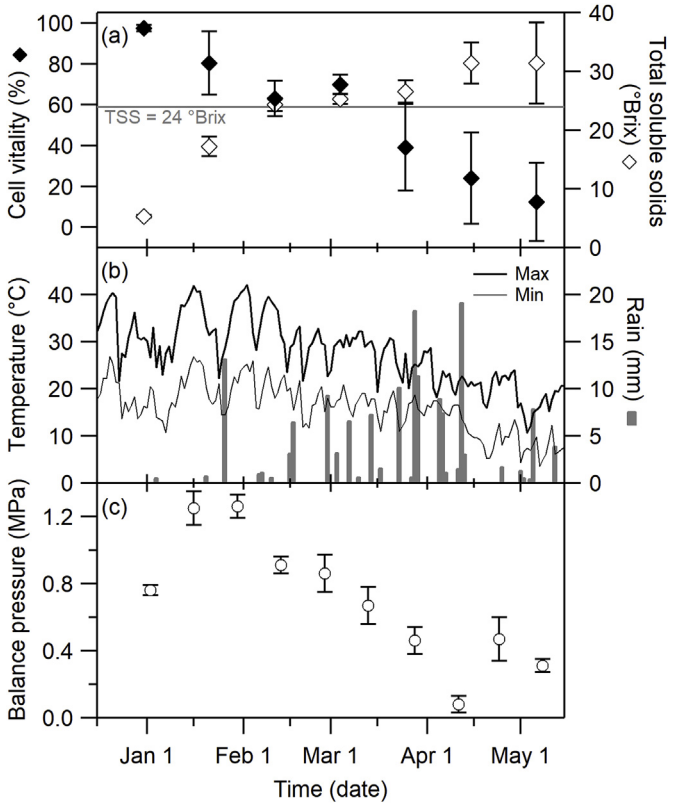

Fig. 1. Berry cell vitality and total soluble solids (a), temperature and rainfall (b), and the midday balance pressure of non-transpiring leaves (c) across the sampling period of preliminary observations made on fruit sourced from a field experiment. Error bars represent ± 1 SE. In (a) the grey reference line indicates the total soluble solids of berries technically fit for harvest.

3.2. Potted vine experiment

3.2.1. Phenology and growing conditions

Mean capfall across the potted vine experiment occurred on October 28, 2015 ($p = 0.041$ for treatment effects but with a negligible difference between means (< 0.2 d)). Precocious-ripening berries were marked on December 18 and late-to-ripen berries were marked on December 27. Anecdotally, there were no obvious gradients in the onset of véraison within bunches, an observation supported by minimal contrasts in the TSS of exposed and sheltered berries early in the ripening period (Table 1). The date late-to-ripen berries were marked is used to demarcate the end of véraison and time zero for the experimental period. Refractometer measurements taken four days later ($n = 15$ per treatment) indicated the TSS was approximately 15.4 °Brix. The same measurements taken again 12 days into the experimental period indicated the TSS had increased to approximately 19 °Brix. The FW of sampled berries increased slightly between these two sampling events, ranging from treatment means of about 1.3–1.7 g, with minor differences between the treatments (Table 1). During these early stages of ripening there was one day when air temperature approached 40 °C and a total of 17 mm of rain, most of which fell two days prior to TSS reaching 19 °Brix (Fig. 2).

Ahead of the first sampling event for cell vitality assessment (day 24), there was little rainfall and a phase of high temperatures: 17 days into the experimental period air temperature reached 42.5 °C. Cell vitality assessments on fruit sampled on day 19 from the buffer plants ($n = 4$) featured the typical symmetrical loss of cell vitality in the inner mesocarp and TSS approaching 20 °Brix (results not shown). At midday on day 23, shrivel was evident on a small number of berries and the tips of new growth had begun to wilt. All plants were immediately given the equivalent of one day of irrigation. The vegetative symptoms of water stress were relieved by early afternoon, did not result in leaf margin burn and were not observed again.

On the sampling date for the first set of samples for cell vitality assessment, the fruit was at oenological maturity because TSS was approximately 24 °Brix (Table 1). The TSS remained at this concentration for the next two weeks. This fortnight included repeated rainfall events in excess of 10 mm d^{-1} (Fig. 2). The mean treatment FW of sampled berries was about 1.6 g at the start of the late ripening period and remained stable at 1.4 g over the next two weeks (Table 1). Upon reviewing the TSS data, a decision was made to collect a final sample of berries for cell vitality assessment two weeks later, 52 days after the end of véraison. The berries of this ultimate sampling event exhibited TSS of approximately 26 °Brix and mean treatment FW below 1.3 g (Table 1). Symptoms of shrivel were more pronounced in berries from the exposed treatment (Fig. 3).

3.2.2. Berry exposure

The mean grape berry surface albedo was 0.093 ± 0.001 (SE) and exhibited no discernible treatment effects (Table 1). Wind velocity recorded at the weather station typically ranged between 0.2 and 0.7 m s^{-1} (Fig. 2c). The translation of wind velocity measured between the replicated rows to the sampling sites was about twice as efficient for exposed (32.7 ± 3.6%) than sheltered (17.0 ± 0.9%) treatments ($p < 0.001$). The differences in exposure to wind reflect the outward location of berries at exposed sampling sites and the obstructions afforded to sheltered berries by leaves and neighbouring fruit. The daily radiation input usually peaked close to 1000 W m^{-2}, being 40–50% lower on overcast days (Fig. 2a). Mean percentage light exposure was much higher and more variable in the exposed (*ca.* 15–35% of controls) than sheltered (< 4% of controls) treatments (Table 1). These contrasts are attributed to the greater degree of direct and reflected light intercepted at exposed sampling sites and the limited but consistent amount of diffuse light reaching sheltered sites.

3.2.3. Berry transpiration

The weight loss of detached berries re-affixed *in situ* was measured on three occasions over the course of the experiment. For the first set of measurements (23 days after véraison) the VPD was about 5 kPa, and in the exposed treatment the transpiration rate was *ca.* 0.3 mmol m^{-2} s^{-1}, which was about 17% higher than the transpiration rate in the sheltered treatment (Fig. 4). No transpiration rate treatment contrasts were observed in the second set of measurements (30 days after véraison) (*ca.* 0.07 mmol m^{-2} s^{-1}) where the VPD was < 2 kPa (Fig. 4). In the third set of measurements (36 days after véraison), the transpiration rate was higher in the exposed treatment even though the flux (*ca.* 0.05 mmol m^{-2} s^{-1}) and VPD were low (1.2 kPa) (Fig. 4). Overall, a strong correlation was observed between *in situ* transpiration and VPD (results not shown), with treatment effects being larger and more significant with increasing VPD.

3.2.4. Grape berry cell vitality

Across the first three sampling events no treatment effects were observed on grape berry cell vitality (Table 1). The treatment means remained in the range of approximately 65–73% across this period. All individual berry cell vitality assessments across this period typically fell within the 55–85% range (Fig. 5). Berries from the exposed treatment appeared more prone to outliers both above and below this range (Fig. 5). It was only at the final sampling event, when mean cell vitality was approximately 50% for the exposed berries and 60% for the sheltered berries, that treatment effects were observed ($p = 0.037$). The range in individual berry cell vitality broadened at this time point, spanning about 30–70% for the exposed treatment and 40–80% for the sheltered treatment (Fig. 5). ANOVA and post-hoc Tukey HSD tests identified a negligible number of plant × treatment effects on cell vitality, amounting to 0.2% of all possible plant × treatment

Table 1
Properties of exposed and sheltered berry treatment groups. Analyses were performed on paired means per treatment per plant across the experiment and are reported as treatment means ± SE. Asterisks on results for the sheltered treatments indicate statistical significance of the treatment effect (see footnote).

Parameter	Treatment	Days after the end of véraison					
		5	13	25	32	39	52
Berry albedo[a]	Sheltered			0.110 ± 0.005	0.082 ± 0.002	0.089 ± 0.002	0.086 ± 0.002
	Exposed			0.115 ± 0.001	0.083 ± 0.002	0.089 ± 0.001	0.086 ± 0.002
Berry cell vitality (%)[a]	Sheltered			65.4 ± 1.4	71.4 ± 1.3	70.1 ± 1.7	61.3 ± 3.4*
	Exposed			65.9 ± 1.7	68.8 ± 2.1	73.3 ± 1.8	49.9 ± 3.0
Berry fresh weight (g)[a]	Sheltered	1.29 ± 0.07*	1.68 ± 0.07	1.59 ± 0.04	1.44 ± 0.05	1.44 ± 0.05	1.23 ± 0.05
	Exposed	1.45 ± 0.07	1.51 ± 0.10	1.66 ± 0.04	1.44 ± 0.05	1.44 ± 0.06	1.28 ± 0.03
Berry total soluble solids (°Brix)[a]	Sheltered	15.4 ± 0.4	18.7 ± 0.3	24.6 ± 0.2*	24.5 ± 0.3**	24.6 ± 0.3**	26.3 ± 0.3
	Exposed	15.4 ± 0.3	19.2 ± 0.4	23.9 ± 0.3	23.5 ± 0.3	23.2 ± 0.3	26.3 ± 0.4

		Batch			
		1	2	3	4
Light exposure (% control)[b]	Sheltered	2.0 ± 0.3***	2.9 ± 0.6***	3.8 ± 0.8***	2.0 ± 0.3***
	Exposed	14.9 ± 2.3	28.5 ± 7.0	33.8 ± 3.6	21.5 ± 2.1

*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.
[a] Analysed with the paired two-sided *t*-test.
[b] Analysed with the Wilcoxon matched-pairs test.

combinations.

4. Discussion

In the potted vine experiment, marked contrasts in light interception as well as wind velocity during berry ripening produced only minor differences in TSS (*ca.* 0.7 °Brix contrast) but no difference in mesocarp cell vitality (mean of *ca.* 65%) at 24 °Brix (Table 1). At this stage of development, the cell vitality of field experiment berries was similar to that of potted vine berries, and shrivel symptoms had not been observed in either setting. The parsimonious explanation for the consistent trends within and between settings is berry ripening and senescence progressed similarly across the settings despite the contrasts in seasons, clones and plant management. Cell vitality measurements taken on Cabernet Sauvignon, Merlot and Sultana berries from the field experiment (results not shown) showed a good correlation ($r^2 > 0.8$) with those of Shiraz. However, the cultivars produced contrasting phenotypes: cell vitality in Sultana berries was strongly bimodal (few observations in the range 5–90%), and in contrast to the other wine grapes, the cell vitality of Merlot berries declined earlier with respect to calendar date, thermal time, and at lower TSS. This is consistent with previous reports of cultivar effects on cell vitality (Fuentes et al., 2010; Krasnow et al., 2008; Tilbrook and Tyerman, 2008, 2009), and suggests caution should be applied when extrapolating the study results to other

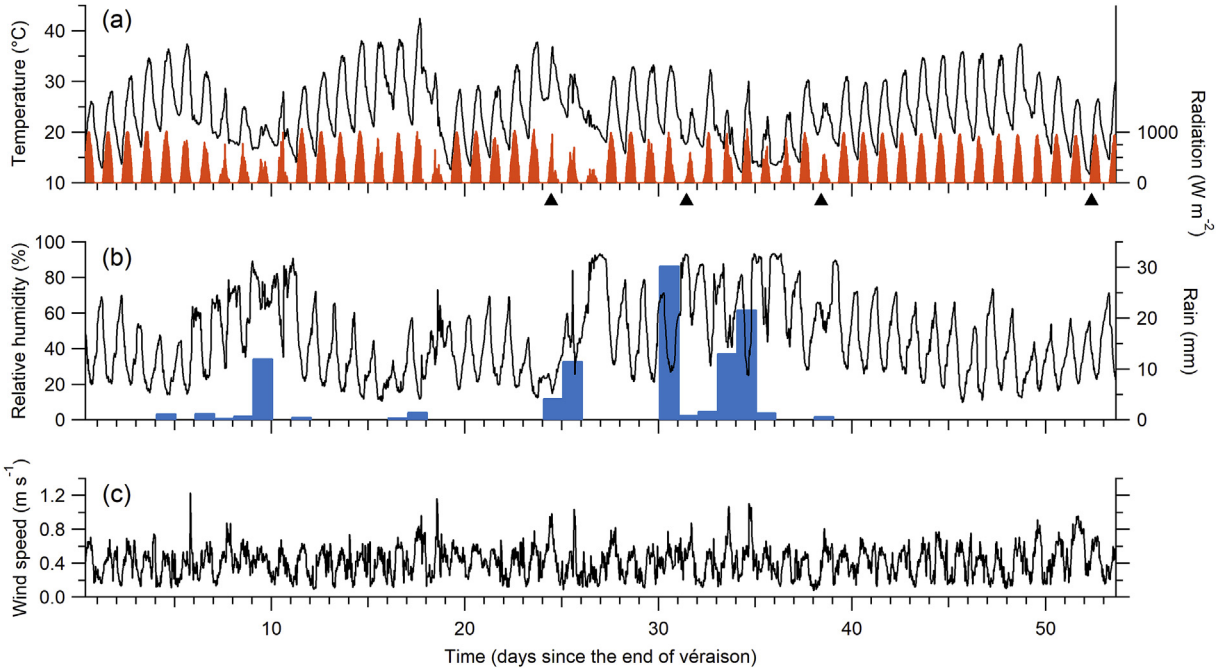

Fig. 2. Weather conditions across the experimental period of the potted vine experiment. Temperature (black line) and radiation (umber fill) are shown in (a), relative humidity (black line) and rain (blue bars) are shown in (b) and wind speed is shown in (c). Triangles in (a) show the timing of cell vitality assessments. For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

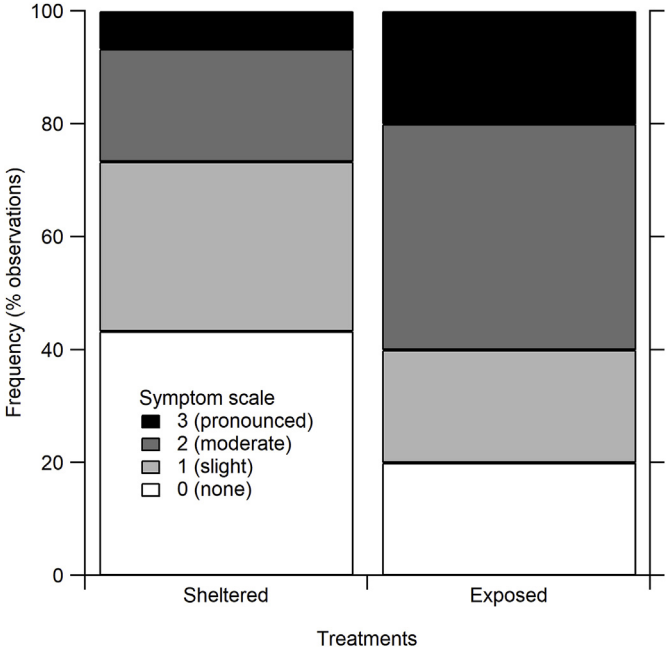

Fig. 3. Extent of shrivel symptoms visible on the surface of grape berries at the final sampling event of the potted vine experiment, 52 days after the end of véraison (n = 30 for each treatment). The scale used to score the symptoms is described in the text.

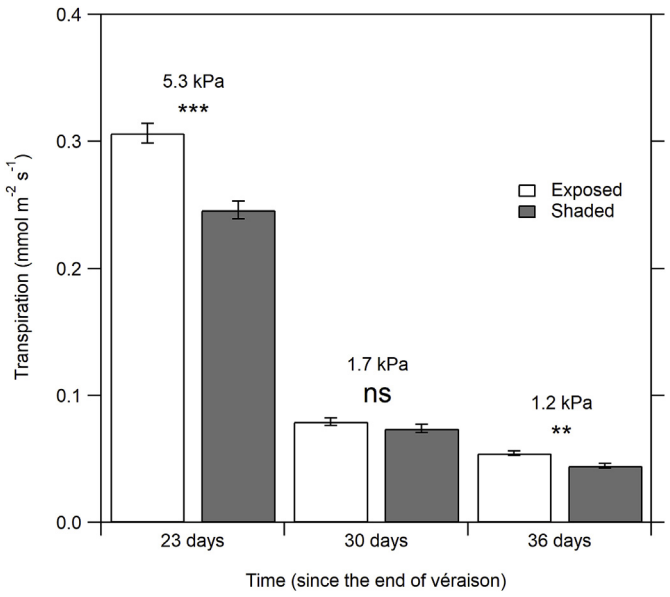

Fig. 4. Transpiration rate of excised grape berries re-affixed *in situ*. The transpiration rate is calculated from the weight loss across the deployment period and expressed with respect to berry surface area. All data points represent the treatment mean (± 1 SE). Asterisks indicate statistical significance of paired *t*-tests, and the vapour pressure deficit is the mean across each deployment period.

cultivars.

In the potted vine experiment, there were significant treatment contrasts in cell vitality 27 d after the fruit was technically fit for harvest, with exposed berries showing lower cell vitality than those in the sheltered treatment (Table 1). However, in both experiments, there was a period lasting for 2–3 weeks from technical ripeness over which berry cell vitality and TSS remained approximately stable. Although this contrasts with the hypothesis that the decline in cell vitality follows a

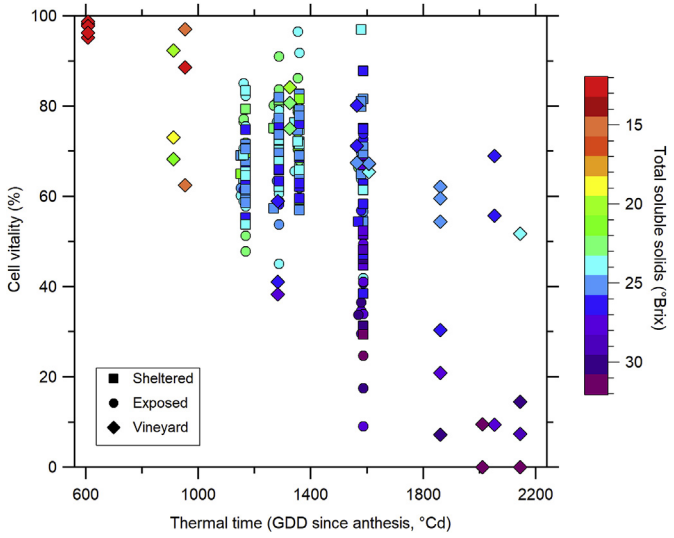

Fig. 5. Individual measurements of grape berry cell vitality as a function of thermal time since anthesis (calculated from air temperature with a base of 10 °C). Symbol shapes correspond to the exposed (squares) and sheltered (circles) treatments of the potted vine experiment, and to the preliminary field experiment observations (diamonds). Symbol colour indicates berry total soluble solids. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

biphasic trend characterised by an increasing rate of decline (Bonada et al., 2013b), there are precedents for this. For example, the decline in cell vitality has been observed to 'stall' for approximately 20 days in the range 60–80% (Caravia et al., 2016; Krasnow et al., 2008; Tilbrook and Tyerman, 2008). In at least one of these examples (Caravia et al., 2016) stable cell vitality was associated with rainfall and stable berry TSS, a characteristic shared with the present study (Fig. 1b and Table 1). Although this may be a developmental trend under genetic control, the coincidence of this stability with rainfall events (Figs. 1 and 2) increases the likelihood that the process that controls the decline in cell vitality interacts with the environment via the berry water balance. Exogenous water on the surface of the berry, receptacle and pedicel readily contributes to the water balance of the fruit (Becker et al., 2012).

In overripe fruit, the berry water balance appears to be a more important control of cell vitality than the grapevine water balance because (1) the latter was held constant in the pot experiment via within-plant statistical tests that showed significant effects at the experiment end (Table 1), and (2) in the field experiment berry cell vitality declined over time despite a continuing improvement in grapevine water status (Fig. 1). Previous work has shown a more rapid decline in cell vitality with deficit irrigation (Bonada et al., 2013a), however the treatments also effected bunch temperature via reduced canopy size, thus it is not clear at what scales (whole plant and/or berry) the treatment had an effect on fruit physiology. The ultimately lower cell vitality in the exposed berries of potted vines may be a result of a lower hydration status produced by increased berry transpiration (Fig. 4). Exposure is expected to increase berry transpiration primarily by increasing radiation interception, thereby elevating the surface temperature and increasing the berry-atmosphere vapour pressure gradient (Smart and Sinclair, 1976). A model used to assess whether this translates to a change in temperature at the centre of a berry (Supplementary Material) suggested the thermal time accumulated by exposed berries over the ripening period was significantly greater than that of shaded berries (Table S1). Nocturnal rehydration is necessary to reconcile the conflict between the greater transpiration flux inferred and observed for exposed berries with the paucity of FW contrasts observed between the treatments (Table 1). Thus, the enhanced shrivel symptoms observed in the exposed berries may be a deformation response to a greater amplitude in

their daily hydration/dehydration.

The potted vine experiment and field observations are consistent with an increased sensitivity of cell vitality to berry water status over the course of ripening and senescence. However, identifying the process that determines this sensitivity is difficult, particularly in the outdoor context of the present work where heat and rainfall events are not controlled. Possible mechanisms include a cell vitality feedback, whereby the loss of cellular membrane competence may impair the ability of the fruit to obtain and retain water, leading to further dehydration-induced loss of cell vitality. An accelerated decline is a common characteristic of many berry cell vitality developmental trends (Fig. 5) (Bonada et al., 2013a; Bonada et al., 2013b; Clarke et al., 2010; Krasnow et al., 2008; Tilbrook and Tyerman, 2008). For Shiraz, this may be exacerbated by a continued hydraulic conductivity to the parent plant, making the fruit susceptible to dehydration via backflow (Tyerman et al., 2004). Another possibility is berry solute concentration acting as a stressor or signal, because cell vitality is seldom maintained above 80% in overripe berries (Fig. 5) (Krasnow et al., 2008). While it remains possible that high TSS leads to a decline in cell vitality, the observation that sheltered berries maintained significantly higher TSS for several weeks after technical ripeness yet ultimately exhibited a lesser degree of cell vitality loss than exposed berries (Table 1), provides evidence there is not necessarily a simple (e.g. linear, or devoid of interactions) or causal effect of TSS on cell vitality. Furthermore, low TSS and reduced cell vitality are associated with 'berry shrivel' (Krasnow et al., 2009), and TSS > 24 °Brix is not a prerequisite for the loss of large areas of vital cells in Shiraz berries (Clarke et al., 2010).

If bunch-scale variations in exposure (or one its components, such as light interception) have an effect on berry cell vitality, and these effects accumulate over time, there is scope for time-dependent variability in cell vitality measurements across a population of berries. This would explain why the cell vitality of field experiment berries became increasingly variable across sampling events (Figs. 1 and 5), and why increased variability with decreasing mean cell vitality is seen in much of the cell vitality research cited above. This heteroscedasticity may require an alternative to the mean ± uncertainty (such as the probability of exceeding a critical cell vitality) to convey, and to render suitable for curve fitting and statistical analyses, the distribution of cell vitality measurements across a population of berries over time.

5. Conclusions

Two sets of observations have verified that technical ripeness of the grape berry is associated with the loss of mesocarp cell vitality, and both provide evidence that late-season shrivel is not synonymous with this early stage of senescence. At technical ripeness, contrasts in exposure to light and wind were not sufficient to produce significant differences in the cell vitality of exposed and sheltered berries of potted vines. However, significant differences were observed 27 d later, with exposed overripe berries showing lower cell vitality than sheltered berries. In the potted vine experiment and preliminary set of observations on field experiment berries, berry cell vitality and TSS remained approximately stable for at least two weeks after the advent of technical ripeness. In both settings, this stability coincided with rain events. These observations support the idea that during development the berry water balance increases in importance as a determinant of the rate at which berry cell vitality declines. In both experiments, the terminal observations showed a decrease and an increase in the berry cell vitality mean and variance, respectively. Due to this heteroscedasticity, an alternative to the mean and its central tendency may be appropriate for describing the extent of cell vitality in a sample of grape berries. As this research is consistent with cultivar and environmental influences on berry ripening and senescence, confidence in the general relevancy of the findings would be enhanced by comparable observations and further experiments designed to identify the primary controls of both processes.

Disclosure statement

There are no conflicts of interest to disclose.

Contributions

Both authors contributed significantly to this manuscript and are in agreement on its content. In consultation with SY, SC designed and performed the research, analysed the data and compiled the manuscript. SR critically reviewed the manuscript.

CRediT authorship contribution statement

Simon J. Clarke: Formal analysis, Writing – review & editing. **Suzy Y. Rogiers:** Formal analysis, Writing – review & editing.

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Appendix A. Supplementary data

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