Elevated temperature and water stress accelerate mesocarp cell death and shrivelling, and decouple sensory traits in Shiraz berries

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Abstract

Both water deficit and elevated temperature are likely to accelerate shrivelling in Shiraz berries with consequences for fruit yield and quality. The process of shrivelling is partially related to mesocarp cell death and it has been proposed that enhancement of berry flavour and aroma also correlates with mesocarp cell death. However, the combined effects of water deficit and elevated temperature on berry shrivelling, mesocarp cell death and berry sensory traits are unknown. We tested the hypotheses that (i) the effects of water deficit and elevated temperature on the dynamics of mesocarp cell death and shrivelling are additive, and that (ii) faster cell death, as driven by warming and water deficit, negatively contributes to grape sensory balance. Using open-top chambers to elevate day and night temperature, we compared heated vines against controls at ambient temperature. Thermal regimes were factorially combined with two irrigation regimes, fully irrigated and water deficit, during berry ripening.

The dynamic of cell death was characterised by a bilinear model with three parameters: the onset of rapid cell death and the rate of cell death before and after the onset of rapid cell death. Statistical comparison of these three parameters indicated that there was not interaction between water and temperature on the dynamics of berry mesocarp cell death. Warming advanced the onset of cell death by ~ 9 days ($P = 0.0002$) and water stress increased the rate of cell death in the period post onset ($P = 0.0007$). Both water stress and elevated temperature increased the proportion of cell death and shrivelling at harvest. An interaction between water deficit and elevated temperature was found whereby the onset of berry net water loss was advanced by elevated temperature under water deficit but not in the fully irrigated treatment. Sensory traits typical of ripened berries were associated with higher cell death; however, warming and water deficit hastened ripening and altered the balance of berry sensory traits.

Introduction

Realised and projected climate change assessments suggest more frequent and severe droughts and warmer temperature in many grape growing regions worldwide (Brunetti et al. 2000, Duchene and Schneider 2005, Webb et al. 2007, Giorgi and Lionello 2008). In this context, this paper deals with grape berry traits in response to the combined effects of water stress and elevated temperature.

Both water deficit and elevated temperature are likely to accelerate shrivelling in Shiraz berries with consequences for fruit yield and quality (Keller et al. 2006, Tilbrook and Tyerman 2009, Bonada et al. 2013). The process of shrivelling is partially related to mesocarp cell death and it has been proposed that enhancement of berry flavour and aroma also correlates with mesocarp cell death (Tilbrook and Tyerman 2008). Recent studies focused on variety and environmental influences on mesocarp cell death in berries and its implications for grape and wine quality (Tilbrook and Tyerman 2008, Fuentes et al. 2010, Bonada et al. 2013). Cell death inception has been linked to berry ontogeny and its relation with berry water loss in later ripening has been elucidated (Krasnow et al. 2008, Tilbrook and Tyerman 2008, 2009). Furthermore, changes in the susceptibility of berries to splitting during late ripening seem to be driven by reduction in mesocarp cell viability (Clarke et al. 2010). While the signalling pathway involved in this genetically controlled process remains to be elucidated, the role of reactive oxygen species triggering cell death is well understood (De Pinto et al. 2012). Furthermore, multiple lipoxygenase isoforms mediate the oxygenation of polyunsaturated fatty acids and lipids in cell membrane and contribute to the enhancement of volatile aroma and flavour compounds on late ripening berries (Podolyan et al. 2010). It has been hypothesized therefore that higher percentage of cell death at harvest would positively contribute to berry flavour and aroma development (Tilbrook and Tyerman 2008, Coetzee and du Toit 2012). The positive association between cell death and desirable berry attributes has been established in comparisons between grapes sourced from low and high grade quality (Tilbrook and Tyerman 2008). We do not know, however, if this positive correlation holds under environmental stresses, including water deficit and elevated temperature.

Moderate water deficit during ripening improves berry quality in red wine varieties (Kennedy et al. 2002, Roby and Matthews 2004, Iland et al. 2011, Basile et al. 2011, Intrigliolo et al. 2012). The effects of water deficit on mesocarp cell death are unknown. However, water deficit often accelerates senescence of other plant tissues (Romero et al. 2010, Prieto et al. 2010). If water deficit improves berry quality but at the same time increases cell death, the positive correlation between cell death and berry quality (Tilbrook and Tyerman 2008, Coetzee and du Toit 2012) may break down under water stress.

The positive association between cell death and quality berry traits may not hold under warming conditions either. On the one hand, direct experimental evidence supports the negative effect of elevated temperature on aspects of berry and wine quality (Sadras et al. 2012c, Sadras and Moran 2012b, Sadras et al. 2013). On the other hand, we recently demonstrated that elevated temperature during berry development anticipates the onset and increased the rate of rapid mesocarp cell death in Shiraz and Chardonnay berries (Bonada et al. 2013). The correlation between cell death and berry quality can therefore depend on the sources of variation affecting both traits.

"Wine balance" has been defined as the "harmonious relationship between acids, fruit, tannin and other natural elements" (Stevenson 2005). Wine balance is partially related to the balance of chemical and sensory traits in berries, and elevated temperature may alter the balance of berry sensory components. The balance of berry sensory traits in response to temperature has been described in terms of two processes: *offset*, which accounts for the average advancement (or delay) across berry traits, and *decoupling*, which measures the scatter in the response of the different traits (Sadras et al. 2013). For example, elevated temperature advanced the ripening of seed more markedly than the ripening of skin and pulp of Cabernet Franc, effectively decoupling the seed from other fruit components (Sadras et al. 2013). Thus, in addition to the effect of elevated temperature and water deficit on individual berry traits, we are interested in the collective effect of these factors on the balance of berry traits.

Here we assessed the effects of elevated temperature, water stress and their interaction on the dynamics of mesocarp cell death and shrivelling in Shiraz grapes. Furthermore we measured the offset and decoupling of berry sensory traits caused by warming and water deficit and the correlation between cell death and berry sensory traits. Our working hypotheses are that: (i) the effects of elevated temperature and water deficit on cell death are additive, and that (ii) faster cell death, as driven by warming and water deficit, negatively contributes to grape sensory balance. The effects of irrigation and temperature on phenology, yield and stomata physiology measured in this trial have been reported elsewhere (Sadras and Moran 2012a, Sadras et al. 2012b, Sadras and Moran 2013).

Materials and Methods

Experiments

A factorial trial of two temperatures and two irrigation regimes was established on October 2010 on own-rooted Shiraz vines (clone 1654) in a vineyard located in the Barossa Valley (34°S, 139°E), **Australia**. Vines were planted in 2004 on a red brown earth soil (Northcote 1979). Row orientation is north-south with 3.0 m between rows and 2.25 m between vines. Vineyard management was the same for all treatments; vines were spur-pruned to 40–50 buds per vine and trained to a single-wire trellis system. The treatments were laid out in a split-plot design with three replicates, where temperature was assigned to main plot and water regime to subplots. Each replicate included nine vines, where the seven central plants were considered for research purposed, while the remaining two were considered as buffer. To avoid interference between treatments, one guard row was left on both sides of the experimental row and replicates were separated by a buffer zone ~ 13.5 m.

Heated plants were compared with control vines at ambient temperature. To increase temperature under realistic field conditions we use open-top chambers (Sadras et al. 2012a). These automated chambers combine passive heating during clear-sky day's hours and active heating, with pressured hot water, during cloudy or night's hours. Automated open-close panels allow for dissipation of heat when temperature exceeds the target and heat irradiates through hot water circulating through polyethylene pipes

at soil level when passive heating is insufficient. Full construction details, performance and limitation are in Sadras et al. (2012a). Water regimens were imposed in two consecutive seasons (2010/11 and 2011/12) based on calendar applications. During season 2011/12, control vines were watered weekly from November (26/11/2011) to February (24/02/2012). In contrast, deficit vines were irrigated only twice (24/01/2012 and 17/02/2012). Vines were drip irrigated at a flow rate of 4.4 L h⁻¹, during approximately 8 hours per irrigation event. Each row had one lateral with compensated button drippers spaced at one meter interval. **Well watered treatments received 152 mm in comparison with their deficit counterparts that received 23 mm.**

Characterisation of thermal and water regimes

Tinytag Ultra2 loggers (Hastings Dataloggers, Port Macquarie, NSW, Australia) were used to continuously record temperature (T°) and relative humidity (RH) at 15 minutes intervals. Loggers were located inside the canopy of the central vines from each replicate at mid canopy level. We also monitored bunch temperature with an infrared camera (FLIR B360, FLIR Systems, Portland USA), with a 320 x 240 pixel resolution. Four pictures of shaded bunches were taken at each sampling date per replicate (4 bunches x 12 replicates x 6 sampling times = 288 pictures). Pictures were acquired at a constant distance of 0.5 m perpendicular to the vertical bunch position. Image analysis was performed using the FLIR QuickReport® software (FLIR Systems, Portland. USA).

Vine water status was assessed using a Scholander type pressure chamber (PMS Instruments, Model 1005, Albany, OR. USA) using the procedures of Hsiao (1990). During both seasons we measured pre-dawn water potential as previously reported in Sadras and Moran (2012a). In 2011-12, we also measured stem water potential at mid- day, between 1 to 3pm, in two selected fully expanded mature leaves from central plants of each replicate. Leaves were enclosed in plastic bags covered with aluminium foil and left on the darkness to stabilise for at least 30 minutes before the measurements.

Vine phenology

Phenology was assessed with the modified E-L system proposed by Coombe (1995) as previously described in Bonada et al. (2013). Growing degree days ($^{\circ}\text{Cd}$) from anthesis onwards were calculated for each replicate using actual temperature at canopy level and a base temperature of 10°C (Williams et al. 1985).

Berry biophysical traits: cell death, berry shrivel and berry water content

Berry sampling, FDA staining and image analysis

Berry biophysical traits were assessed in 2011-12. In mid December, when berries were still hard and green, a total of 10 bunches per vine were selected at a similar stage of development (E-L 33). Bunches were chosen from the shaded part of the canopy to avoid direct sunlight damage and were marked for further observations. From December 22nd to March 7th, we sampled 70 berries per replicate at weekly intervals. To increase uniformity, berries were only taken from the proximal part of the bunch by cutting with scissors through the pedicel as close as possible to their point of attachment at the rachis. Zip-lock plastics bags with berries were immediately placed into an ice-cooled container in the field.

A subsample of 15 berries was randomly collected for each replicate and processed for fluorescein diacetate (FDA) staining and microscopy imaging acquisition as described in Bonada et al. (2013). Image processing for berry tissue vitality and morphometric analysis including berry shrivel was performed using a customised code in MATLAB® R2010b (MATHWORKS inc., Natick, Ma, USA) (Fuentes et al. 2010). Shrivel index calculation and a full discussion about its use have been recently covered (Fuentes et al. 2010, Bonada et al. 2013). Briefly, this normalized index takes into consideration changes in berry diameter:perimeter ratio; it ranges from 1 (maximum turgor) to 0 (maximum shrivel) according to the developmental stage and the cultivar.

The dynamics of cell death were characterised with the bilinear model described in Bonada et al. (2013). The model has three biologically relevant parameters: the onset of rapid cell death, the rate of cell death before (b) and after (b') rapid cell death.

Analysis of variance and analysis of residuals were used to test for treatment effects (Bonada et al. 2013).

Using a subsample of 10 berries per treatment, berry water content was estimated as the difference between fresh and dry weight, after drying berries until constant weight in the oven at 75°C.

A peak lognormal equation with four parameters (Fowler 2003) was fitted to estimate the time of maximum berry water content for each replicate and treatment (SigmaPlot version 11.0, Systat Software Inc., San Jose, California):

$$y = y_0 + \frac{a}{x} \exp \left[-0.5 \left(\frac{\ln(x/x_0)}{b} \right)^2 \right] \quad eq.(1)$$

The dynamics of berry water content (%) were characterised with a concentration-response type curve as described in Sadras et al. (2008).

Berry sensory traits

Bunch sampling and preparation for testing

Sampling date for sensory assessment was specific for each thermal x water regime in order to achieve a similar sugar concentration in berries. To meet this objective we monitored sugar concentration weekly as previously described in Sadras et al. (2012b).

Sensory traits were assessed in the 2010-11 and 2011-12 seasons. At harvest, one bunch per plant per each replicate (7 bunches x 3 replicates) was sampled from the shaded part of the canopy (~ 1 kg). Bunches were collected early in the morning when air temperature remained low, placed immediately in plastic bags and kept in ice-cooled containers to be transported to the sensory laboratory for further conditioning. Berries from the same replicate-treatment were cut off from the rachis with their stalk in place and mixed in a plastic container. Samples were stored in a cool room (2°C) for assessment on the following day.

Sensory assessment

A panel of 12 people was trained in both seasons previous to berry sensory evaluation. During 2010/11, a trained sensory panel from the South Australia Research and Development Institute (SARDI) was used for the assessment. On season 2011/12, panellists were selected for their interest and availability from a group of scientist and postgraduate student from SARDI and The University of Adelaide. Eight out of 12 testers in 2012 had previous experience and training on sensory evaluation, while five recruited panellist had previously performed berry sensory evaluation.

For both seasons, training was carried out in two 1-h sessions following the protocol established by Winter et al. (2009). The first training session was used to check the sensorial capability of each individual to characterize a set of standard attributes, including solutions of: sugar between 20 and 26 °Brix for sweetness, tartaric acid between 0.5 and 2 g l⁻¹ for acidity, quinine sulphate between 10 to 20 mg l⁻¹ for bitterness and grape seed tannin (GrapExTM, Tarac Technologies, Nuriootpa, SA, Australia) between 0.5 and 3 g l⁻¹ for astringency. The concentration of intensity standards was adjusted according to published references (Winter et al. 2009, Lohitnavy et al. 2010). The second session was to familiarise the panellist with the scoresheet and descriptors to be used in the evaluation session. It also included a preliminary assessment of Shiraz berries from the same experiment and with the same maturity level. This session allowed panellists to agree on the scaling of each descriptor and to standardise the vocabulary used to describe the grape sensory attributes.

The actual assessment was conducted in one session of approx. two hours. The sensory room was equipped with 10 independent booths, in line with requirements from ISO 8589 (2007). Panellists evaluated five berries per replicate using defined attributes summarised in the sensory criteria chart in Winter et al. (2009). To avoid palate fatigue, panellists were asked to take 10-15 minute breaks between evaluations. The scoresheet included 20 descriptors which comprise berry parts (whole-berry, pulp, seed and skin) with 3 to 6 traits for each part. The type and length of the scales varied between assessment years. In 2011 the score sheet included a check-box scale

for each attribute from 1 to 4, from unripe to fully ripe as recommended by Winter et al (2009). In 2012, we used a line scale to increase the resolution (Greene et al. 2006, Park et al. 2007). The line scale was 15 cm long, with four word anchors from 0 = unripe to 15 = fully ripe (Lawless and Heymann 2010, Lohitnavy et al. 2010). Berries were served in a randomised order in paper cups; mineral water and unsalted water crackers were provided as palate cleansers during evaluation.

Statistical analyses of sensory traits

Two multivariate analysis methods were used to examine panellist performances (Malundo and Resurreccion 1992, Dijksterhuis 1995, Kennedy and Taylor 2003, Robinson et al. 2011). Principal component analysis (PCA) and cluster analysis were carried out on the matrix between panellist correlation coefficients, resulting from the attribute score arrays between berry traits (rows) and panellists (columns) (King et al. 2001). Ward method was used to assess Euclidean distances on the regression coefficients among testers (Robinson et al. 2011). These methods discriminate the level of agreement among panellist to characterise the same berry attribute and to exclude outlier testers before further statistical comparisons.

Two complementary methods were used to quantify the effects of key sources of variation on berry sensory traits, as further explained in Sadras et al. (2013). First, we used ANOVA and Fisher's PLSD to assess the effects of temperature, water regime, replicate and judge, and their interactions. Second, we assessed the offset and scatter of berry development in two steps. First, we fitted least squares regressions between scores for treatments and a forced slope = 1. Positive intercept was associated to advance ($a > 0$), while negative represent delayed development ($a < 0$). We then calculated the root mean square error:

$$RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^n (M_i - E_i)^2} \quad eq.(2)$$

were $n (= 20)$ is the total number of traits, M_i is the measured score and E_i is the score derived from the fitted model. Higher scatter around the fitted line ($> RMSE$), was indicative of higher decoupling among traits.

Results

Growing conditions: thermal and water regimes

Mean maximum temperature was higher in 2011/12 than in 2010-11; maximum temperature during the growing period September to March was $\sim 1.5^{\circ}\text{C}$ higher in the second season. Differences in mean minimum temperatures were negligible between seasons ($< 0.02^{\circ}\text{C}$). The accumulated rainfall during the season 2010-11 (September to March) was 509 mm, which compares with the 257 mm in the same period during season 2011/12.

Performance of the heating system, including potential artefacts, and thermal regimes during the experiments have been fully described in previous works (Sadras et al. 2012b, Sadras and Moran 2012b). Across two growing seasons and the intervening winter, from September 2010 to March 2012, mean daily temperature at canopy level in the heated treatments was 1.31°C ($SD = 0.63$) higher than in controls (Sadras and Moran 2012a). The full trajectory of temperatures during the experiment is shown in Supplementary Fig. S2. Temperature of shaded bunches tracked ambient temperature during the season ($R = 0.99$, $P < 0.001$); it was approximately 1.5°C lower than temperature at mid canopy level. Water deficit reduced canopy size in the second season, and more exposed bunches had higher temperature than in well watered vines (0.78°C , $SD = 0.53$; $P = 0.007$).

Plants received ~ 79.2 L per vine per irrigation event. Well watered plants were irrigated on weekly bases from setting to harvest (Fig. 1). Only two supplementary irrigations were applied in deficit treatments in the period veraison-harvest to maintain stem water potential between -1.2 and -1.5 MPa (Acevedo-Opazo et al. 2010). Temperature treatments did not affect pre-dawn leaf water potential; the data pooled across temperature regimes showed significant differences in pre-dawn leaf water potential between irrigated and deficit treatments in both seasons

(Supplementary Fig. 1 in Sadras and Moran 2012a). Briefly, differences started to develop shortly before veraison and lasted for 3 weeks in 2010-2011 and shortly before harvest in 2011-12. Measurements of mid-day stem water potential in 2011-12 showed that control treatments were maintained at or above -1.0 MPa from pea size to harvest. Severe stress developed in the water deficit treatments from veraison to harvest (> -1.2 MPa). Heated vines had lower stem water potential (more negative) compared to vines at ambient temperature ($P = 0.02$).

Dynamics of mesocarp cell death

Onset and rates

Fig. 2 shows the dynamics of mesocarp cell death on chronological and thermal time scales, and Table 1 summarises the parameters of the fitted model.

Differences in temperature between treatments shifted the onset of mesocarp cell death in chronological scale (Fig. 2c). Warming brought forward the onset by nine days ($P < 0.001$), from 91 to 82 DAA. There was undetectable water and water x temperature effect on the onset ($P > 0.05$). On thermal time scale, differences in the onset were statistically undetectable for the three sources of variation.

The rates of mesocarp cell death between anthesis and the onset of rapid cell death were unaffected by temperature, water and temperature x water interaction, independently of the scale.

After the onset of rapid cell death, rates varied with both the treatment and the scale. As showed in Fig. 2c the rates of cell death on chronological scale were unaffected by temperature ($P = 0.64$); the fitted lines progressed in parallel, from the onset to harvest. The rates in the same period were higher for the water deficit treatment than in well watered plants ($P < 0.001$, Fig. 2b).

The offset between control and heated treatments disappeared on a thermal time scale, and cell death progressed at similar rates (Fig. 2f). However, water-induced effects on

the rate persisted and berries from water deficit vines showed an accelerated loss of mesocarp cell vitality in a thermal time scale (Fig. 2e).

Additional comparison of the fitted models was carried out with the analysis of the residuals between fitted curves and data as explained (Bonada et al. 2013) (insets Fig. 2a and d). Berries in control-irrigated treatments had the highest proportion of living tissue and the heated-water deficit treatment the lowest in chronological scale ($P < 0.001$); the other two treatments were in between. On thermal scale, residuals of the proportion of living tissue clearly separated the irrigated and water deficit treatments ($P = 0.02$).

Shrivelling and berry water content

Shrivel index declined non-linearly after the onset of rapid cell death (Fig. 3a and b). The statistical analysis of the residuals in chronological scale, showed significant differences between temperature ($P = 0.01$) and water treatments ($P = 0.03$). There was no detectable water x temperature interaction. Shrivel was smallest in berries from irrigated controls while highest shrivel symptoms were in berries grown under elevated temperature and water deficit, ($P = 0.002$) (inset Fig. 3a).

The apparent enhancement of shrivelling with warming disappeared when data was plotted as a function of thermal time (Fig. 3b). Analysis of residuals around the fitted function showed no differences among treatments. However, residuals were negative for water deficit treatments and positive for their irrigated counterparts. This indicated higher berry shrivelling under water deficit at the same stage of development ($P = 0.04$).

There was a positive correlation between the percentage of living tissue and shrivel index ($R = 0.87$, Fig. 3c). Shrivel index was tightly correlated with the mass of water per berry ($R = 0.81$, Fig. 3d) and with the relative content of water ($R = 0.73$, $P < 0.001$, not shown). Lower shrivel index was associated with lower water content in berries, hence, supporting the use of shrivel index as a tool to assess shrivelling during ripening (Fuentes et al. 2010, Bonada et al. 2013).

Peaks of water content were estimated using Eq. 1, applied to each replicate-treatment ($0.87 \leq \text{adjusted } R^2 \leq 0.99$). Peak water content ranged from $1.23 \pm 0.009 \text{ g berry}^{-1}$ in water deficit treatments to $1.44 \pm 0.024 \text{ g berry}^{-1}$ in irrigated treatments ($P < 0.001$), with undetectable effects of temperature and water x temperature interaction. The timing of peak water content was taken as the onset of berry net water loss; Fig. 4a shows the relationship between the onset of rapid cell death and the onset of berry net water loss. In control treatments the onset of cell death matched the onset of water loss independently of water regime; however, in heated treatments, the response varied within water regime (water x temperature interaction, $P < 0.005$). While in berries from deficit plants both processes were approximately synchronized, in the irrigated treatment the onset of mesocarp cell death was ~ eight days earlier than the onset of berry net water loss. The onset of berry net water loss was unaffected by temperature in well watered berries ($P = 0.44$), while the onset was accelerated when berries were exposed to warmer conditions under water deficit ($P < 0.001$) (Fig. 4b). Interestingly, heated berries had higher relative water content at the time of the onset of cell death than their counterparts at ambient temperature ($P = 0.01$, Fig. 4c).

Berry sensory assessment

We aimed at sampling berries with similar total soluble solid (TSS). This was achieved in 2010-11; the actual TSS at the time of sampling was $23 \pm 0.13 \text{ }^\circ\text{Brix}$ without differences between treatments. In 2011-12, TSS in water deficit berries was $27 \pm 0.27 \text{ }^\circ\text{Brix}$ compared with $26 \pm 0.19 \text{ }^\circ\text{Brix}$ in their irrigated counterparts ($P < 0.001$); differences were undetectable, however, for temperature and water x temperature effects.

We used PCA and cluster analysis to probe for bias associated with panellists in the assessment of sensory attributes (Supplementary Fig. S1). In 2011-12, these analyses detected three panellists who disagreed with the remaining testers in the scoring of some berry sensory attributes; their scores were therefore excluded from further analysis (Malundo and Resurreccion 1992, King et al. 2001) without compromising panel size (Heymann et al. 2012, Olarte Mantilla et al. 2012). No apparent bias was found in 2010-11; hence we used the data from all the panellists.

Sensory traits in 2010-11

There was a significant effect of panellist on sensory trait score ($P < 0.001$), and significant interactions between panellist and water ($P = 0.04$), panellist and temperature ($P = 0.01$) and panellist, water and temperature ($P = 0.04$). On the contrary, a replicate effect was found to be non significant ($P = 0.76$).

Ten out of 20 attributes were affected by treatments (Table 2). Eight attributes were affected by temperature, two by water regime, and three traits responded to temperature x water interaction.

Berries under warmer conditions were less turgent, more likely to deform permanently (higher softness score), and had their stalks more strongly attached. Higher temperature advanced the development in one pulp trait: acidity, and in three seed traits: crushability, flavours and tannic intensity. Two traits in seeds responded to the effects of water: colour and crushability. In both cases water deficit advanced ripening. Two traits in pulp, detachment and juiciness, and one in skin, disintegration, responded to the water x temperature interaction. In all these cases berries from well watered plants had a higher response to temperature.

Sensory and analytical methods agreed on the assessment of berry sugar content. There was not effect of temperature, water and temperature x water interaction on either pulp sweetness ($P > 0.10$) or TSS.

Sensory traits in 2011-12

There was a highly significant effect of panellist on sensory trait score ($P < 0.001$) and a higher order interaction between panellist, water and temperature ($P = 0.01$). We found, however, no effect of replicate ($P = 0.13$) neither interaction between panellist and water regime ($P = 0.69$) or panellist x temperature interactions ($P = 0.13$).

Six out of 20 attributes were affected by treatments (Table 3). Five attributes were affected by temperature and two by water regime. No interactions between water and temperature were detectable for any of the 20 berry descriptors.

Temperature advanced development of some traits in pulp, seed and skin. Berries from this treatment were softer and pulp separated easily from the seeds and skin. Phenolic extractability in seeds was reduced, reflected in higher astringency and tannic intensity scores. Also, skin was less acidic in heated berries. Two seed traits responded to effects of water: astringency, which was higher in well watered plants, and flavours, with more toasted character found under water deficit. In both cases, water deficit advanced ripening.

Sensorially, there was an undetectable effect of temperature, water and water x temperature interaction on pulp sweetness. However, the analysis of the variance yielded significantly higher TSS in water deficit treatments as previously explained.

Offset and decoupling in sensory traits

Here we analysed two aspects in berry sensory traits that accounted for differences between treatments: the offset in berry traits, which indicates whether treatments advanced or delayed berry development; and the scatter around the fitted curve, accounting for variation among and within berry parts (Fig. 5).

In both seasons warmer temperature and water deficit conditions advanced berry development (intercept > 0). Temperature offset was ~ 10 fold higher, from 0.04 to 0.51, in the hotter season 2011-12 than in the mild 2010-11 (Fig. 5a and b). Similarly for water regime, the offset ranged from 0.03 in 2010-11 to 0.39 in the drier season 2011-12 (Fig. 5c and d). The decoupling among traits associated with temperature was higher in 2011-12 (RMSE = 0.59) than in 2010-11 (RMSE = 0.15) and the decoupling associated with irrigation was higher in 2011-12 (RMSE= 0.39) than in 2010-11 (RMSE=0.12).

In both years, thermal-driven decoupling (RMSE 0.15 – 0.59) was higher than water-driven decoupling (RMSE 0.12 – 0.39). In 2010-11 the decoupling caused by both temperature and water was mainly associated with the enhanced ripening of seeds in comparison to other berry parts (insets Fig. 5a and c). In 2011-12 both temperature and water decoupled whole-berry traits (mainly softness) and seed traits from other berry traits (inset Fig. 5b and d).

As shown in Fig. 6, berry sensory score was tightly coupled with the progression of mesocarp cell death. More advanced development in berry traits (higher score) was associated with lower percentage of living tissue, e.g. HD and CD.

Discussion

Climate models predict drier and warmer environments for important grape growing regions worldwide. Here we used a factorial experiment to partially account for water, temperature and water x temperature interactions on berry biophysical and sensory traits under realistic field conditions.

Effects of temperature and water on mesocarp cell death and shrivelling

The link between berry water loss and the onset of mesocarp cell death adds a functional explanation to our first assumption that warming and water deficit will offset berry mesocarp cell death during berry ontogeny, accelerating the associated physiological process. Integrity of semi-permeable membranes plays a crucial role driving flows in and out of the berry (Tilbrook and Tyerman 2008, 2009). Recent studies have demonstrated that warming accelerated the onset of mesocarp cell death and increased the rate of cell death after the onset of rapid cell death, driving and anticipated shrivelling in Shiraz berries (Bonada et al. 2013). Here we found the same offset on the onset of mesocarp cell death by temperature and an effect of water deficit increasing the rates of rapid cell death. Furthermore, growing conditions that combined warming and water stress resulted in berries with the highest shrivelling at harvest. These observations together with the lack of interaction between these two factors support our first hypothesis about additive effects of water and temperature in the offset of mesocarp cell death and shrivelling.

Berry susceptibility to shrinkage and recovery from drought changes during berry development in relation to changes in berry hydraulic connection with the plant (Keller et al. 2006, Bondada and Shutthanandan 2012). Shrinkage is less prone to occur in post-veraison berries as phloem flow, predominant at that stage, is less sensitive to plant and soil water status than xylem flow (Keller et al. 2006, Keller 2010). Membrane disintegration, however, may result in an accelerated back flow of water and rapid shrinkage. As membrane semi-permeability is lost, mesocarp cells are not longer effective in balancing xylem tension generated by leaf transpiration and water backflow from the berry to the plant are more likely (Tilbrook and Tyerman 2008). The less water conservative behaviour of Shiraz under soil water deficit and evaporative demand implies larger fluctuations in plant water potential (Prieto et al. 2010, Lovisolo et al. 2010) that are transmitted from the leaves to the berry (Keller et al. 2006, Tilbrook and Tyerman 2009). This explanation is consistent with the response that we found in berries ongoing high evaporative conditions and water stress, where the onset of net water loss coupled with the onset of cell death. However, even when rapid onset of cell death has started, berries from well watered plants maintained water content for a short period of time. As it has been noticed in grapevines and other fruits, it is likely that xylem flows will continue in response to transpiration, keeping a net water gain at that stage (Greer and Rogiers 2009, Clearwater et al. 2011). From the viticultural point of view, this plastic behaviour of shrivelling in Shiraz under different plant water potentials may represent a valuable physiological advantage toward the design of irrigation strategies aiming to reduce the thermal offset of shrivelling.

Sensory decoupling by temperature and water

Berry sensory assessment is being widely used by viticulturist and winemakers to assess ripening and allocate grapes to different wine styles (reviewed by Olarte Mantilla et al. 2012). Here we used it to assess the offset and decoupling on berry sensory traits by temperature and water (Sadras et al. 2013) and to correlate sensory traits with mesocarp cell death.

Standardised practices in sensory evaluations may minimise bias; but many aspects inherent to human responses cannot be completely controlled (Lawless and Heymann 2010). Here we found in both seasons differences between panellists to grade the same attributes. This panellist effect has been widely reported in sensory studies (Le Moigne et al. 2008a, Le Moigne et al. 2008b, Tardaguila et al. 2008, Baiano et al. 2011, Sadras et al. 2013), mainly due to the different use of the scale (Nurgel et al. 2004). The lack of significant replicate effect, however, confirms that panellists were consistent in their rating between seasons and samples (Tardaguila et al. 2008, Baiano et al. 2011). The high order interaction between panellist and treatments may suggest some grade of bias attributed either to the high grade of similarity between samples or the different criteria of scaling used by panellist (Zamora and Guirao 2002, Tardaguila et al. 2008). To elucidate this implicit training effect, we confronted panellists' assessments. Only three panellists were excluded in 2011-12 due to the degree of dissimilarity, the remaining panellists seemed to grade similarly the same trait-intensity. On 2010-11, however, while dissimilarities among panellists were lower, the high dispersion shown in the PCA (Supplementary Fig. S1) could suggest a degree of bias associated with the use of a four point scale (Olarde Mantilla et al. 2012) which was also reflected in the higher number of traits with water x temperature interactions. Based on this observation, we used a line scale 15 cm long in the second season.

Inter-seasonal variation in growing conditions strongly interact with management practices (Keller 2009). Here we found that hotter and drier conditions on season 2011-12 caused large offset and decoupling on berry sensory traits than in the milder 2010-11. Thermal effect, however, was higher than water effect. The large decoupling caused by elevated temperature was mainly associated to differences within berry parts. Berry softness was the trait more sensitive to temperature (open triangle in Fig. 5b); this is consistent with the higher percentage of mesocarp cell death in the heated treatment (Fig. 2, Table 1). In contrast, decoupling associated with water regime was mainly related to differences between berry parts (whole-berry, pulp, seed and skin) (insets Fig. 5c and d); water deficit advanced development of seed and whole-berry traits (mainly softness) in relation to skin and pulp traits. This asynchrony in seed development in relation of the rest of berry was previously reported and their oenological implication were highlighted (Sadras et al. 2013). More advanced seed

ripening could represent a higher relative proportion of skin derived tannins and general improvement in tannin quality (Ristic et al. 2010, Kassara and Kennedy 2011). In this sense, the potentially positive effect associated to an advance in seed ripening could be offset by the simultaneous decoupling in berry softness. More shrivelled berries may increase concentration of sugar and potential alcohol at harvest and alter the relation sugar vs. rest of the berry compounds, e.g. sugar:anthocyanins and sugar:acids ratios (Tilbrook and Tyerman 2008, Petrie and Sadras 2008, Sadras and Petrie 2011b, a, Sadras and Moran 2012b). Lower offset and decoupling were associated with mild and wet conditions during ripening of 2010-11. Consistently for both water and temperature effects, seed ripening was advanced in relation to the rest of berry parts, however only significantly for water effect (insets Fig. 5a and c).

For the first time we showed that more advanced sensory traits were associated with lower percentage of mesocarp cell vitality (Fig. 6). This is consistent with the practice of letting grapes hang on the plants beyond maximum physiological sugar content, waiting for the development of flavours and aromas (Tilbrook and Tyerman 2008, Keller 2009). The potential beneficial effect on the sensory profile of grapes associated with this practice, however, could not be hold under environmental conditions that offset berry development as previously explained. Warming and water deficit accelerated mesocarp cell death (Fig. 2, Table 1); however, this advance in ripening was coincidental with the offset and decoupling in berry sensory traits (Fig. 5). Consistently with our hypothesis the positive association between berry aroma and flavour development and cell death did not hold when environmental stresses drive cell death. Further investigation is needed to elucidate the implications in wine sensory profile.

Implication for irrigation management

Trade-offs between high yield and berry traits related to wine quality are not universal but are common and may impose constraints to the dual maximisation of volume and value per unit volume of production; this trade-off also underlies regulations in some European countries in which the irrigation of vineyards was forbidden or prescribed (Sadras and Schultz 2012). A critical question is how to manage irrigation to capture the benefit of high yield and a level of quality that maximises returns to the

grapegrower. Whereas diversity of environments, wine styles and production objectives (i.e. high volume vs. high quality) do not allow for generalisations, some degree of water deficit during berry ripening is considered beneficial for production of quality grapes in red wine varieties (Kennedy et al. 2002, Roby and Matthews 2004, Iland et al. 2011, Basile et al. 2011, Intrigliolo et al. 2012). However, under warmer conditions, the intensity and timing of water deficit might need to be revised to account for deleterious effects of elevated temperature in water-stressed plants.

Cell death is necessary but not sufficient for shrinkage to occur in Shiraz berries (Tilbrook and Tyerman 2009, Bonada et al. 2013). We found here that cell death and shrivelling could be uncoupled by irrigation regimes and that warming temperatures are expected to interfere with their inception and severity. In the light of these findings, palliative measures to counteract thermal stress may be necessary that reduce energy load in the canopy, maintain plant water status and reduce heat damage in critical periods (Sadras et al. 2012b, Sadras et al. 2013). In this sense, timing and extent of regulated deficit irrigation need to be carefully combined to account for increasing evaporative demand resulting from higher temperature (Schultz and Stoll 2010), to maintain water supply in vines facing short spells of high temperature (Sadras and Soar 2009, Webb et al. 2010) and to counteract thermal offset on mesocarp cell death and berry sensory traits. The trade-off between water deficit required to achieve quality grapes and the need to maintain vine water status to reduce thermal stress needs to be solved locally against the aims of the production system and wine style imperatives.

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Table 1: Rates of mesocarp cell death before (b) and after (b') the onset of rapid cell death in chronological (DAA) and thermal ($^{\circ}\text{Cd}$) scales for Shiraz, season 2011-12. Slopes b and b' represent rates of change on the percentage of living tissue per day after anthesis ($\% \text{ day}^{-1}$) and per in growing degree day ($\% \text{ }^{\circ}\text{Cd}^{-1}$). The thresholds and slopes were estimated from curve fitting of bilinear segments model (Bonada et al. 2013). Data are means $\pm$ s.e. and P values are from analysis of variance (ANOVA), accounting for: temperature, water and temperature x water interaction.

Scale	Variable	Treatment										P value				
		Control-Deficit			Control-Irrigated			Heated-Deficit			Heated-Irrigated			Temperature	Water	Interaction
DAA	b	-0.32	±	0.046	-0.24	±	0.043	-0.33	±	0.008	-0.30	±	0.033	0.27	0.16	0.51
	Onset	90	±	1.53	91	±	1.33	81	±	1.00	82	±	1.52	0.0002	0.60	0.96
	b'	-1.89	±	0.139	-1.21	±	0.066	-1.68	±	0.110	-1.33	±	0.040	0.64	0.0007	0.12
°Cd	b	-0.02	±	0.003	-0.02	±	0.004	-0.02	±	0.000	-0.02	±	0.002	0.43	0.33	0.80
	Onset	1032	±	11.04	1018	±	23.79	994	±	20.74	990	±	23.09	0.14	0.66	0.81
	b'	-0.14	±	0.007	-0.10	±	0.004	-0.13	±	0.01	-0.10	±	0.002	0.71	0.0002	0.11

Table 2: Effect of temperature, water regime, and their interaction on Shiraz berry sensory traits, season 2010-11. Scores range from 1, less developed trait, to 4, more developed trait. Filled cells indicate effects in black ($P < 0.05$) and grey ($0.05 < P < 0.10$).

Part	Trait	Deficit		Well-watered		P values		
		Control	Heated	Control	Heated	Water	Temperature	Interaction
Berry	Colour	4.00	3.97	4.00	3.97	0.980	0.157	0.984
	Softness	2.92	3.08	2.75	3.00	0.177	0.025	0.652
	Stalk removal	3.03	2.86	3.11	2.94	0.339	0.053	0.993
Pulp	Acidity	3.08	3.28	3.22	3.50	0.126	0.046	0.723
	Detachment	2.67	2.75	2.86	2.44	0.570	0.090	0.011
	Fruity aromas	2.83	3.06	2.92	2.92	0.815	0.349	0.349
	Herb. Aromas	3.50	3.39	3.50	3.36	0.903	0.272	0.903
	Juiciness	2.44	2.61	2.81	2.36	0.810	0.150	0.004
	Sweetness	2.78	3.00	2.86	2.78	0.602	0.602	0.252
Seed	Astringency	2.80	2.89	2.87	2.69	0.107	0.565	0.763
	Colour	2.51	2.69	2.39	2.39	0.051	0.412	0.412
	Crushability	2.86	3.25	2.83	2.86	0.081	0.075	0.122
	Flavours	2.74	3.03	2.64	2.83	0.187	0.035	0.688
	Tannic intensity	2.86	2.92	2.58	2.92	0.103	0.020	0.103
Skin	Acidity	3.22	3.22	3.31	3.33	0.404	0.905	0.905
	Astringency	3.06	3.14	3.08	3.31	0.384	0.173	0.534
	Disintegration	2.33	2.36	2.42	1.94	0.180	0.074	0.045
	Fruity aromas	2.31	2.42	2.44	2.53	0.391	0.504	0.924
	Herb. aromas	2.92	2.83	2.97	2.94	0.441	0.607	0.797
	Tannic intensity	2.19	2.42	2.25	2.33	0.893	0.139	0.500

Table 3: Effect of temperature, water regime, and their interaction on Shiraz berry sensory traits, season 2011-12. Scores range from 0, less developed trait, to 15, more developed trait. Filled cells indicate effects in black ($P < 0.05$) and grey ($0.05 < P < 0.10$).

Part	Trait	Deficit		Well-watered		P values		
		Control	Heated	Control	Heated	Water	Temperature	Interaction
Berry	Colour	13.74	14.38	13.84	14.15	0.868	0.204	0.664
	Softness	10.79	13.19	10.22	12.39	0.192	0.000	0.824
	Stalk removal	11.33	11.83	10.88	10.89	0.136	0.586	0.595
Pulp	Acidity	10.57	10.91	10.57	10.49	0.726	0.829	0.728
	Detachment	9.70	10.50	9.04	10.64	0.691	0.069	0.542
	Fruity aromas	10.84	10.84	10.33	10.89	0.727	0.665	0.667
	Herb. Aromas	11.36	11.44	10.78	11.31	0.561	0.621	0.714
	Juiciness	9.82	10.24	9.07	10.45	0.671	0.163	0.451
	Sweetness	11.43	11.88	10.48	11.40	0.166	0.190	0.653
Seed	Astringency	7.87	8.75	5.86	8.18	0.066	0.023	0.139
	Colour	10.02	10.40	9.19	9.75	0.208	0.423	0.875
	Crushability	11.52	11.87	11.06	11.06	0.251	0.748	0.753
	Flavours	10.75	10.99	8.75	9.94	0.017	0.255	0.446
	Tannic intensity	7.38	8.03	6.05	7.64	0.156	0.064	0.435
Skin	Acidity	10.65	11.47	9.03	11.45	0.130	0.003	0.137
	Astringency	8.48	9.34	9.65	9.46	0.331	0.614	0.424
	Disintegration	8.30	9.29	8.87	8.93	0.841	0.350	0.410
	Fruity aromas	8.05	9.28	8.14	8.39	0.592	0.323	0.509
	Herb. aromas	9.22	9.68	8.63	9.36	0.533	0.414	0.851
	Tannic intensity	6.70	6.19	6.37	6.21	0.785	0.554	0.753

Caption of figures

Fig. 1 Seasonal mid-day stem water potential of Shiraz vines in a 2 x 2 temperature and water factorial experiment in Barossa Valley. Data are mean $\pm$ s.e. of six measurements. Diamonds indicate irrigation (full) and rainfall (empty) events, vertical bars indicate supplementary irrigation in water deficit treatments, and squares indicate key phenological stages (Mean $\pm$ SD): anthesis (A), pea size (PS), veraison (V) and harvest (H) along the season (DAA).

Fig. 2 Dynamics of mesocarp living tissue (%LT) in (a, b and c) chronological and (d, e and f) thermal time scales, during season 2011-12. Figures a and d show progression of %LT for the combined effects of temperature and water treatments. Each point corresponds to the mean $\pm$ s.e. Lines correspond to the bilinear fitted model for each data set (Bonada et al. 2013). Insets in a and d compare the regression residuals for temperature x water treatments (mean $\pm$ s.e.). Figures on the right hand size represent fitted models accounting separately for effects of (c and f) temperature: control (black) and heated (grey), and (b and e) water: irrigated (dash-dot) and deficit (dashed).

Fig. 3 Progression of shrivelling in (a) chronological and (b) thermal time scales during the stage of rapid cell death. Each point corresponds to the mean $\pm$ s.e., for the combined effects of temperature: control and heated, and water: irrigated and deficit treatments. Dashed lines represent the best fit regression for the complete set of data (2nd order polynomial). Insets compare the regression residuals for each data set with the fitted curve (mean $\pm$ s.e.). Relationship between Shrivel index with: (c) percentage of living tissue, and (d) berry water content, from the onset of rapid mesocarp cell death. Solid lines are fitted regressions.

Fig. 4 Relationship between the onset of mesocarp cell death and the onset of berry net water loss, both expressed in days after anthesis. Each point corresponds to the mean $\pm$ s.e. Dotted black lines represent $y = x$. (b) Elevated temperature advances berry net water loss in berries from water deficit plants but not in berries from well irrigated vines. (c) Berry water content (%) at the onset of rapid mesocarp cell death. Vertical dotted lines are mean water contents for control and heated berries.

Fig. 5 Comparison of scores for sensory traits in (a and b) control and heated treatments, and (c and d) irrigated and water deficit treatments over two seasons. Note that the scale used was from 0 to 4 in 2010-11 and from 0 to 15 in 2011-12. The solid lines are least squares regressions with slope = 1 and intercept = a. The dotted lines are $y = x$. RMSE is the root mean square error (eq. 2). Insets are the score residuals (mean $\pm$ s.e.) for whole-berry (white), pulp (black), seed (red) and skin (blue).

Fig. 6 Relation between berry sensory score and percentage of living tissue at harvest for the combined effects of temperature and water. Points represent the mean score of each replicate for 20 berry attributes and 9 panellists

Supplementary figures

Fig. S1 PCA and cluster analyses of the correlation factors among tester and trait scores. Correlation matrices on both seasons were based on scores of 12 panellist evaluating 20 berry sensory traits. The bias associated to these methods to assess panellist performances is reduced when use in conjunction (King et al. 2001). The lack of agreement between them in 2010-11 did not support any decision to exclude panellist. In 2011-12 both discriminated panellists F, G and I from the rest, with a notorious dissimilarity of these panellists to score the same attributes

Fig. S2 (left) Comparison of mean daily temperature in heated and control treatments in two growing seasons (2010–11 and 2011–12) and the intervening winter period. (right) Frequency distribution of temperature difference between heated and control treatments; SD is the standard deviation. Temperature was measured at canopy level. Adapted from Figure 1 in Sadras and Moran (2012a).

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