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Effects of timing and intensity of elevated temperatures on reproductive development of field-grown Shiraz grapevines

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Abstract

Aim: To investigate whether timing and duration of exposure to elevated temperatures impact the reproductive development of field-grown Shiraz grapevines.

Methods and results: The reproductive responses of Shiraz grapevines (*Vitis vinifera* L.) to two levels of elevated temperatures at budburst and flowering were investigated in an irrigated vineyard in the Barossa Valley (South Australia) over two consecutive growing seasons. Custom-built under-vine 'tents' and closed flow-through chambers enclosing a set of grapevines in the field were used to raise canopy temperatures above ambient. Higher temperatures at flowering resulted in lower yields due to decreased fruit set in 2007-08, while yield was virtually unaltered the following year despite the lower fruit set. Two indicators of grapevine reproductive performance, Coulure Index and Millerandage Index that quantify abscised and underdeveloped berries, respectively, were calculated to be higher as a result of the heat treatments in both seasons. Stigma receptivity, pollen germination, and pollen tube kinetics were generally lower in vines grown under the tents.

Conclusion: Flowering and fruit set are strongly influenced by temperature changes during this period of development.

Significance and impact of the study: This is one of the first field based studies to demonstrate that extreme temperatures (>35°C) during the flowering period detrimentally effect fruit set and final yield, thus providing critical knowledge for managing vineyards in a changing climate.

Keywords: flowering, fruit set, climate change, heat stress, millerandage, coulure

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OENO One, 2017, **51**, 4, 409-421

Introduction

Temperature is a primary environmental factor that plays a key role in affecting several plant physiological processes including phenology, vegetative growth, flowering and fruit set, crop development, yield and quality. The relatively recent trend towards higher average growing season temperatures in premium viticultural regions worldwide has heightened the importance of understanding the viticultural consequences of these temperature shifts (Schultz, 2000; Soar *et al.*, 2008; Webb *et al.*, 2007). When extreme temperatures (daily maximum temperatures greater than 35°C) were included in one study predicting the effects of future climate change on winegrape production in the United States, areas currently marginally suitable to grapevine production were almost eliminated while the acreage of premium grape growing regions decreased up to 81% (White *et al.*, 2006). While an increase in the average growing season temperature is likely to have negative effects on crop yield and quality, short episodes of extreme temperatures are expected to be even more detrimental particularly at key phenological stages of plant development such as flowering and fruit set (Ferris *et al.*, 1998; Hedhly *et al.*, 2005; Prasad *et al.*, 1999).

Studies on grapevine reproductive development in controlled environments have shown that high temperatures are detrimental to reproductive performance and consequently yield. Comparing pre- and post-budburst exposures to elevated temperatures (>30°C) for approx. two weeks per period, Petrie and Clingeleffer (2005) found that flower number per inflorescence was 18% lower than at ambient conditions as a result of the pre-budburst heat treatment than in the post-budburst treatment, where a 15% reduction was observed. Similar results were obtained by Keller *et al.* (2010), who found nearly a one-third reduction in flowers per inflorescence when buds were exposed to warmer conditions (up to 40°C) from pre-bud swell to when the flowers were first visible. Higher temperatures applied after budburst (until fruit set) advanced the phenology of berry set and had a negative effect on flower number per inflorescence (Buttrose and Hale, 1973). High daytime temperatures of 35-40°C around flowering were also detrimental to fruit set and ovule fertility, and resulted in fewer berries per cluster (Ebadi *et al.*, 1995a; Ewart and Kliewer, 1977; Kliewer, 1977). In Pinot Noir, there was a 23% decrease in fruit set and 21% decrease in ovule fertility when the maximum day-time temperature was 35°C compared to a maximum day-time temperature of 25°C (night temperatures were held constant at 20°C; (Kliewer, 1977)). Keller *et al.* (2010) reported a 6% increase in fruit set when the ambient temperature at budburst of 10°C was increased to 15°C, whereas a similar temperature increase from 14°C to 19°C resulted in a 12% increase in fruit set.

Pollen germination is a temperature-sensitive process in most fruiting plants. In grapevines, Staudt (1982) found that pollen germination *in vitro* was reduced at a temperature of 15°C, while at a higher temperature of 28°C, pollen germination and subsequent tube growth was at its optimum. This temperature optimum for pollen germination was also found in Cabernet Sauvignon (Rajasekaran and Mullins, 1985). Pollen tube growth commences approx. 30 min after pollination (Staudt, 1982) and is also a temperature-dependent process. Studies on a number of horticultural crops have shown that pollen tube growth rate is positively-correlated with temperature (Hedhly *et al.*, 2005; Jefferies *et al.*, 1982; Sorkheh *et al.*, 2011). Snider *et al.* (2011) suggested that high temperatures (~ 35°C) negatively affect the soluble carbohydrate contents of the pistil leading to decreased pollen tube growth through the style and lower fruit set.

Although several studies have investigated the response of grapevine flowering and fruit set processes to elevated temperatures in controlled environments such as glasshouses, only a few have studied these responses in a field-setting. Using custom-built under-vine 'tents' and whole-vine chambers to elevate the ambient temperature of field-grown Shiraz grapevines, we investigated grapevine reproductive responses within a season to elevated temperatures at key phenological stages of vine development. The goal of the present study was to determine whether timing and duration of exposure to elevated temperatures impact the reproductive development of field-grown Shiraz grapevines.

Materials and methods

1. Plant material and study site

The trial was initiated in 2007-08 in a well-established experimental vineyard of the South Australian Research and Development Institute (SARDI) in Nuriootpa (Barossa Valley), South Australia (34° 29' S, 139° 0' E). Details of the site and climatic conditions of the region are given in Sadras and Soar (2009). Briefly, the own-rooted grapevines (*Vitis vinifera* L. cv. Shiraz) were planted in 2004 in rows oriented east-west, trained to a single bilateral cordon, and spur-pruned to approx. 40-50 buds per vine. Spacing was 3.0 m × 2.25 m (row × vine). The soil type in this vineyard is a Light Pass

fine sandy loam, classified as a red brown earth (Seeliger and French, 1971). The vines were drip-irrigated at approx. 21 L vine^{-1} weekly starting in mid-December.

2. Experimental design and heat treatments

Under-vine 'tents' (UVT; open heating system) and chambers (closed heating system) were built in-house at the SARDI research station, Nuriootpa for the purpose of elevating the ambient temperature of field-grown Shiraz grapevines (Figure 1). Details of the two heating systems, experimental design, treatments, and weather data are provided in Sadras and Soar (2009) and Soar *et al.* (2009). Briefly, the two heating systems were made using polycarbonate sheets attached to steel frames. The polycarbonate material blocked ultraviolet (UV) radiation (light wavelengths below 400 nm) but uniformly transmitted approx. 90% of light between 400-1600 nm (Sadras and Soar, 2009). The experimental design was a randomized complete block consisting of three blocks each for the chambers and tents. For the chambers, each block consisted of one panel of three vines that was heated approx. 6-8°C above ambient temperature and two panels of three vines each that were at ambient temperature. For the UVTs, each of the three blocks consisted of nine contiguous vines per treatment replicate of which only the middle seven vines were used for a total of 21 experimental vines per treatment; the two end vines were used as buffers. Average temperature increases relative to ambient were between 2-4°C within the tents. In the first season of the trial, 2007-08, only the tents were available for use and were applied around flowering for a two-week period. In the 2008-09 season, the tents were applied for a three-week period while the chambers were applied for three days at each phenological period. In both seasons, the same set of vines were used for the study. Elevated temperature treatments were imposed at two different developmental stages, budburst (E-L Stage 4) and flowering (E-L Stage 19; Eichhorn and Lorenz, 1977). Day-night temperatures tracked each other closely in both the tents and chambers (Sadras and Soar, 2009; Soar *et al.*, 2009). Air flow in the whole-canopy chambers was set such that no condensation occurred inside the chambers to artificially increase relative humidity around the vines.

3. Reproductive measurements

In order to determine the percentage of flowers set into fruit, basal inflorescences of each shoot were bagged in a 20 x 30 cm white nylon mesh bag and labelled prior to flowering. After flowering was completed, the bags were collected and the flower caps counted as an estimate of flower number per inflorescence. The inflorescences were marked and left to develop into clusters. During the growing season, all marked clusters were collected and the following information collected for each cluster: cluster weight, number of seeded berries, number of seedless (berries that fail to fully develop), live green ovaries (LGOs), and rachis weight. Percent fruit set was calculated by counting the total number of berries set on each cluster, including seedless, and dividing that by the total number of flowers in each inflorescence. From the berry count data, two indices of reproductive performance were calculated as per Collins and Dry (2009): (i) Coulure Index (CI), an indicator of berry abscission; and (ii) Millerandage Index (MI), an indicator of improperly formed berries, either seedless berries or LGOs. Higher index values indicate greater severity of the condition. Average berry weight was calculated using

(a) (b)

Figure 1. (a) Under-vine tents and (b) whole-canopy chambers used to elevate the temperature of field-grown Shiraz grapevines.

OENO One, 2017, **51**, 4, 409-421

the difference between cluster weight and rachis weight and the total berry number. Fruit set data was collected from three clusters per vine.

4. Pollen tube observations

Pollen tubes in the pistils of flowers during flowering were observed using fluorescence microscopy. Briefly, 10 ovaries from the inflorescences of each treatment vine were collected at anthesis and fixed in Carnoy's fluid (absolute ethanol:chloroform:acetic acid, 60:30:10). The fixed ovaries were hydrated in a series of increasingly polar solvents (70% EtOH, 30% EtOH, distilled water), softened in 0.8 N NaOH at 60°C for approx. 20 minutes, and rinsed with water. The ovaries were then stained with 0.1% aniline blue for at least 10 minutes prior to observation using a Carl Zeiss Axiophot photomicroscope (Carl Zeiss, Inc., Göttingen, Germany) with fluorescence filters. The fluorescence microscope was connected to a Nikon DXM1200F digital camera (Nikon Corporation, Japan) attached to a computer with Nikon imaging software. Stigma fluorescence, pollen tube number and length, and ovule fertilization were observed for 10 flowers per vine. Stigma fluorescence intensity and pollen tube growth were scored from 1 (low intensity; pollen tube length < one-third length of style) to 3 (high intensity; pollen tube length ~ style length).

5. Pollen germination in vitro

Prior to anthesis, anthers were collected randomly from the flowers of vines of the individual treatments and stored in 20 mL glass vials. The anthers were left to dry for 24-48 hours at room temperature. To isolate the pollen, glass vials containing the anthers were gently tapped for 15 sec to ensure the shedding of pollen from the anther sacs and the separation of pollen from other flower parts; it also mixed the pollen grains to give a homogenous sample. All of the debris was discarded. The pollen grains left at the bottom of the vial were then used for the pollen germination and tube growth assays. Pollen was transferred using a fine brush onto cavity slides containing 1 mL of pollen germination medium. The pollen germination media consisted of sucrose (0.44 M), boric acid (1.62 mM), calcium nitrate (1.27 mM), magnesium sulphate (0.81 mM), and potassium nitrate (0.99 mM) in 1% agar (w/v), modified from Brewbaker and Kwack (1963). The glass slides were placed in a container lined with warm, moistened tissue paper to create a humid environment to promote germination, and left at room temperature under fluorescent lights. After 48 hours, the percentage of germinated pollen was counted under a microscope with 10x magnification. Pollen was considered to have germinated, if the length of the pollen tube was greater than the diameter of the pollen grain.

6. Yield components

Vine yield components data was collected following harvest in both seasons for the under-vine tents. Data collected included yield per vine, cluster weights of three randomly selected clusters per vine, berry number from the same three clusters per vine, and berry weight. Average cluster number per vine was calculated from the vine yield and average cluster weight.

7. Berry and seed development

For each of 10 randomly selected berries per cluster, berry weight, seed number, and total seed weight was determined. Average seed weight of each berry was calculated by dividing total seed weight by seed number. Pericarp weight of each berry was calculated by subtracting total seed weight from berry weight. In order to determine seed viability, the number of seeds that were 'floaters' (seeds with empty cavities) and 'sinkers' (seeds with intact endosperm) in every berry was determined by a floatation technique (Bordelon and Moore, 1994).

8. Statistical analysis

One-way analysis of variance (ANOVA) was performed on the measured or calculated treatment means using SPSS statistical software (v.22, IBM Corp.). Prior to ANOVA, the data was tested for normality and homogeneity of variance; based on these tests, no transformations were required. A generalized linear model procedure was used in which the fixed effect was 'treatment'. The error term was treatment × block × vine for all the data analyzed. Differences between treatment means and control means were determined at the 5% significance level using Tukey's HSD test.

Results

1. Reproductive performance

In 2007-08, under-vine tents were installed at flowering on the field-grown Shiraz grapevines to study the effects of elevated temperature on Shiraz grapevines. Higher temperatures did not affect flower numbers but altered berries per cluster and fruit set (Table 1). Berry numbers were approx. 27% lower in the heat-treated vines compared to control vines, while fruit set decreased significantly from 48% to 38%. This trend was consistent with fruit set and

berry counts in the following year, 2008-09, where similar decreases were observed (Table 2). Coulure Index (CI) values were consistently higher in the UVT vines compared to control vines, indicating that more flowers abscised under elevated temperatures. Given the similar Millerandage Index (MI) values of the two treatment groups, there did not appear to be a different proportion of berries on each cluster that were either seedless or LGOs. While cluster number per vine did not differ, cluster weight was lower in the UVT vines due to significantly fewer berries per cluster and lower fruit set. Berries that developed normally had an average weight of 0.7 g in both treatments, indicating that yield compensation did not occur at the level of the cluster I.e. lower fruit set and fewer berries per cluster did not result in larger berries. The lower (~ 23%) vine yields in the UVT vines could be attributed to the lower cluster weights (cluster numbers per vine were similar between treatments) and fewer berries per cluster.

In 2008-09, the study utilized under-vine tents and whole-vine chambers to impose two levels of elevated temperatures at two phenological stages: budburst and flowering. Results of the reproductive effects of these treatments are given in Tables 2 and 3. In the under-vine tents, flower numbers per inflorescence were unaltered in vines that experienced higher temperatures either at budburst or flowering compared to control vines at ambient temperature (Table 2). Higher temperatures at flowering negatively impacted the number of seeded berries as well as fruit set. CI was not different in the budburst treatment, however, the significantly higher CI value at flowering in the UVT treatment indicated a higher level of abscission I.e. flowers that did not get fertilized. Elevated temperatures at budburst resulted in higher MI values in the heat-treated flowers compared to the 'control' flowers, indicating that there were a higher number of seedless berries and LGOs compared to seeded berries. No differences in MI values were observed when temperatures were elevated at flowering. Higher temperatures at flowering resulted in consistently fewer berries per cluster. There were no differences in cluster weight or berry weight as a result of the elevated temperatures. No differences were observed in either clusters per vine or yield.

When temperatures were increased at flowering using the whole-vine chambers in 2008-09, the number of seeded berries and fruit set were both significantly lower compared to control, but not when the same treatment was applied at budburst (Table 3). Berry number per cluster decreased by 14% in the budburst treatment and by 19% in the flowering treatment. Fruit set was 10% lower with the heat treatment at flowering, resulting in fewer berries per cluster and, consequently, a nearly 20% reduction in cluster weight. Berry weights, CI, and MI values were similar between the different treatments.

Pollen viability and growth

Temperature of grapevines around budburst using under-vine tents did not alter pollen viability and pollen tube growth (Table 4). Pollen receptivity on the stigma, as indicated by the level of stigma fluorescence, decreased significantly as a result of higher temperatures at both budburst and flowering in the UVTs in 2008-09. Higher temperatures at budburst and flowering resulted in an approx. 35% reduction in pollen tube number. *In vitro* pollen

Table 1. Effects of under-vine tents (UVT) applied at flowering on the reproductive performance of Shiraz grapevines, 2007-08. Means ± SE.

	Treatment		*P* value	Significance
	Control	UVT		
Flower cap number (inflorescence^{-1})	253.4 ± 14.5	255.8 ± 15.4	0.910	ns
Seeded berry number (cluster^{-1})	118.9 ± 4.8	86.4 ± 5.1	0.000	***
Fruit set (%)	48.2 ± 2.2	38.1 ± 2.4	0.003	**
Coulure Index	4.2 ± 0.3	5.5 ± 0.3	0.002	**
Millerandage Index	1.7 ± 0.2	1.7 ± 0.2	0.998	ns
Cluster weight (g cluster^{-1})	86.6 ± 4.1	64.1 ± 4.4	0.001	***
Berry weight (g berry^{-1})	0.70 ± 0.03	0.68 ± 0.03	0.553	ns
Cluster number (vine^{-1})	60.1 ± 2.4	62.5 ± 2.6	0.498	ns
Yield (kg vine^{-1})	5.07 ± 0.23	3.89 ± 0.25	0.001	***

*****, *** and 'ns' indicate significantly different at $P \leq 0.01$, $P \leq 0.001$, and non-significant, respectively (Tukey's HSD test).

OENO One, 2017, **51**, 4, 409-421

Table 2. Effects of under-vine tents applied at budburst and flowering on the reproductive performance of Shiraz grapevines, 2008-09. Means ± SE.

	Treatment			*P* value	Overall significance
	Control	Budburst	Flowering		
Flower cap number (inflorescence^{-1})	211.5 ± 12.9	213.7 ± 12.8	207.2 ± 12.8	0.932	ns
Seeded berry number (cluster^{-1})	116.6 ± 5.0 a	112.8 ± 4.9 a	97.7 ± 5.0 b	0.024	*
Fruit set (%)	60.8 ± 2.2 a	59.5 ± 2.2 a	50.7 ± 2.2 b	0.005	**
Coulure Index	3.0 ± 0.3 b	2.7 ± 0.2 b	3.9 ± 0.3 a	0.040	*
Millerandage Index	1.5 ± 0.16 b	2.1 ± 0.16 a	2.0 ± 0.16 ab	0.025	*
Cluster weight (g cluster^{-1})	102.8 ± 5.0	100.3 ± 4.9	91.5 ± 5.0	0.244	ns
Berry weight (g berry^{-1})	0.80 ± 0.03	0.84 ± 0.03	0.85 ± 0.03	0.361	ns
Cluster number (vine^{-1})	85 ± 4.0	85 ± 6.4	85 ± 3.9	0.890	ns
Yield (kg vine^{-1})	5.8 ± 0.56	6.2 ± 0.77	6.3 ± 1.12	0.770	ns

Means followed by different letters indicate significantly different at $P \leq 0.05$ (Tukey's HSD test).
*, ** and 'ns' indicate significant at $P \leq 0.05$, $P \leq 0.01$, and non-significant, respectively.

Table 3. Effects of whole-vine chambers applied at budburst and flowering on the reproductive performance of Shiraz grapevines, 2008-09. Means ± SE.

	Treatment			*P* value	Overall significance
	Control	Budburst	Flowering		
Flower cap number (inflorescence^{-1})	222.7 ± 13.5	213.7 ± 14.3	187.9 ± 13.5	0.181	ns
Seeded berry number (cluster^{-1})	132.3 ± 7.7 a	117.5 ± 8.2 ab	96.0 ± 7.7 b	0.004	**
Fruit set (%)	63.2 ± 2.6 a	59.5 ± 2.8 ab	53.9 ± 2.6 b	0.037	*
Coulure Index	2.8 ± 0.3	3.0 ± 0.3	3.6 ± 0.3	0.158	ns
Millerandage Index	1.6 ± 0.1	1.7 ± 0.1	1.9 ± 0.1	0.179	ns
Cluster weight (g cluster^{-1})	127.8 ± 7.9 a	112.2 ± 8.4 ab	89.4 ± 7.9 b	0.003	**
Berry weight (g berry^{-1})	0.88 ± 0.02	0.88 ± 0.02	0.85 ± 0.02	0.447	ns

Means followed by different letters indicate significantly different at $P \leq 0.05$ (Tukey's HSD test).
*, ** and 'ns' indicate significant at $P \leq 0.05$, $P \leq 0.01$, and non-significant, respectively.

germination and pollen tube length in the style were not affected by the heat treatment using the tents at either of the two phenological stages. When ambient temperatures were raised using the whole-canopy chambers, stigma fluorescence, pollen tube number, and pollen tube length of the heat-treated vines at both times were virtually unchanged compared to control vines (Table 5). *In vitro* germination of pollen showed that germination increased markedly as a result of the heat treatment at flowering but not at budburst.

Berry and seed development

The development of grape berries and their seeds in response to elevated temperatures was evaluated in the 2008-09 season. No differences were observed in average berry weight, seed weight per berry, or seed number per berry in response to the higher temperatures in either the under-vine tents (Table 6) or whole-vine chambers (Table 7) compared to control. The average weight of the berry pericarp was calculated to be significantly higher when heat treatments were applied at flowering in the UVTs. The average proportion of 'sinkers' in each berry was found to be lower as a result of the under-vine tent heat treatment at flowering compared to that at budburst but not compared to control. Differences in the proportion of 'sinkers' in each berry were not apparent in berries obtained from the whole-vine chambers. 'Floaters' were significantly higher in the

Table 4. Stigma receptivity and pollen performance in response to elevated temperatures in the under-vine tents, 2008-09. Numbers reported are per pistil. Means ± SE.

	Treatment			*P* value	Overall significance
	Control	Budburst	Flowering		
Stigma fluorescence (1=low; 3=high)	2.05 ± 0.10 a	1.51 ± 0.09 b	1.61 ± 0.09 b	0.004	**
Pollen tube count (pistil^{-1})	12.6 ± 1.0 a	7.9 ± 1.0 b	7.9 ± 1.0 b	0.003	**
Pollen tube length (mm)	1.51 ± 0.11	1.19 ± 0.11	1.20 ± 0.11	0.108	ns
Pollen germination (%)	28.8 ± 6.4	11.3 ± 6.4	16.0 ± 5.8	0.167	ns

Means followed by different letters indicate significantly different at $P \leq 0.05$ (Tukey's HSD test).
** and 'ns' indicate significant at $P \leq 0.01$ and non-significant, respectively.

Table 5. Stigma receptivity and pollen performance in response to elevated temperatures in the whole-vine chambers, 2008-09. Numbers reported are per pistil. Means ± SE.

	Treatment			*P* value	Overall significance
	Control	Budburst	Flowering		
Stigma fluorescence (1=low; 3=high)	1.52 ± 0.16	1.76 ± 0.14	1.65 ± 0.13	0.476	ns
Pollen tube count (pistil^{-1})	6.16 ±1.40	9.59 ± 1.30	6.65 ± 1.20	0.156	ns
Pollen tube length (mm)	1.52 ± 0.24	1.62 ± 0.22	1.30 ± 0.20	0.563	ns
Pollen germination (%)	4.3 ± 1.5 b	6.0 ± 2.1 ab	12.5 ± 1.5 a	0.014	*

*Means followed by different letters indicate significantly different at $P \leq 0.05$ (Tukey's HSD test).
* and 'ns' indicate significant at $P \leq 0.05$ and non-significant, respectively.

Table 6. Berry and seed development in response to elevated temperatures in the under-vine tents, 2008-09. Means ± SE.

	Treatment			***P* value**	**Overall significance**
	Control	**Budburst**	**Flowering**		
Berry weight (g berry^{-1})	0.75 ± 0.01	0.76 ± 0.02	0.91 ± 0.02	0.919	ns
Total seed weight (mg berry^{-1})	51.4 ± 1.4	55.1 ± 1.9	52.0 ± 2.2	0.346	ns
Seed number (berry^{-1})	1.83 ± 0.05	2.02 ± 0.07	1.83 ± 0.08	0.084	ns
Average seed weight (mg seed^{-1})	28.3 ± 0.4	27.5 ± 0.5	28.8 ± 0.6	0.207	ns
Pericarp weight (g berry^{-1})	0.70 ± 0.01 b	0.71 ± 0.02 b	0.86 ± 0.02 a	0.000	***
'Sinkers' (seeds berry^{-1})	1.77 ± 0.05 ab	1.92 ± 0.07 a	1.67 ± 0.08 b	0.046	*
'Floaters' (seeds berry^{-1})	0.07 ± 0.02 b	0.10 ± 0.03 ab	0.16 ± 0.03 a	0.033	*
'Sinkers' (% berry^{-1})	96.4 ± 1.1 a	94.7 ± 1.6 ab	90.9 ± 1.8 b	0.023	*

*Means followed by different letters indicate significantly different at $P \leq 0.05$ (Tukey's HSD test).
* and 'ns' indicate significant at $P \leq 0.05$ and non-significant, respectively.

flowering treatment in the UVTs but not in the chambers.

Discussion

1. Reproductive performance

Using under-vine (open-top) tents and closed-top heated chambers, we were able to investigate the effects of two levels of elevated temperatures on the reproductive performance of Shiraz grapevines. Projections of regional warming in a premium wine region of South Australia indicate growing season temperature increases of 1 to 4°C from 2030 to 2070 (Suppiah et al., 2010; Webb et al., 2013), underscoring the need for physiological studies on grapevine reproductive biology as influenced by temperature, which is still not well-understood. Field-grown Shiraz grapevines showed an overall

OENO One, 2017, **51**, 4, 409-421

Table 7. Berry and seed development in response to elevated temperatures in the whole-vine chambers, 2008-09. Means ± SE.

	Treatment			*P* value	Overall significance
	Control	Budburst	Flowering		
Berry weight (g berry^{-1})	0.74 ± 0.02 ab	0.79 ± 0.02 a	0.72 ± 0.02 b	0.018	*
Total seed weight (mg berry^{-1})	57.6 ± 21.9	61.5 ± 27.1	63.7 ± 25.4	0.231	ns
Seed number (berry^{-1})	1.99 ± 0.09	2.07 ± 0.07	2.15 ± 0.08	0.314	ns
Average seed weight (mg seed^{-1})	29.5 ± 0.8	30.0 ± 0.6	30.2 ± 0.7	0.820	ns
Pericarp weight (g berry^{-1})	0.68 ± 0.02 ab	0.72 ± 0.02 a	0.65 ± 0.02 b	0.025	*
'Sinkers' (seeds berry^{-1})	1.87 ± 0.09	1.98 ± 0.07	2.11 ± 0.08	0.092	ns
'Floaters' (seeds berry^{-1})	0.11 ± 0.03	0.08 ± 0.02	0.04 ± 0.03	0.141	ns
'Sinkers' (% berry^{-1})	95.4 ± 1.4	96.2 ± 1.1	98.2 ± 1.2	0.246	ns

Means followed by different letters indicate significantly different at $P \leq 0.05$ (Tukey's HSD test).
* and 'ns' indicate significant at $P \leq 0.05$ and non-significant, respectively.

higher sensitivity to elevated temperatures at flowering compared to at budburst in both seasons of this study. Temperature is a key environmental factor that influences inflorescence development, which commences at budburst (May, 2004). Our results indicate that flower number was unaltered when the vines were exposed to higher temperatures at budburst, which was in contrast to other studies that found fewer or smaller inflorescences when maximum daily budburst temperatures were between 15 to 28°C compared to 10 to 12°C (Ezzili, 1993; Keller et al., 2010; Pouget, 1981). Pouget (1981) reported that flowers per inflorescence increased from 434 to 997 when the mean air temperature at budburst was lowered from 25°C to 12°C, therefore a linear relationship between flower number and mean air temperature would equate to approx. 43.3 flowers °C^{-1}, as suggested by Dunn and Martin (2000). This sensitivity is much higher than our observations and likely attributable to the large temperature range over which the Pouget study was conducted. Pouget (1981) conjectured that lower temperatures at budburst favored inflorescence development over shoot growth. This hypothesis has been supported by recent field studies that found lower temperatures around budburst correlated with higher numbers of inflorescences (Dunn and Martin, 2000; Petrie and Clingeleffer, 2005). Differences in climate, cultivar, stored carbohydrate reserves, water and nutrient availability are amongst the factors that may have contributed to the contrasting results of the present study compared to those previously published.

The fruit set numbers in the present study were found to be higher than those reported in the literature for Shiraz (Ebadi *et al.*, 1995a) as well as other red grape varieties such as Cabernet Sauvignon, Zinfandel (Ewart and Kliewer, 1977), and Pinot Noir but similar to Carignan (Kliewer, 1977). However, fruit set varies significantly between different cultivars and regions.

Higher temperatures resulted in a higher rate of flower abscission, as indicated by the higher CI values. Coulure may be caused by a number of factors including climate (Carbonneau and Ollat, 1993){Carbonneau, 1993 #0;Carbonneau, 1993 #6}, vine physiology and cultural practices (Vergnes, 1982){Vergnes, 1982 #0;Vergnes, 1982 #46}, water status (Smart and Coombe, 1983){Smart, 1983 #41;Smart, 1983 #41}, vine nutrition (Delas *et al.*, 1991; Glad *et al.*, 1994; Zapata *et al.*, 1999), hormonal balance (Bernard, 1986; Bessis and Fournioux, 1992), and nitrogen status around flowering (Glad *et al.*, 1994). Millerandage or 'hen and chickens', the occurrence of berries of varied size on a cluster (May, 2004), has been attributed to several factors including nutrient deficiencies, environmental conditions, and imperfect inflorescence development (May, 2004).

The lower (~ 23%) vine yields in the UVT vines in the first season (2007-08) could be attributed to the lower cluster weights (cluster numbers per vine were similar between treatments) and fewer berries per cluster. In the second season (2008-09), however, the vines appeared to compensate yield I.e. lower fruit set resulted in larger berries, in the flowering treatment. This result was consistent with that of Bowen and co-workers in British Columbia (Canada). Using transparent polyethylene enclosures around the canopy to elevate the temperature of 'Merlot' grapevines for a seven-week period at three weeks pre-budburst at three sites, Bowen et al. (2004) found no consistent differences in yield and yield components between heated (daily maximum

temperature ~ 22°C) and control (daily maximum temperature ~ 17°C) vines. Spayd et al. (2002) reported that heating shaded clusters resulted in slightly but non-significantly smaller berries (by weight). Given the similar cluster numbers between treatments in both seasons that had similar environmental conditions (Figure 2), we hypothesize that yield compensation occurred in the second season due to enhanced cluster primordia development resulting in a lower drop in berry number from the elevated temperatures compared to the first season (-27% in Y1 vs -16% in Y2). Higher temperatures during shoot growth have been previously observed to increase cluster primordia differentiation (Buttrose, 1969). As shown in Figure 2, maximum temperatures during the flowering period in 2007 were significantly higher (~ 33°C) compared to 2008 (~ 25°C) which supports the significant reduction in fruit set and hence vine yields in 2007 as maximum temperatures on average in the under-vine tents were approx. 35°C; known to be detrimental to fruit set (Snider *et al.*, 2011).

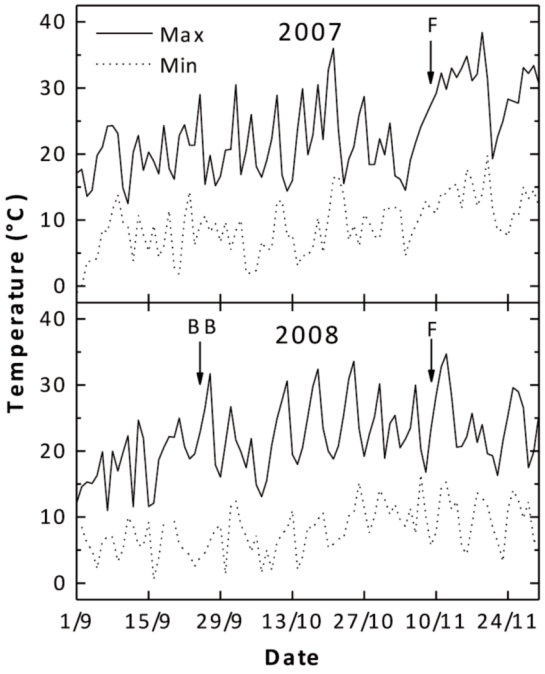

Figure 2. Daily maximum and minimum ambient air temperatures from September through November in 2007 and 2008 reported from an on-site weather station (Bureau of Meteorology, Nuriootpa-PIRSA). 'BB' and 'F' shown with arrows indicate the approx. start dates of budburst and flowering, respectively.

2. Pollen viability and growth

In the present study, higher temperatures with the UVTs around budburst had a detrimental effect on the number of viable pollen on the stigma (indicated by stigma fluorescence) and pollen tube number compared to control; no differences were observed in pollen tube growth in the style or pollen germination. Pollen germination rates were higher with elevated temperatures under the chambers, which was expected given that maximum daily canopy temperatures during flowering were around 30°C in the chambers whereas control vines were 2 to 8°C cooler. One study found that the optimum temperature for walnut (*Juglans regia* L.) pollen germination positively correlated with the mean daily integrated temperature (degree days) during pollen development, indicating a degree of adaptability of pollen germination to temperature (Polito *et al.*, 1991). Temperature is a key factor influencing pollen tube growth. Warm conditions facilitate rapid penetration of the pollen tube through the style leading to the micropyle (Vasconcelos *et al.*, 2009). Ebadi and colleagues reported that cooler temperatures adversely affected pollen tube growth in the pistil, particularly when the inflorescences were exposed to lower temperatures on the day of flowering compared to a couple of days before flowering (Ebadi *et al.*, 1995b). Our results indicate that pollen tube growth was impaired by high temperatures in the chambers, and it is possible that cultivar-specific differences exist since other studies did not investigate this phenomenon in Shiraz grapevines. Higher temperatures reportedly shorten the duration of stigmatic receptivity while hastening the rate of pollen tube growth, both of which in effect maintain a level of synchrony between male and female parts of the flower (Hedhly *et al.*, 2005). Our results are consistent with Hedhly *et al.* (2005), who define pollen tube kinetics as the rates of pollen germination and pollen tube growth, which decreased under conditions of elevated temperature. A plausible explanation for the decrease in pollen tube kinetics is a diminished signal, hormonal or other, from the ovule to the growing pollen tubes due to compromised embryo sacs. Another possibility put forth by Snider *et al.* (2011) is that high temperatures result in reduced soluble carbohydrates in the pistil leading to decreased pollen tube growth.

The success of fertilization depends on pollen viability, germination, and embryo sac viability. Our results of *in vitro* germination of pollen grains indicate increased pollen germination in the chambers at flowering (but not in the UVTs) and therefore viability at higher temperatures suggesting

OENO One, 2017, **51**, 4, 409-421

that decreased fertilization is not due to pollen viability but possibly due to decreased viability of the embryo sac. Both warm and cool conditions have been reported to result in underdeveloped or missing embryo sacs in the ovules (Ebadi et al., 1995b; Kozai et al., 2004). Degenerative embryo sacs may reduce pollen tube growth due to reduced attraction to the ovule (Ebadi *et al.*, 1995b). However, in peach (*Prunus persica* Batch.), high temperatures (>25°C) pre-flowering resulted in embryo sac degeneration but did not decrease pollen tube growth (Kozai *et al.*, 2004).

3. Berry and seed development

In our study, the number of functional seeds ('sinkers') resulting from the elevated temperature treatment with the UVTs at flowering decreased significantly compared to those at budburst while 'floaters' showed the opposite trend compared to control. Empty seeds ('floaters') are the result of either imperfect embryo sac development pre-flowering, failure to fertilize the ovule, or, after flowering, imperfect development of zygote or endosperm that may result in seed abortion and seedless berries (stenospermy; Ebadi *et al.*, 1996b). The higher percentage of 'floaters' and lower percentage of 'sinkers' observed in the under-vine tents in the present study indicate that elevated temperatures at flowering may have an adverse effect on ovule fertilization and/or endosperm development. Ebadi *et al.* (1996a) observed a lower percentage of 'sinkers' per berry as well as a higher percentage of berries that had at least one 'floater' when temperatures were lowered from 25°/20°C day/night to 17°/14°C or 12°/9°C. 'Floater' seeds generally result in berries of reduced size (Ebadi et al., 1996b). More work needs to be done to determine the effects of specific environmental conditions pre- and post-flowering on seed development and factors that result in their abortion.

An important aspect that needs to be considered in the analysis of our results is the effect of reduced UV radiation exposure (due to the UVTs and chambers) on reproductive processes of grapevines. Previous research has shown that high UV-B exposure negatively affects pollen germination *in vitro* (Caldwell *et al.*, 1979; Campbell *et al.*, 1975), however, very few *in vivo* studies have been done on the effects of low (or no) UV on the reproductive biology of flowering plants. Using partial (~ 20%) UV-blocking film, one study on bush bean (*Phaseolus vulgaris* L.) found that flowering was delayed by one day and one species had more flowers under low UV compared to normal (ambient) UV light (Saile-Mark and Tevini, 1997). The lack of *in vivo* studies on the effects of UV exposure on reproductive organs in plants may be since pollen within anthers is well-protected from UV-B and that anther walls reportedly absorb over 98% of UV radiation (Flint and Caldwell, 1983). Furthermore, the walls of pollen grains filter UV-B radiation and ovules may be similarly well-protected (Caldwell *et al.*, 1998; Tevini and Teramura, 1989). However, after the pollen has been transferred to the stigma, the processes of germination and pollen tube growth may be vulnerable to UV-B radiation (Flint and Caldwell, 1984; Torabinejad *et al.*, 1998). In the present experiment, we were unable to separate the effects of (reduced) UV from those of (elevated) temperature under either the UVTs or chambers. Considering the significantly higher viability of pollen (indicated by higher pollen germination) under elevated temperatures in the chambers of the present study and the above-mentioned reports of pollen being relatively well-protected against UV-B, we may surmise that the effects observed in the present study were primarily temperature-related. This hypothesis needs to be validated with controlled experiments of temperature and UV in the future.

Conclusion

Flowering and fruit set processes are complex and highly sensitive to environmental influences. Higher average growing season temperatures, temperature spikes during grapevine reproductive development, as well as temperature-associated shifts in phenology occurring in many premium viticultural regions worldwide has increased the importance of understanding their effects on grapevine physiological performance, both vegetative and reproductive. Elevated temperatures at budburst and flowering resulted in varying grapevine reproductive performance ranging from reductions in fruit set to decreased pollen viability. Our data suggests that temperature is a key factor dictating the viability of reproductive organs and processes in grapevine, particularly around flowering. Future work in this area could address questions relating to the roles that water and light availability during the budburst to fruit set period play in influencing flowering and fruit set, as well as their effects on inflorescence primordia initiation and possibly differentiation prior to dormancy. Cultivar-specific differences in reproductive responses to elevated temperatures may also exist. The future work mentioned above would inform cultivar selection in future vineyard plantings particularly in regions situated in marginal climates that are becoming increasingly warmer. Furthermore, with the relatively recent availability of the whole

genome sequence of grapevine as well as high throughput sequencing technologies, opportunities exist to identify specific genes involved in flowering and fruit set, and how the expression of these genes might be altered under varying environmental conditions.

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