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Establishing the temperature dependency of vegetative and reproductive growth processes and their threshold temperatures of vineyard-grown *Vitis vinifera* cv. Semillon vines across the growing season

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Abstract. A hydrocooling system provided canopy temperature control of *Vitis vinifera* L. cv. Semillon vines at set points of 30, 35 and 40°C. The impacts on vegetative and reproductive growth over the growing season were assessed. Dynamics and rates of leaf expansion, bunch biomass and sugar accumulation were strongly affected by canopy temperatures – being highest at 30°C and lowest at 40°C. Leaf and stem biomass accumulation at 40°C was detrimentally affected but was otherwise little affected by temperature. Leaf expansion was earliest, leaf sizes greatest and rates of expansion all optimal at 30°C and all were strongly temperature dependent. Bunch biomass accumulation was earliest at 35°C but amount of biomass in bunches and rates were both highly temperature dependent and optimal at 30°C. Rates of sugar accumulation and total amounts accumulated at harvest were both highly temperature-dependent processes: fastest and greatest at 30°C. Many of the temperature-dependent processes decreased in rates and amounts linearly between 30 and 40°C. Despite the effects of temperature on bunch and berry growth, there were no treatment effects on the yield per vine. The study confirms that the threshold temperature for most processes was 35°C, where some depreciation in dry matter and sugar accumulation occurred, whereas 40°C was detrimental to all growth processes.

Additional keywords: berry ripening, dry matter accumulation, growth dynamics, hydrocooling, rates of accumulation.

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Introduction

Reducing canopy and fruit temperatures of grapevines and other crops is an important strategy in mitigation of the negative effects of recurrent high summer temperatures that pervade many of the grape growing regions of Australia and elsewhere (Matsui *et al*. 1986; Gladstones 1992; Greer and Weedon 2013). High temperatures, above ~35–40°C, have many deleterious effects on plants including reductions in photosynthesis, stomatal conductance, leaf expansion, stem growth, flowering (Greer and Weston 2010*b*), berry development (Sepúlveda and Kliewer 1986) and yield (Lomas 1992). Thus, there is a need to find strategies and management practices to alleviate the effects of heat events on the productivity of grapevines, especially in the light of climate-change induced increases in incidence and intensity of summer high temperatures (Jones *et al*. 2010; Tomasi *et al*. 2011).

One strategy of covering whole vines with shade cloth has the benefit of reducing the radiant energy over the whole vine and has the potential to reduce the canopy temperatures by as much as 6–10°C (Morrison and Noble 1990; Rana *et al*. 2004; Greer *et al*. 2010). However, for Semillon vines at least, reducing the radiant energy by 70% also had an impact on photosynthesis, the canopy carbon budget and biomass accumulation (Novello *et al*. 1999; Greer *et al*. 2011). Similar results were observed for cv. Sangiovese vines treated with 40 and 70% shade cloth (Cartechini and Palliotti 1995). However, plastic covering, which reduced photon flux densities (PFD) by 57% over the table grape cv. Matilde, caused a significant increase in both shoot length and shoot leaf area (Novello *et al*. 1999). In contrast, the shade cloth over cv. Sangiovese vines had no effect on shoot length but significantly increased individual leaf areas and delayed berry ripening (Cartechini and Palliotti 1995). Berry ripening was also delayed when Semillon vines were shaded (Greer and Weedon 2012*a*). Short-term coverage of vines with shade covering also has had variable effects. For example, a range of shade covers (decreasing PFDs by 50 to 90%) over the grapevine cv. Aglianico for short periods (15–27 days) after flowering caused no impact on vegetative growth, however, did reduce fruit set and bunch compactness (Basile *et al*. 2015). A similar short-term shade treatment, which reduced PFDs by 80% for 22 days from flowering, caused an increase in leaf size but a decrease in leaf dry weight of both cv. Grenache and Muscat but had no effect on berry weight (Coombe 1959). Thus, covering vines with shade cloth for heat protection can

have negative effects on vegetative and reproductive development through reduced irradiance, but the impact is dependent to some extent on the grapevine cultivar.

An alternative strategy that does not affect irradiance is to use the latent heat of evaporation of water to cool the canopy for a whole vine in a comparable manner to individual leaves being cooled by transpiration. For grapevines, this has been assessed in several studies; most notably Kliewer and Schultz (1973), who used overhead water sprinklers above several grape cultivars to intermittently spray the vines whenever the air temperatures exceeded 30°C. This treatment reduced leaf and berry temperatures by 5 to 20°C. A similar reduction in temperature was observed for the leaves and berries of other water sprinkled grape cultivars when temperatures exceeded 32°C in work by Aljibury *et al*. (1975) and Gilbert *et al*. (1970). The practice of using hydrocooling in table grapes has, therefore, been adopted by some growers in California (Wallender *et al*. 2007). Hydrocooling has also been used in reducing fruit drop of oranges (Brewer *et al*. 1979), maintaining fruit colour of apples (Iglesias *et al*. 2002) and pears (Dussi *et al*. 1997), controlling apple sunburn (Gindaba and Wand 2005) and reducing cranberry yield losses (Pelletier *et al*. 2016). More recently, Greer and Weedon (2014*a*) applied water to Semillon vines in an automated cycle of short pulses coupled with a drying period to maintain the canopy temperatures at or below 35°C across the growing season. In all these studies, beneficial effects of hydrocooling on growth and reproductive development were observed. This included delayed leaf expansion in cv. Semillon (Greer and Weedon 2014*a*), faster rates of shoot growth in cvv. Chardonnay, Chenin Blanc and Semillon (Aljibury *et al*. 1975), faster rates of berry dry matter accumulation in cvv. Cardinal, Carignane and White Riesling (Kliewer and Schultz 1973), higher berry or fruit weights in cv. Semillon (Iglesias *et al*. 2002; Greer and Weedon 2014*a*), Chenin Blanc and Carignane (Kliewer and Schultz 1973) and a higher yield in Semillon (Greer and Weedon 2014*a*). Thus, there is no doubt that intermittent spraying and evaporation of water in high temperature climates is a successful strategy to cool the canopy and to avoid deleterious effects of the high temperatures (Liu and Kang 2006; Pelletier *et al*. 2016).

Many plant processes are affected by temperature and most processes are negatively impacted on by temperatures above ~35°C. For example, photosynthesis of C_3 species such as grapevines and other horticultural crops are known to be markedly reduced at such high temperatures (Kriedemann 1968; Ferrini *et al*. 1995; Greer and Weedon 2012*b*; Greer 2015; Pelletier *et al*. 2016). High temperature-induced reductions in photosynthesis and/or stomatal conductance can lead to insufficient carbon to meet berry growth requirements (Sepúlveda and Kliewer 1986; Greer and Weston 2010*b*). In addition, berry expansion, sugar and dry matter accumulation of grapevine cultivars are all highly temperature-dependent processes (Radler 1965; Buttrose *et al*. 1971; Hale and Buttrose 1974; Kliewer 1977) but the responses are curvilinear. For example, rates of sugar accumulation of Chardonnay grapevines were optimal at 25°C and markedly reduced at temperatures above 35°C whereas for Semillon berries, the optimum was at 35°C but rates were reduced at 40°C (Yamane *et al*. 2006; Greer and Weedon 2014*b*). Other processes in vines known to be affected by temperature include budbreak (Moncur *et al*. 1989), leaf appearance (Moncur *et al*. 1989; Greer *et al*. 2004), leaf expansion and stem extension (Ferrini *et al*. 1995; Greer and Jeffares 1998) and shoot DW and length (Buttrose 1969). Yields of apple trees have also been shown to be dependent on temperature in a curvilinear fashion (Greer *et al*. 2002). Although it is clear that high temperatures impact a wide variety of plant processes, the question of how high the temperatures have to be during the growing season to affect grapevine performance remains unanswered. However, this is an important question for grapevines and other economically important crops as costs of protecting the crops becomes increasingly significant as heat events increase in intensity and frequency.

Accordingly, the objective of the present study was to adopt the hydrocooling system developed by Greer and Weedon (2014*a*) to control the canopy temperatures of vineyard-grown *Vitis vinifera* L. cv. Semillon grapevines, and to extend their study to control the canopy at selected set points across the growing season. These set points (30, 35 and 40°C) were chosen to represent the upper range for many of the plant processes listed above and enabled determination of the threshold temperatures which caused an impact on these processes. The processes of vegetative and reproductive growth and development were assessed in vines across the whole growing season.

Materials and methods

This study was undertaken on a commercial vineyard in the Riverina region of NSW, Australia over the 2010–11 growing season. Vines were planted in 2003 from cuttings and grown on own roots. The rows were orientated in a north–south direction at 1.8 m spacing between vines and 3.5 m between the rows. Vines were trained to grow on a vertically shoot positioned (VSP) trellis and spur pruned. The cordon was 1.2 m high and catch wires were 50 cm above the cordon. The wires were lifted when canopy growth was completed after fruit set had occurred. Vines were drip irrigated with drippers at 0.6 m spacing and delivered 2.4 L h^{-1} for 12 h $week^{-1}$ until veraison, after which the irrigation was increased to 24 h $week^{-1}$. Nutrition was supplied through the dripper system. Midday water potential measurements were conducted in midsummer and averaged -1.16 ± 0.1 MPa but otherwise there were no visible symptoms of water stress apparent on the vines. Budbreak occurred in late September, flowering in early November and fruit set in mid-November.

Hydrocooling treatments

Across two whole panels, each of three vines, for each of three treatments, a 19 mm irrigation pipe was suspended with wire across the entire 6 m in length of the two panels in each treatment. At 60 cm intervals, micro misters (0.4 micron orifice, 4 L h^{-1}) were inserted into the irrigation line and directed downwards to the vines. The sprayer system and the details of the microprocessor control of the system were previously reported by Greer and Weedon (2014*a*) except that finer micro misters were used and the system ran for 60 s with misting and less water was used. However, another innovation for the present study was to invoke a set point of the control system such that

water was transiently sprayed by the pulsed wetting and drying system onto the vines when the canopy temperatures reached the set points. These were set at 30, 35 and 40°C. Two similar panels of vines in the same row but without the hydrocooling system were used as controls and all treatments were replicated once, also in the same row. This hydrocooling system was implemented at 43 days after budbreak (DAB).

Temperature and irradiance

On each side (east and west) of the centre vine of one panel in each treatment and replicate, an infrared temperature sensor with a 22° view (IRRP, Apogee) was located 1.2 m above the ground and 0.3 m out in the inter row space and pointed horizontally towards the canopy. The sensors were connected to a datalogger (CR1000, Campbell Scientific Australia) and hourly averages recorded. Air temperature and relative humidity (HMP50, Vaisala) placed within a protected white screen as well as irradiance measured with a quantum sensor (LI190s, Li-Cor) were placed 500 mm above the canopy and measured with the datalogger at similar intervals.

Vine measurements

Shoot growth

Two shoots on each vine in each treatment and replicate and on either side of the vine (total of 12 shoots per treatment) were tagged at budbreak (25 September) and shoot lengths measured at ~7 day intervals from the 11 October until harvest on the 22 February. Leaf dimensions (width and length) of each leaf on these shoots were also measured at the same time once the leaf width was ~15–20 mm in diameter. These data were then used to determine the time of leaf appearance and the leaf areas according to Greer and Weston (2010*a*). The number of lateral shoots on each of the selected shoots was assessed in midsummer. At regular intervals (1–2 times per week), whole untagged shoots were detached from some of the vines (two from each of three vines) in each treatment and taken back to the laboratory and oven-dried for 2 weeks at 60°C for biomass determinations.

Leaf area and internode distribution

At the time of the fruit harvest, all of the selected shoots were destructively harvested and taken back to the laboratory. Main stem and lateral leaves were removed from each shoot and counted then areas of individual main stem leaves and total lateral shoot leaf area was determined with a leaf area meter (LI-3000, Li-Cor). Internode lengths along each shoot were also measured as well as the overall stem length.

Berry diameter and soluble solids

From ~90 DAB, berry diameters on three berries from the upper, middle and basal segments of two bunches on one shoot of three vines of each treatment and replicate (total of 36 berries) were measured using a microcaliper at regular intervals. These berries were destructively harvested and soluble solids concentration measured with a digital refractometer (PR-101, Atago). Berry sugar content was calculated using the berry diameters to estimate berry volume and converting the Brix measurement to sugar concentration according to Greer and Weedon (2014*a*). In addition, berries from other bunches were similarly sampled and taken back to the laboratory where diameter, soluble solids and DW were determined.

Yield

At harvest, all bunches were removed from all vines in each treatment and replicate and counted and then the total bunch FW vine^{-1} determined. These bunches were transported to the laboratory where bunches on the selected shoots were separated into the components (berries, rachis) and the number of berries counted and berry FW determined.

Biomass determination

All leaves (main stem and laterals) and stems of each shoot as well as the lateral stems and the selected whole bunches were dried for 2 weeks at 60°C and then weighed to determine the biomass. Individual berries, along with the bunch rachis, were also dried at the same conditions. Biomass of the leaves, stems and bunches across the growing season were also determined in part from samples collected during the growing season but also from allometric relationships between leaf area and leaf DW, between stem length and DW and between bunch length and DW (Greer and Sicard 2009).

Data analysis

All data were analysed using generalised linear models (GLM) with SAS ver. 9.3 (SAS Institute) and least-squares means and standard errors determined. All data were analysed assuming a fully-randomised design and statistical significance was assessed at the 5% level. Leaf, stem, bunch biomass accumulation and berry ripening dynamics were assessed by applying the Boltzmann sigmoid function to the data using OriginLab's Origin 8 Pro (OriginLab) software following Seleznyova and Greer (2001). The parameters of the function included the timing of expansion, determined from the inflexion point of the function, duration of maximum growth, the initial and final size determined as when the growth has reached 20 and 80% of the maximum. The expansion/accumulation rates were also determined using SAS to fit a linear function to the data over the duration of when the rates were maximal.

Results

Canopy and air temperatures across the growing season

The mean air temperatures across the day were generally maximal in the range from ~27 to 32°C in the first half of the growing season (Fig. 1*a*). However from ~80 DAB, the maximum air temperatures were commonly above 35°C and on a few occasions, exceeded 40°C. The daily temperature minima on occasions went as low as 10°C, but typically were between 15 and 20°C. However, in the later part of the growing season (after 90 DAB), the minima were more frequently around 20°C. The growing season was also characterised by relatively high photon flux densities (Fig. 1*b*), with only a few cloudy days.

For the untreated Semillon grapevines, the canopy temperatures followed a similar pattern (Fig. 2*a*) to the air temperatures except that the canopy exceeded 40°C on several occasions. However, for the canopy with the hydrocooling set point at 30°C (Fig. 2*b*), there was only a slight reduction in the

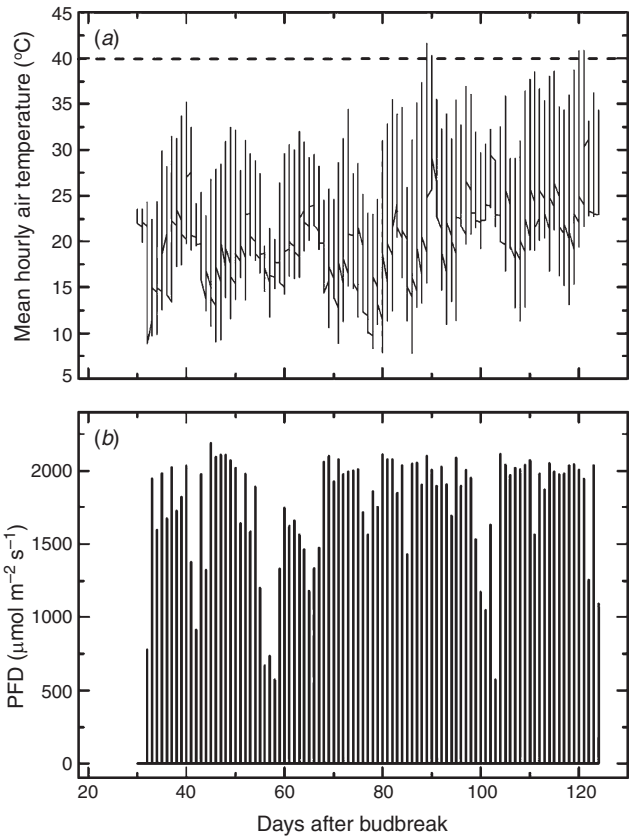

Fig. 1. (*a*, *b*) Changes in air temperatures and photon flux densities (PFD) across the 2010–11 growing season. In each case the data are mean hourly averages and the dotted line indicates 40°C to demonstrate when these high temperatures occurred.

number of days over 30°C but there was a reduction in the canopy temperatures by 2−3°C over the warm temperature period. In contrast, the canopy with the cooling at the set point of 35°C (Fig. 2*c*) had a much lower frequency of temperatures above 35°C (12 compared with ~30 for the control canopy) and again a slightly lower temperature with the cooled canopy. The canopy at the set point of 40°C (Fig. 2*d*) had a temperature range comparable with the control canopy given that the set point was only briefly reached but the hydrocooling kept the canopy at or below 40°C.

To assess the effects of the cooling treatments on the canopy temperatures more closely, diurnal air and canopy measurements and the duration of hydrocooling were determined for selected days (Figs 3, 4). On the first occasion at 102 DAB (Fig. 3*a*), the air temperature reached a maximum of 33°C and the treated canopy at the 30°C set point was maintained relatively constant at or close to the set point and up to 4°C below the air temperature. Hydrocooling was required at 1200 hours (Fig. 4*a*) and peaked at 1500 hours with 35 min h^{-1} of water sprayed on the vines. In contrast, the canopies at a set point of 35°C (and 40°C, data not shown) increased by several degrees above air temperature and no hydrocooling was required.

About 10 days later (Fig. 3*b*), the air temperature reached a maximum of 38.6°C but from ~1000 hours, the canopy temperatures at the 30°C set point treatment began to deviate from the set point and tracked air temperatures despite hydrocooling commencing from 0900 hours (Fig. 4*b*). However, with over 30 min of hydrocooling each hour from 1400 to 1600 hours, the canopy temperatures eventually decreased by more than 3−4°C and were maintained 5−7°C below air temperature although not at the set point. In contrast, the canopy temperatures of the 35°C set point treatment were maintained at 35°C from 1300 to 1800 hours. About 10 to 15 min h^{-1} were required to maintain the set point temperature (Fig. 4*b*). Canopy temperatures for the 40°C set point (data not shown) more or less tracked air temperature and no hydrocooling was required.

The next day, the air temperatures were similar, though peaked much earlier in the day, at around noon (Fig. 3*c*). Again the canopy temperatures of the 30°C set point treatment were unable to be controlled, despite nearly 40 min h^{-1} of water spray commencing from 1000 hours (Fig. 4*c*). However, the cumulative effect of the hydrocooling up to 1500 hours was again eventually effective and the canopy temperatures subsequently dropped by 6−7°C and the temperatures were thereafter maintained at the set point up to 1900 hours Although the canopy temperatures of the 35°C set point treatment were controlled over the period of high temperatures, they were typically closer to 36°C and up to 20 min h^{-1} of hydrocooling occurred from 1100 to 1700 hours. The canopy temperatures of the 40°C set point treatment more or less tracked the air temperatures but were up to 3°C below air temperatures in the late afternoon (1700 hours).

On one of the hottest days in the latter part of the growing season (Fig. 3*d*), the air temperatures peaked at 40.8°C at 1500 hours but remained close to 40°C right up to 1900 hours. In addition, the air temperature had reached 30°C by 0830 hours. On this occasion, and again despite large amounts of hydrocooling (Fig. 4*d*), which started at 0800 hours, there was no apparent control of canopy temperatures in either the 30°C set point or the 35°C set point treatments and both tracked at or slightly above air temperature, at least up to 1500 hours. Some effectiveness of the hydrocooling was apparent from then on; however, when the canopy temperatures for these two treatments began to deviate by 6−7°C below air temperature they were still above the set points. It was also notable on this occasion that the canopy temperatures of the 40°C set point treatment were maintained at or just below 40°C and the hydrocooling was invoked for this treatment, with nearly 20 min h^{-1} of water spray at 1400 hours and small amounts to 1600 hours.

Across the period of hydrocooling from 80 DAB to harvest, the average maximum temperatures were 31.3, 32.7, 33.1 and 35.3°C for the hydrocooled and control vines compared with air temperature at 35.0°C.

Leaf area distribution along the shoot

Early in the growing season, leaf areas distributed across the shoot (Fig. 5*a*) increased from node position 1 to 5, where the leaf area was maximal (0.0086 m^2), with a subsequent decline to those leaves recently emerged and still expanding up to node position 7. In the mid-spring period (Fig. 5*b*), there was a similar pattern of leaf area distribution along the shoot for all

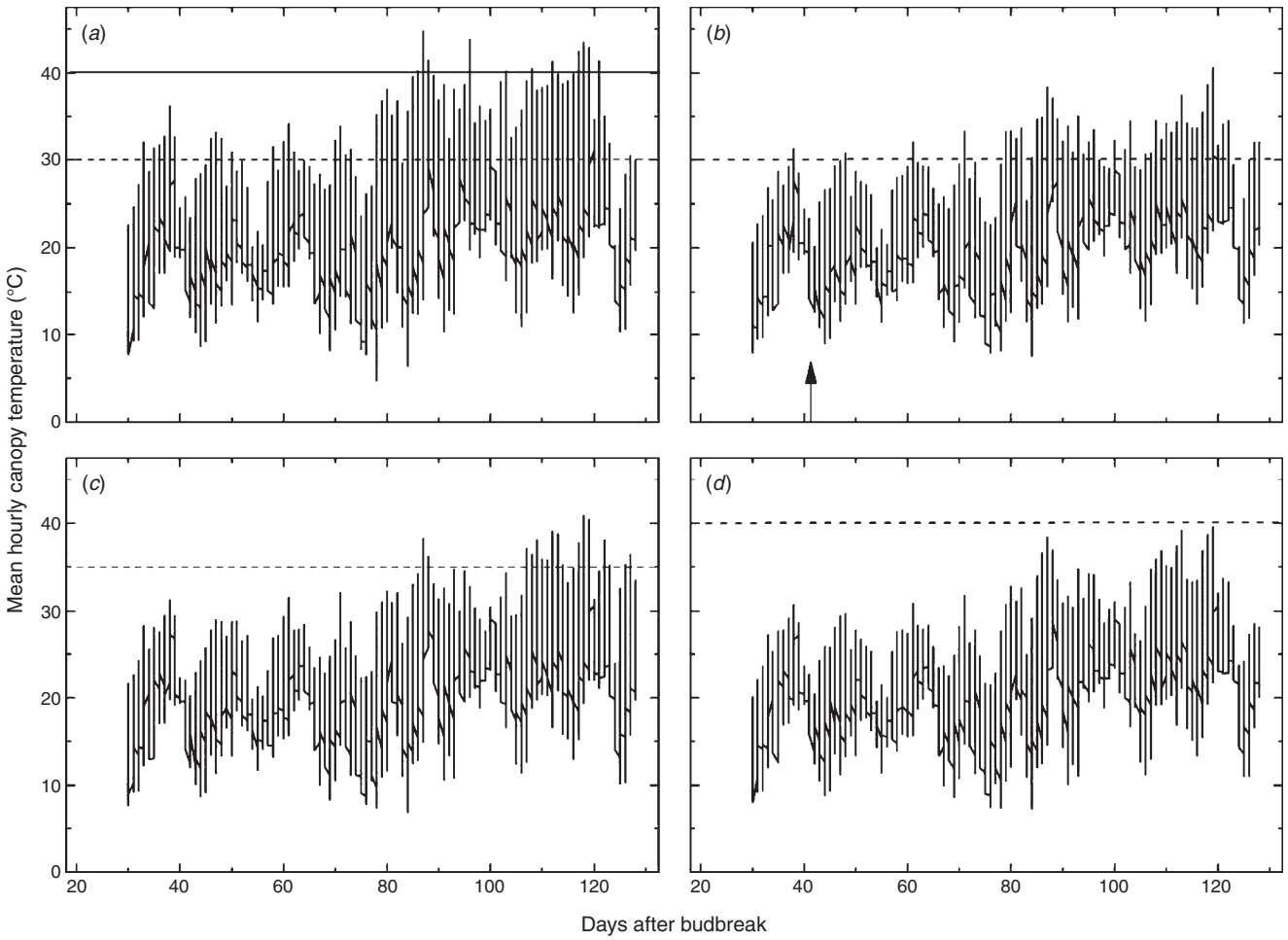

Fig. 2. (*a–d*) Canopy temperatures measured over the 2010–11 growing season on Semillon vines in the control treatment (*a*), 30°C set point treatment (*b*), 35°C set point treatment (*c*) and the 40°C set point (*d*). In each case, lines represent the set point temperatures except for the control treatment where the lines indicate the low and high set points to compare the other treatments against. The hydrocooling treatment commenced 43 days after budbreak (DAB) as indicated by the arrow in (*b*).

treatments, with maximum leaf areas in the range of 0.015 to 0.017 m^2 at nodes 5–6. There were also some significant differences for the recently expanding leaves (nodes 10–20), with the control vines with largest sized leaves and those for the 30°C set point treatment larger than those for the 35 and 40°C set point treatments. These differences were perhaps more likely to reflect variation between vines than attributable to the treatments as temperature differences were not strong at this time.

By early summer when the temperatures had started to rise (Fig. 5*c*) and hydrocooling was required, at least for the 30°C set point treatment, the pattern of distribution of leaf areas along the shoot remained comparable with that occurring earlier, except that leaf expansion had occurred in all but the youngest few leaves. The leaves at node positions 5–7 were generally the largest in all cases (0.018–0.019 m^2). Those later emerging leaves on the vines in the 30°C set point treatment, especially those at nodes 10 to 15, were significantly larger than comparable leaves in the other hydrocooling treatments (~0.014 cf. 0.011 m^2). Furthermore, these leaves in the 30°C set point treatment had expanded more than the leaves of control vines from 61 to 81 DAB and were larger, though not significantly so, compared with leaves on control vines.

This trend of the leaves on the vines in the 30°C set point treatment having the largest leaves was still evident at the end of the growing season (Fig. 5*d*). Most of the leaves up to node 20 had completed expansion but those on vines in the control and the 35°C set point treatment were generally similar in size. However, the leaves on vines of the 40°C set point treatment were significantly smaller compared with the other treatment. It would appear that the hydrocooling the vine canopy to a set point of 30°C was beneficial for leaf expansion.

Leaf appearance

For the first 7–8 leaves, the leaf appearance rate was negligible and these leaves came out over ~1.5–2 days (Fig. 6). Thereafter, the leaves appeared at a constant rate, at least until node 20, with each leaf with a phyllochron of 3.4 ± 0.9 days (at a rate of 0.29 leaves day^{-1}) to appear. However, there was a marked

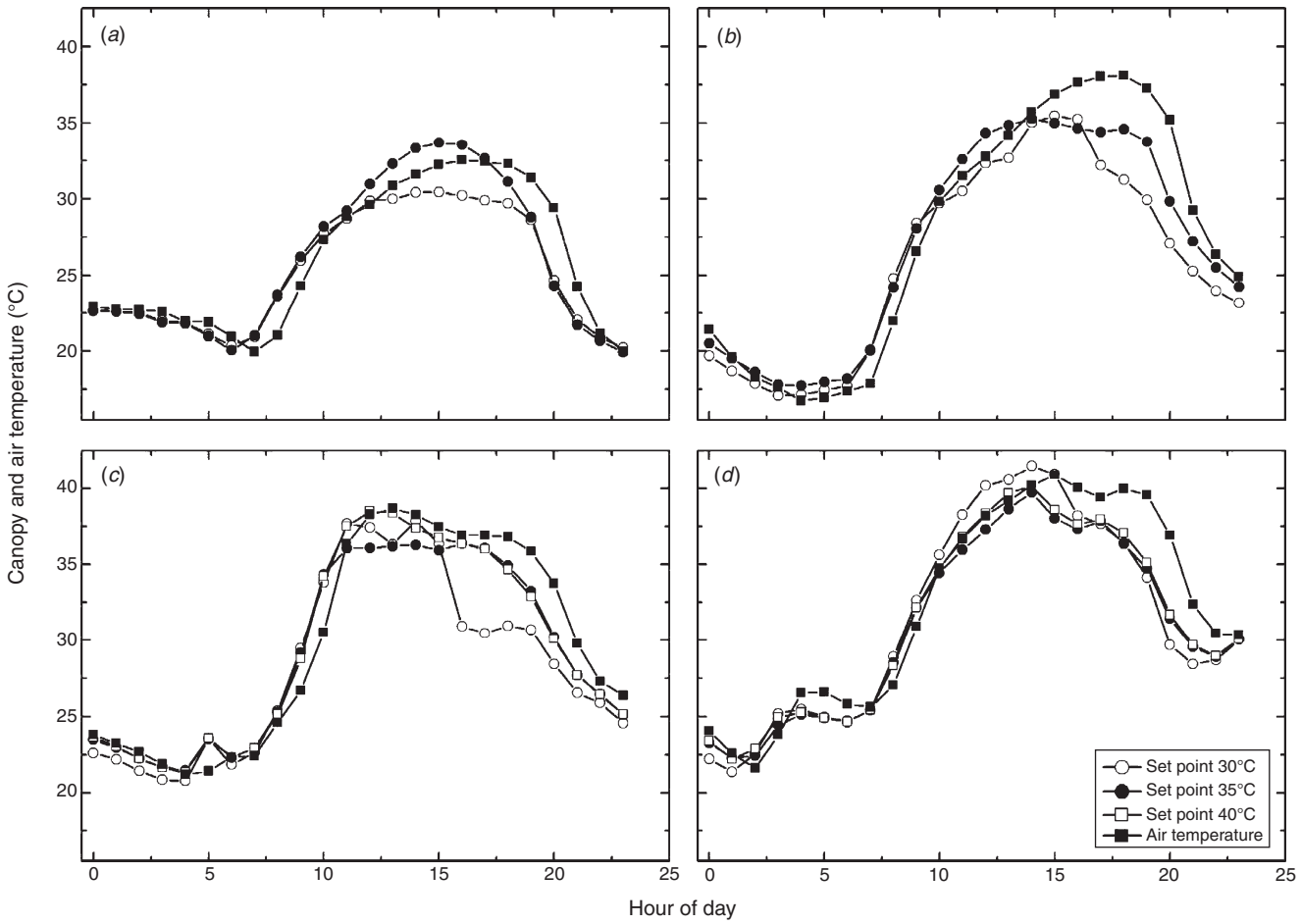

Fig. 3. (*a–d*) Diurnal changes in mean hourly air and canopy temperatures of Semillon vines in the different hydrocooling treatments at selected days over the growing season: (*a*) 102 days after budbreak (DAB), (*b*) 112 DAB, (*c*) 113 DAB and (*d*) 119 DAB. Some data for the set point 40°C in (*a*) and (*b*) has been omitted for clarity as it is comparable with the 35°C data.

increase in the phyllochron for leaves at node position 21 along the shoot to node 26 at 5.6 ± 0.2 days (rate of 0.18 leaves day^{-1}). Thus, most leaves had appeared well before the high temperatures had started to occur.

Dynamics of leaf expansion

The expansion of selected leaves along the shoot over time (Fig. 7) indicated marked differences in times of emergence, with the leaves at nodes 2 and 5 appearing 9 and 10 DAB whereas the leaves at nodes 10 and 15 appeared at 15 and 32 DAB and the leaf at node 20 emerged at 55 DAB. In each case, all leaves expanded in a sigmoid pattern and the analyses of the expansion dynamics are shown in Table 1. There were highly significant differences in the maximum leaf areas, with leaf 5 largest in size and leaf 20 smallest in size, with leaves 2 and 10 similar and intermediate in size. The time of maximum expansion (i.e. point of inflexion) shifted progressively along the shoot, with leaf 2 being earliest at ~15 DAB and leaf 5 at ~50 DAB. Notably, leaves 10 and 15 were also delayed to reach maximum expansion at about the same time at 59–62 DAB whereas leaf 20 was considerably more delayed at 77 DAB, and consistent with the shift in phyllochron from about node 19.

However, the duration of maximum expansion also varied along the shoot, but was longest for leaf 2 (15 days) and shortest for leaves 10, 15 and leaf 20 (8 days). Consistent with these differences were the expansion rates, which increased progressively from 1.7 cm^2 day^{-1} for leaf 2 to 4.3 cm^2 day^{-1} for leaf 10 and 1.0 cm^2 day^{-1} for leaf 20, thus, for this leaf the expansion rate was considerably slower than all other leaves. Thus, although leaf 2 emerged earliest in the season, the expansion process was relatively slow whereas leaf 10 emerged only a few days later and moderate in size but had the fastest rate of expansion. In contrast, leaf 5 was the largest leaf in size but intermediate in expansion rate. It would appear, therefore, that leaf size along the shoot was not dependent on the dynamics of expansion but rather the position along the shoot, hence more ontogenetically determined.

To assess the impact of the cooling treatment on the dynamics of leaf expansion, leaves 17–20 were chosen as these leaves all emerged and expanded when the hydrocooling treatments were active. For all dynamic attributes, there were highly significant ($P < 0.001$) treatment effects, which accounted for

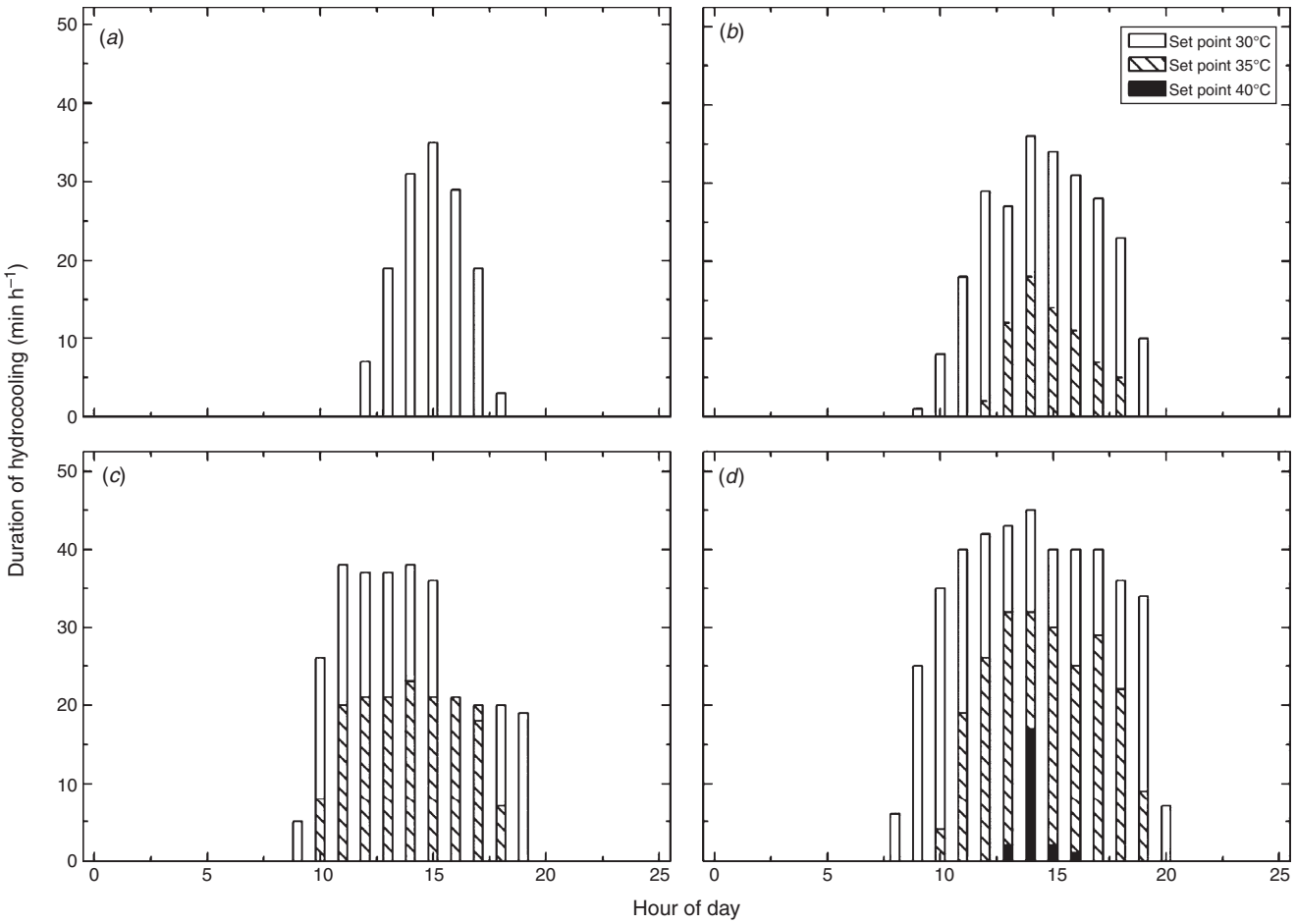

Fig. 4. (*a–d*) The amount of water used per hour across the day in the hydrocooling system over the Semillon vines for each of three set point treatments as indicated on the selected days as shown in Fig. 3: (*a*) 102 days after budbreak (DAB), (*b*) 112 DAB, (*c*) 113 DAB and (*d*) 119 DAB.

75–95% of the variance. The dynamics of leaf expansion of leaf 18, as an example, are shown in Fig. 8 and the dynamic attributes averaged over leaves 17–20 are shown in Table 2. There were marked treatment effects on the maximum size of leaf 18, with those vines treated at the 30°C set point having the significantly largest leaves at 0.0101 ± 0.0001 m^2 and the leaves on control vines were also large at 0.0090 ± 0.0001 m^2 but for the 35 and 40°C set point treatments, the leaves were significantly smaller at 0.0059 ± 0.0001 and 0.0049 ± 0.00003 m^2, respectively, and consistent with Fig. 5 and the average of the four leaves (Table 2).

For the vines in the Control and 30°C set point treatments, the times of maximum expansion for the four leaves were 33 to 40 ± 2 DAB (Table 2) and significantly earlier for the 30°C treatment leaves. For the leaves on vines in the 35 and 40°C set point treatments, the time of maximum expansion was significantly delayed to $53–55 \pm 1.5$ DAB. The duration of maximum expansion was shortest at 11.9 ± 0.7 days for vines in the control treatment, and for the other treatments the duration of maximum expansion was significantly longer at 14 to 17 ± 1.5 days. However, leaf expansion on average started consistently earlier for these four leaves on vines in the 30°C set point treatment by 5–9 days compared with all other treatments. In addition, it was leaves on the control vines that finished expansion earliest, though not significantly so compared with vines in the 30°C set point treatment, by 5–13 days compared with the two other treatments. However, the expansion rates for the leaves in each treatment occurred in two groups; ~2.5 cm^2 day^{-1} for the control and 30°C set point treatment and less than 1 cm^2 day^{-1} for the 35 and 40°C set point treatments. Although not linear, the average change in leaf expansion from 30 to 40°C occurred at average of 0.19 cm^2 day^{-1} °C^{-1}. From these results, it would appear that the dynamics of leaf expansion of this leaf cohort were sensitive to temperature and optimal at ~30°C and impaired at temperatures at and above 35°C.

Dry matter accumulation

The accumulation of DW of the leaves and stems across the growing season (Fig. 9) followed a sigmoidal pattern, at least for some time, with no treatment differences apparent until ~70 DAB. Thereafter, average leaf DWs continued to increase in the control, 35°C and 40°C set point treatments for a short period but then the leaves began to decrease in DW in the 40°C set point treatment and this continued more dramatically (62%)

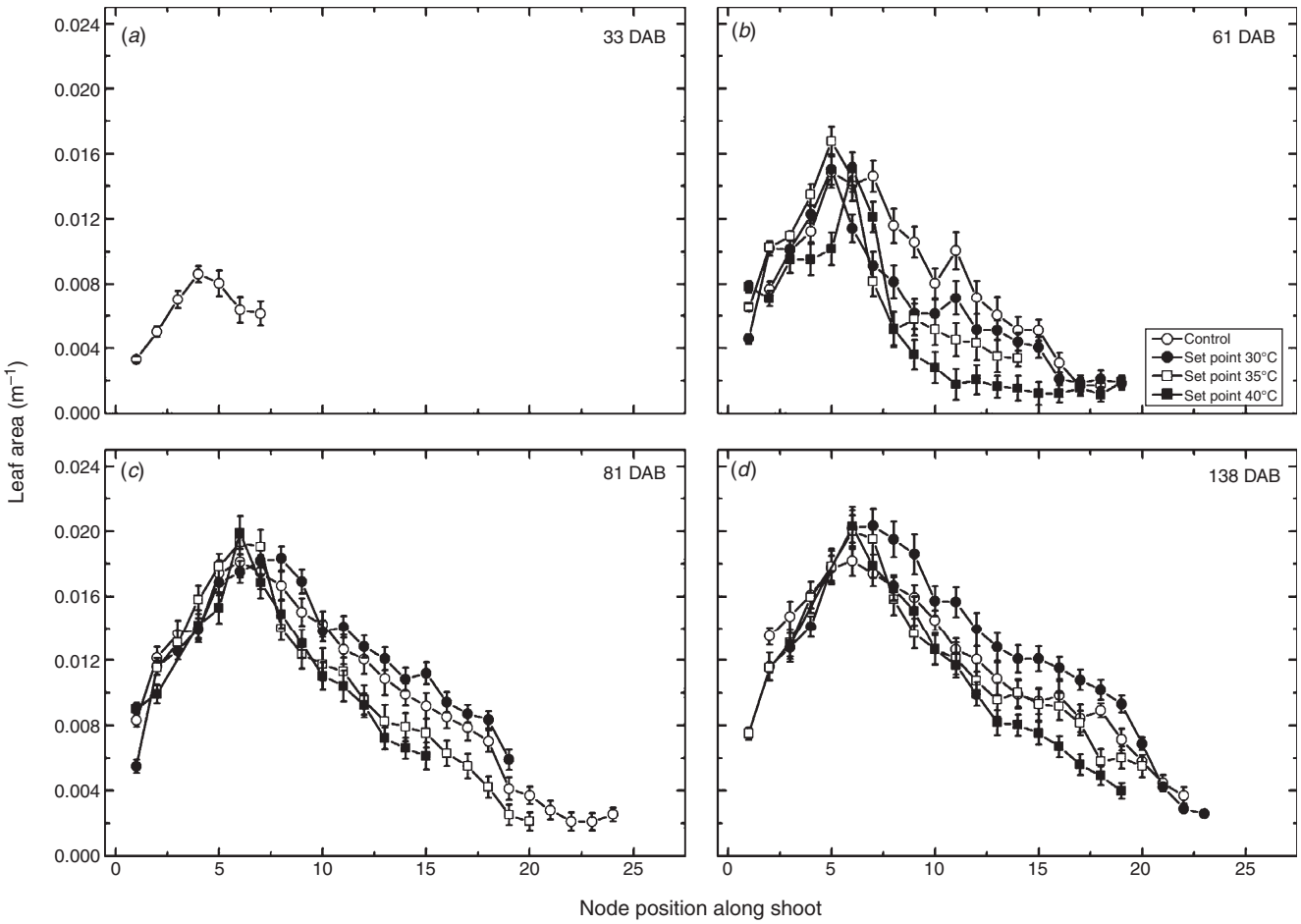

Fig. 5. (*a–d*) Changes in the leaf area (mean ± s.e., $n = 8$) at different node positions along the shoot of Semillon vines in each of four hydrocooling treatments as indicated on selected days across the growing season: (*a*) 33 days after budbreak (DAB), (*b*) 61 DAB, (*c*) 81 DAB and (*d*) 138 DAB. Note, not all leaves were measured on all occasions.

from ~80 DAB. A similar decrease also occurred in the control leaves but later (>90 DAB) and less so (21%). In contrast, the leaves in the 30°C set point treatment appeared to stop accumulating dry matter from ~60 DAB and remained relatively constant in dry matter across the growing season as did those in the 35°C set point treatment. For the latter treatment, leaf DW remained significantly higher than leaves in all other treatments, suggesting 35°C was the optimal temperature for leaf dry matter accumulation, whereas 40°C was clearly detrimental. There were no significant differences in the dynamics of leaf dry matter accumulation and no treatment differences in the rates of accumulation, which averaged 0.235 ± 0.021 g day^{-1} over all treatments (data not shown).

There was a different response for the accumulation of stem DW of shoots in all treatments, which reached maximum weights at ~90 DAB and did not change for the remainder of the growing season. However, there were some treatment differences apparent, with those shoots in the control, 30 and 35°C set point treatments having the highest stem DW, with few differences between them (18.9–19.9 ± 1.7 g). However, there was a significantly lower stem DW in the 40°C set point treatment compared with all other treatments. Thus, an optimum temperature for stem dry matter accumulation was not readily discernible except that 40°C was also too high for this process. Consistent with the accumulation process of leaves, there were also no treatment effects on the dynamics of stem dry matter accumulation and maximum rates averaged 0.373 ± 0.017 g day^{-1} across all treatments (data not shown). However a comparison of the accumulation rates during the period from 61 to 76 DAB, that is, when the stem accumulation process was approaching saturation, indicated that rates declined for the control treatment to the 40°C set point treatment from 0.265 ± 0.019, 0.250 ± 0.016, 0.228 ± 0.019 and 0.189 ± 0.013 g day^{-1} and the 25% reduction between the 30°C set point treatment and the 40°C set point treatment was significant ($P < 0.01$). Thus, the rates of extension during the mid- to late stage of stem development, especially of the vines in the 40°C set point treatment, conformed to the reduced total dry matter accumulation in the stems of this treatment. It was notable that, on average, dry matter accumulation in leaves of all treatments reached 20% of the total at 27.8 ± 0.8 DAB and reached 80% of the total at 55.2 ± 5.5 DAB, whereas for the stems, the comparable dates were 40.1 ± 0.2 DAB and 64.5 ± 0.6 DAB. Despite the stem dry matter accumulation process

lagging behind that of the leaves, the durations of growth were comparable at 27.4 ± 4.8 and 24.4 ± 0.8 days.

The accumulation of total dry matter of the shoots and bunches across the growing season (Fig. 10) indicated that the bunch DW dominated the biomass of the shoot once flowering and pollination had occurred in all treatments. Bunch dry matter accumulated from ~70 DAB and generally followed a sigmoid pattern in each treatment and exceeded stem biomass after ~90–95 DAB. There were significant treatment differences in the dynamics of bunch dry matter expansion (Table 3) with the bunches on vines at the 35°C set point reaching the time of maximum accumulation significantly earlier, by 11–16 days, than those at 30 and 40°C set points; however, the time was most delayed for the control bunches. Similarly, the duration of maximum accumulation was shorter for the bunches on vines in the 35°C compared with those in the 30°C set point treatment but not so when compared with those in the 40°C set point treatment but these differences were not significant. The time to reach 20% of total accumulation did not vary between treatments and averaged 90.2 ± 3.7 days. However, the time to reach 80% of the total accumulation was significantly earlier (by 22 days on average) for the bunches on vines in the 35°C set point treatment compared with all other treatments. These data suggest the dynamics of dry matter accumulation were optimal at 35°C, and exposure to 40°C conditions did not appear too detrimental to the dynamics of dry matter accumulation. We note that rates of bunch dry matter accumulation were significantly ($P < 0.01$) different between all treatments except between the control and 35°C treatments. There was a clear temperature dependency, with the rates optimal at 30 and lowest at 40°C, with a linear ($P = 0.043$, $r^2 = 0.787$) decrease in rates by 0.12 ± 0.02 g day^{-1} °C^{-1} between the 30 and 40°C set point treatments. Thus, the rate of biomass accumulation was clearly optimal at 30°C, in contrast to the dynamics of accumulation, which were clearly optimal at 35°C.

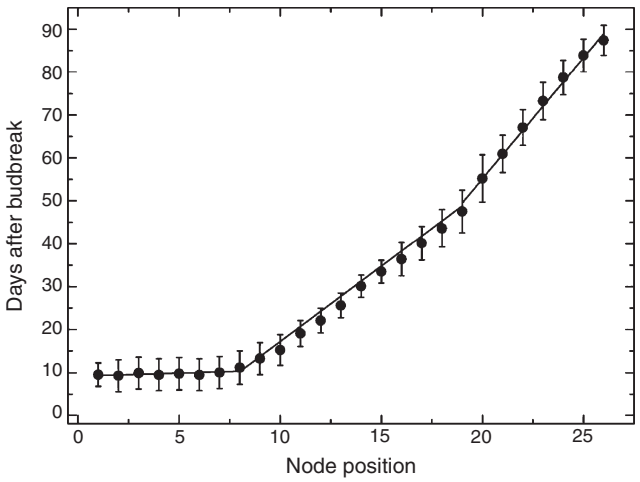

Fig. 6. Times of leaf appearance (mean ± s.e., $n = 40$) in days after budbreak at different node positions along the shoots of Semillon vines, averaged over all hydrocooling treatments. In each case, the lines are the best fit to a linear function and with the exception of that for nodes 1–7, the linear functions fitted to the data for nodes 8–19 and 20–26 were highly significant ($P < 0.001$, $r^2 = 0.99$).

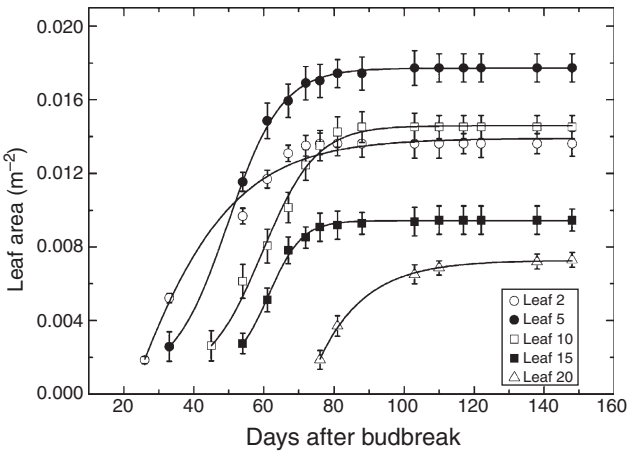

Fig. 7. Changes in leaf expansion (mean ± s.e., $n = 10$) of selected leaves along the shoots of Semillon vines as indicated. In each case, the lines are the fit to a Boltzmann sigmoid function and all highly significant ($P < 0.001$, $r^2 = 0.98$). The parameters of the fits to the function are shown in Table 1.

Table 1. Dynamics of leaf expansion of selected *Vitis vinifera* cv. Semillon leaves of vines grown in vineyard conditions as indicated

In each case, the Boltzmann sigmoid function was fitted to the expansion pattern as shown in Fig. 7. The exception is the expansion rate which was derived from a linear fit to these data from when the expansion was linear (equivalent to the duration of maximum growth). In each case, the linear functions were highly significant ($P < 0.001$, $r^2 = 0.904$–0.984). All the data are means ± s.e. ($n = 8$). Also shown is the probability (P) that the differences between treatments were significant. DAB, days after budbreak

Leaf	Maximum area (m^2)	Timing of maximum growth rate (DAB)	Duration of maximum growth (Days)	Expansion rate (cm^2 day^{-1})
2	0.0139 ± 0.0003	14.7 ± 2.5	15.1 ± 1.6	1.7 ± 0.2
5	0.0179 ± 0.0004	40.1 ± 3.4	11.6 ± 1.3	2.1 ± 0.3
10	0.0138 ± 0.0007	59.6 ± 3.5	8.3 ± 1.7	4.3 ± 0.3
15	0.0095 ± 0.0007	61.4 ± 4.9	7.8 ± 1.5	3.0 ± 0.2
20	0.0065 ± 0.0007	77.2 ± 3.5	8.1 ± 1.8	1.0 ± 0.4
P	<0.0001	0.032	0.045	0.0008

There were also some treatment differences in the total bunch biomass accumulated at harvest, the control and 30°C set point treatments averaged ~97 ± 2 g whereas in the other two treatments, the bunch final weights ranged from 80.5 ± 1.4 g to 73.5 ± 1.6 g, and these differences were significant ($P < 0.05$). Furthermore, there was a linear ($P < 0.05$, $r^2 = 0.927$) decrease in the final bunch DW at an average of 2.3 ± 0.4 g °C^{-1} between 30 and 40°C. As a proportion of the total biomass, the control and 30°C set point treatment vines had 77% of biomass as bunches whereas the 35°C treatment vines had 73% biomass as bunches and the 40°C set point treatment had 78% of the biomass as bunches. A slightly higher bunch allocation in this latter treatment occurred because of the much lower allocation to leaf biomass. These data certainly confirm that 30°C was optimal for the total bunch biomass accumulated and that the process was temperature dependent.

There were comparable changes in the dynamics of total shoot dry matter accumulation compared to those for the bunches and therefore not reported here. The rates of total shoot biomass accumulation ranged from 1.03 ± 0.02 to 2.28 ± 0.10, 1.18 ± 0.08 and 0.91 ± 0.03 g day^{-1} for the control, 30, 35 and 40°C treatments, respectively, with all regressions highly significant ($P < 0.001$; $r^2 = 0.995$). There were also significant ($P < 0.01$) treatment differences between all the set point temperature treatments, thus confirming that dry matter accumulation of the Semillon shoots was optimal at 30°C.

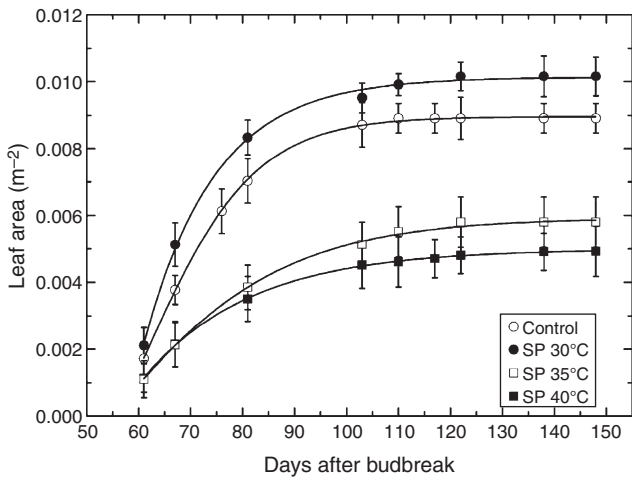

Fig. 8. Changes in the expansion across the growing season of the leaf at node position 18 (mean ± s.e., $n = 10$) along the shoot of Semillon vines in each of four treatments as indicated. In each case, the lines are the fit to a Boltzmann sigmoid function and all highly significant ($P < 0.001$, $r^2 = 0.98$). The parameters averaged over leaves 17–20 for each treatment of the fits to the function are shown in Table 2.

Sugar accumulation, berry and bunch attributes and yield

There were no treatment effects on berry ripeness and the total soluble solids concentration averaged 23.7 ± 3.8 °Brix across all treatments. However, the accumulation of sugar in the berries (Fig. 11) was significantly different ($P < 0.05$) from ~125 DAB in that the vines in the 30°C set point treatment had a significantly higher amount of sugar (402 ± 16 mg berry^{-1}) compared with the vines in the control and 35°C set point treatments and highly significantly different from the 40°C set point treatment, which had the lowest (328 ± 17 mg berry^{-1}) at harvest. In addition, there was a significant ($P = 0.0114$, $r^2 = 0.999$) linear relationship between berry sugar content and treatment temperature, with a slope of 7.4 ± 0.1 g °C^{-1}. In all cases, the sigmoidal function closely fitted the patterns of sugar accumulation and the attributes of the fit are shown in Table 4.There were no treatment differences in the timing of the maximum accumulation, which occurred at 120 DAB. However, there were significant treatment effects on the duration of maximum accumulation, with a significantly shorter duration for the 30°C set point treatment compared with the control and 40°C set point treatments but not significantly so with the 35°C set point treatment. Conforming to these differences, the rates of sugar accumulation were optimal in the 30°C set point treatment and significantly lower in both the 35 and 40°C set point treatments. Across these temperature treatments, the rates of berry sugar accumulation decreased in a linear ($P = 0.045$, $r^2 = 0.76$) pattern between 30 and 40°C, by an average of 0.33 ± 0.11 g day^{-1} °C^{-1}.

Berry FWs at harvest between the treatments were not significantly different and averaged 1.77 ± 0.11 g berry^{-1} but there were significant ($P = 0.0305$) treatment differences for the berry DWs, which averaged 404, 428, 404 and 352 ± 17 mg berry^{-1}, for the control, 30, 35 and 40°C set point treatments respectively. In contrast to the sugar content, the relationship between berry dry matter and treatment temperature was not significant. There were also no treatment effects on the berry diameters, which averaged 14.3 ± 0.09 mm.

Table 2. Average dynamics of leaf expansion of leaves 17–20 of *Vitis vinifera* cv. Semillon of vines grown in vineyard conditions

In each case, the Boltzmann sigmoidal function was fitted to the expansion pattern as shown in Fig. 8. The exception is the expansion rate which was derived from a linear fit to these data from when the expansion was linear. In each case, the linear functions were highly significant ($P < 0.01$, $r^2 = 0.924$–0.999). All the data are means ± s.e. ($n = 4$). Also shown is the probability (P) that the differences between treatments were significant. DAB, days after budbreak

Treatment	Final leaf area (m^2)	Timing of maximum growth (DAB)	Duration maximum growth rate (days)	Days to reach 20% expansion	Days to reach 80% expansion	Expansion rate (cm^2 day^{-1})
Control	0.0082 ± 0.0003	44.5 ± 2.1	11.9 ± 1.5	29.8 ± 1.1	62.8 ± 1.8	2.37 ± 0.04
30°C set Point	0.0107 ± 0.0009	46.1 ± 1.2	15.2 ± 1.9	25.2 ± 1.3	67.3 ± 1.6	2.68 ± 0.21
35°C set point	0.0065 ± 0.0006	52.6 ± 1.3	14.1 ± 1.5	33.9 ± 1.1	73.2 ± 1.8	0.96 ± 0.15
40°C set point	0.0044 ± 0.0006	68.1 ± 1.3	17.1 ± 1.5	29.5 ± 1.1	75.5 ± 1.8	0.83 ± 0.14
P	0.0001	0.020	0.0134	0.021	0.029	<0.02

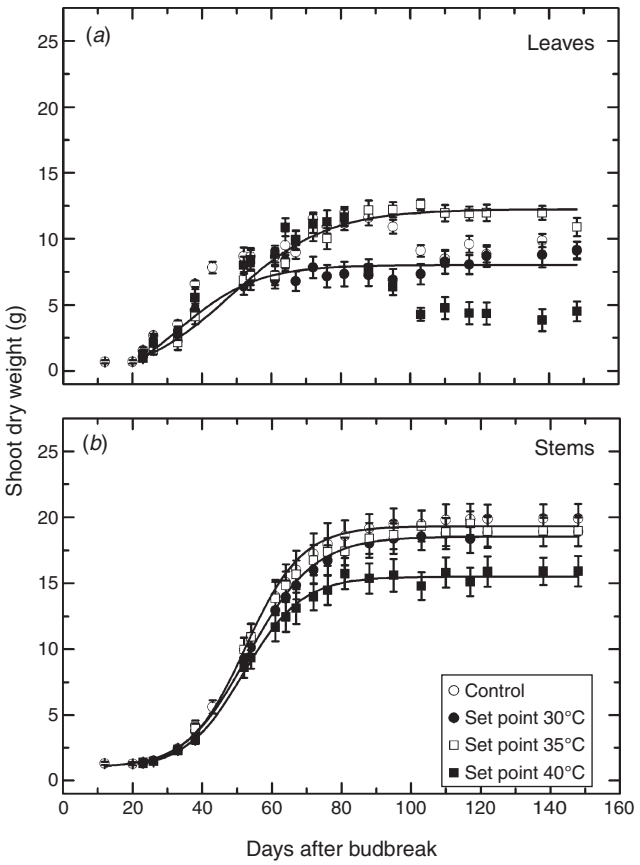

Fig. 9. (*a*, *b*) Changes in total leaf (*a*) and stem (*b*) DW (mean ± s.e., $n = 6$) across the growing season of shoots of Semillon vines in each of four treatments as indicated. In each case, the lines are the fit to a Boltzmann sigmoid function and all highly significant ($P < 0.001$, $r^2 = 0.98$). However, no lines were fitted to the leaf DW accumulation in the control and 40°C set point treatments as these did not follow sigmoidal kinetics. The differences in sigmoidal parameters were not significant (data not shown).

There were treatment effects on the FWs of the bunches (Table 5): bunch FW declined in a significantly linear ($P = 0.0466$, $r^2 = 0.978$) pattern from the 30°C treatment to the 40°C treatment at an average of 11 g °C^{-1}. There were also similar significant effects on bunch length and bunch volume as there was on the bunch DW (cf. Table 3). However, there was no treatment effect on the number of bunches per vine and similarly no effect on the yields per vine, which averaged 13.8 ± 0.9 kg vine^{-1} across all treatments.

Discussion

The hydrocooling system was designed to control the canopy temperature through a cycle of misting of the canopy and allow the latent heat of evaporation to cool the vine over a short drying time. It was evident that the system was generally able to control the canopy temperatures at the set points; however, across the growing season, the system was somewhat compromised when the heat load was large. This was, in part, because the finer water droplets tended to be blown away from the canopy by the wind, which is in contrast to the slightly larger droplets that were used in previous work (Greer and Weedon 2014*a*). This compromise was most apparent at the 30°C set point and when temperatures exceeded ~35–36°C, the hydrocooling control was only successful after a sustained cooling effect had occurred. Nevertheless, canopy temperatures were reduced well below air temperature (by 6–8°C) but were still above the set point. Similar differences between air and canopy temperatures were observed in several other grape cultivars when pulsed with water and drying (Gilbert *et al*. 1970; Kliewer and Schultz 1973). In contrast, the set point at 35°C in the present study was maintained proportionately more but was also compromised for similar reasons when the air temperatures were around 40°C. However, hydrocooling control at the set point of 40°C was successful in maintaining the temperature at 40°C, but this took only a small amount of hydrocooling capacity given that it was needed on only a few occasions.

In summary, across the warm part of the growing season (>80 DAB), the 30°C set point treatment maintained canopy temperatures for 27% of the days below 32°C and 60% below 34°C with an average maximum temperature of 31.3°C. The 35°C set point treatment maintained canopy temperatures below 37°C for 45% of days and below 39°C for 77% of days with an average maximum temperature of 32.7°C. For the set point at 40°C, 100% of days were below the set point at an average of 33.1°C, whereas air temperatures were above 40°C for 11% of the days. These data confirm the sophisticated hydrocooling control was able to keep the canopy temperatures to within 2–4°C of the set point for most of the time (Greer and Weedon 2014*a*). In contrast, the cooling system used by Aljibury *et al*. (1975) was highly effective at maintaining leaf temperatures of Semillon and Chenin Blanc vines at a threshold of 32°C across most of the day when air temperatures peaked at ~40°C. However, considerably more water was used in the earlier study compared with the present study. In other studies, hydrocooling reduced pear fruit temperatures by 5–7°C (Dussi *et al*. 1997), apple fruit temperatures by 4–9°C (Iglesias *et al*. 2002) and leaf temperatures of cranberry plants by 5–10°C (Pelletier *et al*. 2016), these being well in accordance with the present study.

It was evident that once the hydrocooling was occurring, there were marked effects of the different canopy temperatures on vegetative growth. Leaf area, especially for those leaves appearing and expanding in the mid growing season, was greatest on vines in the 30°C set point treatment and least on vines in the 40°C set point treatment. Those leaves on vines in the 35°C set point were closest in size to the 40°C leaves. This suggests that leaf expansion is highly sensitive to temperature and appeared to be optimal at ~30°C. This was confirmed when leaves at nodes 17 to 20 – known to appear and expand when the temperature control was active – were examined. The differences in leaf size across the treatments confirmed the conclusion above. Consistent with this, in terms of expansion dynamics, the 30°C leaves reached the time of maximum expansion significantly earlier, by ~16 days, than for those leaves in the 35 and 40°C treatments, but about the same as the control leaves. Furthermore, the maximum expansion rates were also highly temperature dependent: highest in the 30°C treatment and lowest in the 40°C treatment. There was curvilinear relationship between the rates and temperatures, with rates

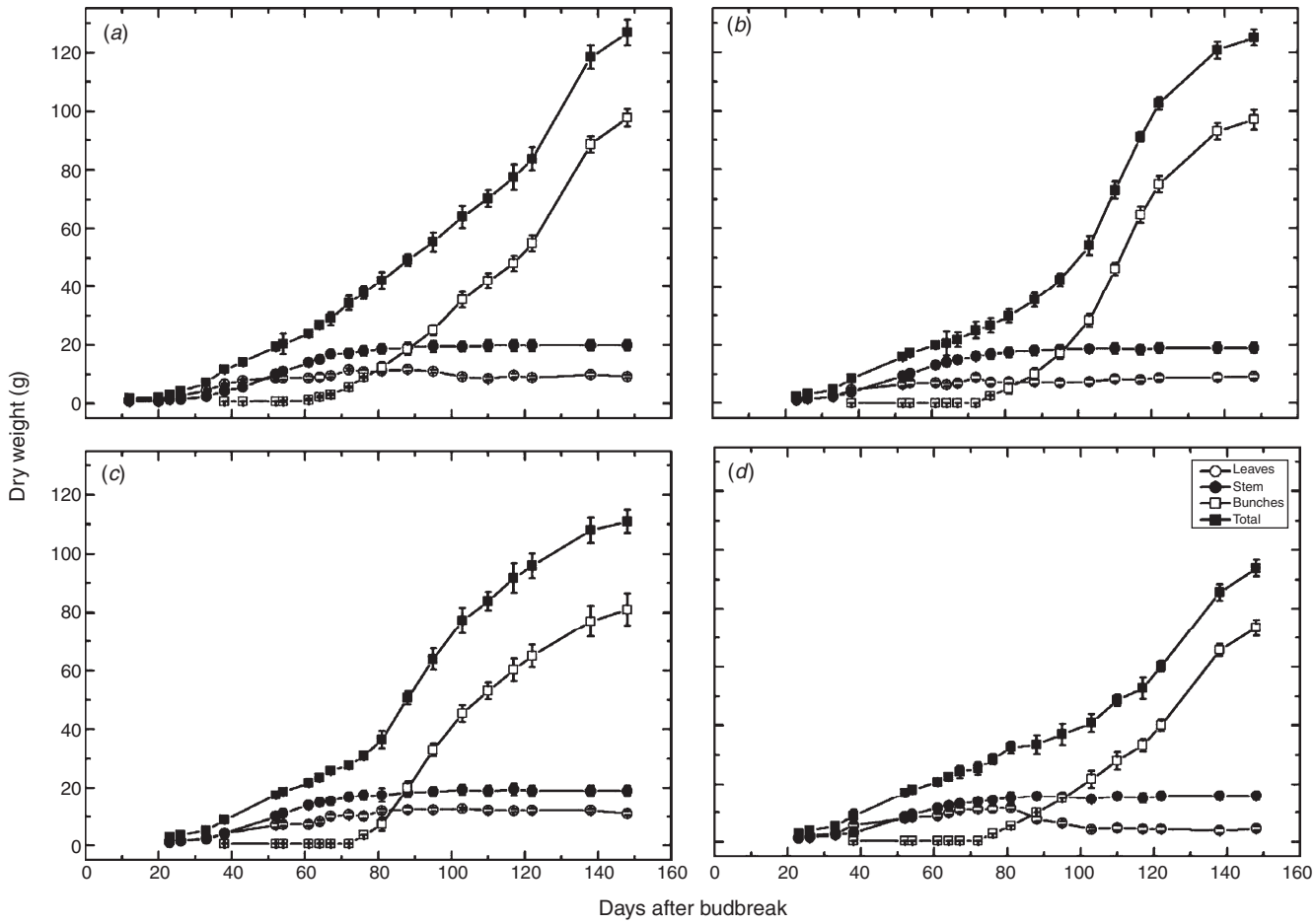

Fig. 10. (*a–d*) Changes in the dry matter content (mean ± s.e., $n = 6$) across the growing season of the different shoot components of Semillon vines in each of four treatments as indicated: (*a*) control treatment, (*b*) 30°C set point treatment, (*c*) 35°C set point treatment and (*d*) 40°C set point treatment. A Boltzmann sigmoid function was fitted to the bunch biomass accumulation (data not shown) and the parameters of the function are shown in Table 3.

Table 3. Dynamics of bunch dry matter accumulation of *Vitis vinifera* cv. Semillon of vines grown in vineyard conditions

In each case, the Boltzmann sigmoid function was fitted to the accumulation pattern as shown in Fig. 10. The exception is the accumulation rate, which was derived from a linear fit to these data from when the expansion was linear. In each case, the linear functions were highly significant ($P < 0.001$, $r^2 = 0.986$–0.996). All the data are means ± s.e. Also shown is the probability (P) that the differences between treatments were significant: ns, not significant

Treatment	Bunch weight (g)	Time of maximum growth (days)	Duration of maximum growth (days)	Days to reach 20% growth	Days to reach 80% growth	Rate of dry matter growth (g days^{-1})
Control	97.7 ± 3.8	119.6 ± 1.3	14.2 ± 0.2	99.9 ± 3.4	139.3 ± 4.5	1.08 ± 0.04
30°C set point	97.0 ± 3.6	113.5 ± 1.9	17.6 ± 0.9	89.1 ± 3.8	137.8 ± 3.8	2.18 ± 0.15
35°C set point	80.9 ± 3.5	97.2 ± 1.3	11.1 ± 0.8	81.8 ± 2.8	112.6 ± 3.1	1.21 ± 0.07
40°C set point	73.4 ± 2.6	108.4 ± 1.1	13.3 ± 0.9	89.9 ± 3.6	126.8 ± 3.7	0.88 ± 0.02
P	0.0066	0.045	ns	ns	0.0023	0.015

declining to a greater extent between 30 and 35°C than between 35 and 40°C. However, with the exception of the 40°C treatment, the differences between treatments in the time of maximum expansion were related less to the rate of expansion and more to the dynamics and an earlier time of appearance of the leaves in the 30°C treatment than in the other treatments. Leaves of both the control and 30°C treatments also finished expansion earlier than leaves of the other two treatments. Thus, the dynamics of leaf expansion were clearly temperature dependent, with an optimal temperature at 30°C.

It was notable for three grapevine cultivars; Semillon, Chardonnay and Chenin Blanc, that maintaining temperatures at or below 32°C had marked effects on shoot length, with 34, 18 and 57%, respectively, longer shoot lengths in cooled than in uncooled vines (Aljibury *et al*. 1975). There was, however, no such effect in the present study on the Semillon shoot lengths

(data not shown, but they were over five times longer than in the earlier study), as there was only an 8% difference between the 30 and 40°C treatments – these treatment differences being not significant. Similarly, it appeared in the study by Aljibury *et al.* (1975) that the shoot extension rates of the three cultivars were faster in cooled than uncooled vines. Again, in the present study, there were no treatment differences in any aspect of stem extension dynamics. It was most likely that shoot extension of the vines in the present study had largely been completed when the temperatures had started to increase, thus accounting for the small response to the differences in canopy temperature.

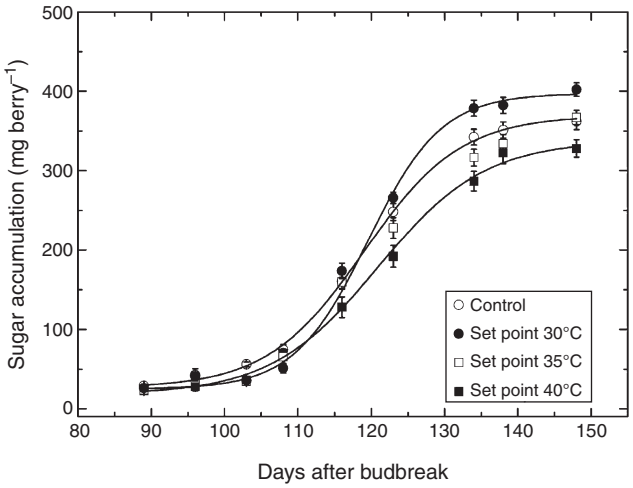

Fig. 11. Accumulation of sugar content (mean ± s.e., $n = 12$) across the growing season of berries on Semillon vines in each of four treatments as indicated. In each case, the lines are the fit to the Boltzmann sigmoid function and all highly significant ($P < 0.001$, $r^2 = 0.98$). The parameters of the fits to the function are shown in Table 4. The line for the 35°C set point treatment has been omitted for clarity.

The accumulation of dry matter, especially in the leaves, revealed a different pattern to that of expansion. The leaves of the 30°C treatment had stopped accumulating dry matter at a time when the leaves in all other treatments continued to accumulate dry matter, as was also shown by Greer and Weedon (2014*a*) for the same cultivar. Furthermore, there were no differences, at least initially, in dry matter accumulation between the other treatments, in that maximum rates of dry matter accumulation were not significant. However, from about when the temperature control was exerted, the dry matter of leaves in the 40°C treatment began to decline. This lasted for ~20 days when the dry matter content then became relatively steady. Consequently, these leaves had an obvious and significantly lower dry matter content than the leaves in the other treatments. It was not clear what caused this decline, but it is possible that the late emerging small leaves may have had a lower dry matter content compared with the larger leaves in the other treatments. A similar though smaller, decline in dry matter content also occurred in the control treatment, although this was not related to smaller leaves. Irrespective of this, it was clear that at harvest, leaf dry matter in the 35°C treated leaves had accumulated 33% more dry matter than the 30°C leaves and three times more than the 40°C leaves and, therefore, dry matter accumulation was optimal at 35°C. This suggests the leaf dry matter accumulation process was somewhat sensitive to temperature but also, and consistent with leaf expansion, that the 40°C treatment was detrimental to both processes.

Table 4. Dynamics of berry sugar accumulation of *Vitis vinifera* cv. Semillon of vines grown in vineyard conditions
In each case, the Boltzmann sigmoid function was fitted to the accumulation pattern as shown in Fig. 11. The exception is the accumulation rate which was derived from a linear fit to these data from when the expansion was linear. In each case, the linear functions were highly significant ($P < 0.002$, $r^2 = 0.986$–0.998). All the data are means ± s.e. Also shown is the probability (P) that the differences between treatments were significant: ns, not significant

Treatment	Maximum sugar content (mg berry^{-1})	Time of maximum growth (days)	Duration of maximum growth (days)	Rate of sugar accumulation (mg berry^{-1} day^{-1})
Control	362 ± 16	119.1 ± 0.9	6.65 ± 0.4	10.8 ± 0.5
30°C set point	402 ± 17	119.4 ± 0.8	4.91 ± 0.3	13.7 ± 0.4
35°C set point	367 ± 13	120.5 ± 1.4	5.72 ± 0.6	10.5 ± 0.3
40°C set point	328 ± 16	120.5 ± 1.4	7.13 ± 0.6	9.6 ± 0.1
P	0.041	ns	0.038	0.017

Table 5. Bunch attributes at harvest of *Vitis vinifera* cv. Semillon vines grown in vineyard conditions
All the data are means ± s.e. Also shown is the probability (P) that the differences between treatments were significant: ns, not significant

Treatment	Bunch FW (g)	Bunch volume (mL)	Bunch length (mm)	Berries bunch^{-1}	Bunches vine^{-1}	Yield (kg vine^{-1})
Control	240 ± 16	224 ± 24	148 ± 4	276 ± 22	50 ± 2	13.4 ± 0.9
30°C set point	315 ± 19	302 ± 26	151 ± 5	260 ± 18	51 ± 4	13.9 ± 0.8
35°C set point	251 ± 15	236 ± 24	130 ± 7	239 ± 25	48 ± 3	14.2 ± 0.7
40°C set point	205 ± 16	195 ± 23	133 ± 7	219 ± 20	55 ± 4	13.7 ± 1.0
P	0.043	0.034	0.016	ns	ns	ns

Compared with the leaves and to stem extension, there was yet a different response to temperature when considering stem dry matter accumulation. There were no differences between the control, 30 and 35°C treatments when shoot growth had stopped in the amounts of dry matter accumulated, averaging 18.9 ± 0.57 g. Consistent with this, there were no differences in the maximum rates of dry matter accumulation, varying from 0.389 ± 0.027 to 0.403 ± 0.010 g day^{-1}. However, consistent with many other aspects of vegetative growth, stem dry matter accumulation at 40°C was significantly reduced, by 21%. Though not significant, there was a 15% reduction in the maximum rate of dry matter accumulation in this treatment which at least conformed to the lower dry matter content of the stems. However, the rates of accumulation in the midsession (60 – 70 DAB) were significantly lower for the 40°C treatment than for the 30°C treatment and this would at least partially account for the lowest stem dry matter. By contrast, there were no effects of temperatures on the stem accumulation dynamics. This suggested the stem dry matter accumulation process was relatively insensitive to temperature compared with leaves and a broad optimum temperature from 30 to 35°C was apparent for this process. No differences in stem dry matter accumulation were observed between hydrocooled and control vines for this cultivar in the Greer and Weedon (2014*a*) study.

In contrast to the leaves and stems, which commenced growth well before the temperature control was exerted, the bunch dry matter accumulation process occurred entirely within the period of hydrocooling temperature control. Accordingly, there were marked temperature effects on the dry matter accumulation process in that at harvest, bunch dry matter at 30°C was highest and lowest for the 40°C treatment. However, in terms of accumulation dynamics, bunches on vines at 35°C reached the time of maximum accumulation significantly earlier, by 11 days, compared with bunches in the 30°C treatment and significantly delayed by 26 and 36 days in the control and 40°C treatments respectively. However, the duration of maximum accumulation was similar at 30 and 35°C at 10 days and longer at 20 days at 40°C and in the control vines. In contrast, the rates of dry matter accumulation were highest at 30°C with the rates significantly lower at 35°C and again so at 40°C. The rates for the control vines were similar to those at 35°C. This confirms that the bunch dry matter accumulation process was optimal at 30°C but also highly sensitive to temperature, with nearly 1 g day^{-1} °C^{-1} reduction in rates of dry matter accumulation between 30 and 40°C. This temperature response agrees with that observed for the maximum rate of berry dry matter accumulation for Semillon vines in the study by Greer and Weedon (2014*b*), although the optimum was at 35°C. In both studies, however, 40°C was too high for dry matter accumulation in berries and bunches of this cultivar, both in terms of rates and dynamics of accumulation.

Similar to results seen in the bunches, the total shoot biomass accumulation was higher in the 30°C vines than the 35°C vines, and the 40°C vines were lowest in total shoot biomass; the differences between the temperatures being significant. In addition, the rates of total biomass accumulation paralleled that of the bunches in that the highest rate occurred at 30°C and the lowest at 40°C, but given that the bunch biomass was 75–80% of the total, this result was not surprising. Thus, there was a clear effect of temperature on shoot biomass accumulation, with 30°C apparently optimal for the rate and amount of biomass accumulated. This is at odds with the leaves and stems, which were both generally optimal at 35°C, although the contributions of leaves and stems were relatively small, and especially so at 40°C.

At harvest, there were indications that the effects of the treatments had been carried over because there were marked treatment effects on several bunch attributes as alluded to above. Notably, bunch FW and bunch architecture were affected, with heavier and more open bunches apparent in the 30°C treatment compared with those in the 40°C treatment. As with the bunch DW, there was a linear decrease in the bunch FW between 30 and 40°C, indicating that this was also a temperature-dependent process, although this may be more a consequence of the bunch dry matter content driving the response. Increases in fruit size were also noted by Iglesias *et al*. (2002) and Gindaba and Wand (2005) for apples. Despite these treatment effects on the Semillon bunch weights, these did not translate to an effect on yield, partly because the numbers of bunches per vine were not affected but also the numbers of berries per bunch were not affected. However, the numbers of berries per bunch were highly variable and this could have attributed to the lack of difference in the yield between treatments. There were also no differences in berries per bunch or bunches per vine in the study of Greer and Weedon (2014*a*) but there was a significantly higher yield in hydrocooled than the control vines. In contrast, Gilbert *et al*. (1970) noted hydrocooling of Tokay vines tended towards a higher yield whereas for wine grape cultivars (not named), there was no effect on yield. However, yields were not determined in most of the other studies using hydrocooling so it remains uncertain how sensitive yield per vine is to temperature.

There was no effect of temperature on the early stages of sugar accumulation in the berries on the vines in the various treatments. However, from about the midpoint of the process, sugar accumulation at 30°C became increasingly and significantly higher than in all other treatments, and at harvest was significantly higher than at 35°C and significantly more than at 40°C. The berries on control vines had the lowest sugar content at harvest. Temperature had no effect on the time of maximum sugar accumulation but did affect the duration of maximum sugar accumulation. This was also evident from a significant effect on the maximum rates of sugar accumulation, with the highest at 30°C and significantly so compared with that at 35°C, with the lowest rate of sugar accumulation in bunches on vines in the 40°C treatment. These data generally conform to effects of temperature on the rates of sugar accumulation in the Semillon berries in the study by Greer and Weedon (2014*b*), except that the rates were maximal at 35°C in the former study. In addition, the rates of sugar accumulation were well in keeping with those reported by Greer and Weedon (2014*b*) and references cited therein. Tighter temperature control in the earlier study compared with that of the present Semillon study might account for the slight differences in temperature optima. However, in the present study, the rates of sugar accumulation were temperature dependent, changing ~0.33 mg day^{-1} for each °C change between 30 and 40°C. Although not presented in the earlier study, a comparable change in the rate of sugar

accumulation for Chardonnay grapes was 0.39 mg day^{-1} for each °C change in temperature from 25 to 40°C (DH Greer unpubl. data) thus, well in keeping with the present study. These data strongly support the conclusion that rate of the sugar accumulation process in grape berries is a highly temperature-dependent process and that 40°C is detrimental to the process. Consistent with this conclusion was the observation that soluble solids concentration was higher in hydrocooled 'Topred Delicious' apples than in control fruit (Iglesias *et al*. 2002) whereas for 'Cripps Pink' apples, hydrocooling appeared to reduce total soluble solids. A similar effect was noted by Aljibury *et al*. (1975) for three grape cultivars and also by Kliewer and Schultz (1973) for Cardinal and Carignane cultivars, whereas hydrocooling caused a significant reduction in total soluble solids in the White Riesling cultivar. These data suggest the benefits of cooling the fruit temperature to improve sugar accumulation may depend on the cultivar.

The FWs of Semillon berries in the different treatments at harvest were not significantly different. This contrasts with work reported by Aljibury *et al*. (1975) and by Greer and Weedon (2014*a*), where Semillon berry FWs were 20–25% higher in hydrocooled vines than control vines. It was also notable in the study by Aljibury *et al*. (1975) that hydrocooling caused an increase in berry FW of three grapevine cultivars, Semillon, Chenin Blanc and Chardonnay and over 2 years. Similarly, hydrocooling increased the berry FWs of White Riesling, Cardinal and Carignane vines (Kliewer and Schultz 1973). In contrast, in the present study there were significant treatment effects on berry DW, in keeping with the sugar content, as was also observed by Greer and Weedon (2014*a*) and also by Kliewer and Schultz (1973). Notably, however, there was no relationship between the berry dry matter content and temperature, suggesting this process is less sensitive temperature than the sugar accumulation process.

Conclusions

The hydrocooling system was able to control the canopy temperatures of Semillon vines across the growing season such that distinct differences in canopy temperatures occurred at or close to the set points. Evidence of the control was apparent from the statistically significant treatment effects on many aspects of vegetative and reproductive growth. The size of leaves, the dynamics of leaf expansion and the rates of expansion were all highly temperature dependent, with an optimum at 30°C. In contrast, stem extension and leaf and stem biomass accumulation were not strongly temperature dependent, but these processes were nevertheless detrimentally affected at 40°C. Further, the dynamics of bunch biomass accumulation were optimal at 35°C but the rates and total amounts of biomass accumulated and bunch FW were optimal at 30°C. The dynamics, rate of accumulation and amounts of sugar accumulated in the berries were all strongly temperature-dependent processes and, in all cases, optimal at 30°C. In contrast, yield per vine was not affected by the treatments and appeared to be insensitive to temperature. Therefore, for almost all vegetative and reproductive growth, there is a threshold temperature for most processes where some depreciation was evident at 35°C, and for all processes exposure to 40°C was distinctly detrimental. It is not clear if other cultivars have similar temperature sensitivities, but the likelihood of summer temperatures reaching above the threshold more often and more severely will most likely have a negative influence on vine performance. Thus, cost-effective strategies to maintain canopy temperatures below the threshold are needed to maintain vineyard productivity, and hydrocooling could be part of the solution, as has already been recognised in some commercial vineyards (Wallender *et al*. 2007).

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