



Research article

Modelling the seasonal changes in the gas exchange response to CO₂ in relation to short-term leaf temperature changes in *Vitis vinifera* cv. Shiraz grapevines grown in outdoor conditions

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ABSTRACT

Effects of temperature on the photosynthetic response of *Vitis vinifera* cv. Shiraz leaves to CO₂ were investigated across the growing season and modelling was used to determine relationships between photosynthesis and seasonal climate. Results indicated that photosynthetic rates declined from spring to summer, conforming to the deciduous habit of grapevines. Rates of ribulose 1,5-bisphosphate (RuBP) carboxylation and regeneration increased in a temperature dependent pattern throughout the season. However, the maximum rates decreased as the season progressed. There were also marked decreases in temperature sensitivity for each of these processes, consistent with the decreases occurring faster at high compared to low temperatures. There were no correlations between the seasonal climate and each of these photosynthetic processes but the effect of day was significant in all cases. CO₂ saturated rates of photosynthesis (A_{max}) across the season were highly correlated with the maximum rates of RuBP carboxylation and regeneration. The transition temperature between RuBP regeneration and RuBP carboxylation-limited assimilation varied across the growing season, from 23 °C in spring, 35 °C in mid-summer and 30 °C at harvest and were highly correlated with mean day temperature. This suggested dynamic control of assimilation by carboxylation and regeneration processes occurred in these grapevines in tune with the seasonal climate.

1. Introduction

The seasonal climate is a major driver of growth and development and also the photosynthetic capacity of deciduous perennial plants. The spring time is especially important for such species, because the entire canopy development is initiated by expansion and extension of preformed leaf primordia and internodes (Gerrath et al., 2001). In many deciduous horticultural plant species, there are a large number of preformed primordia, upwards of 15 for vines and over 20 for trees (Greer, 2018a) and the appearance of these leaves is highly temperature-dependent (Moncur et al., 1989). The resources to enable this early development are largely derived from carbohydrates mobilised from root reserves (Bennett et al., 2005). It is generally recognised that this heterotrophic stage of growth can last as much as 6 weeks before the canopy becomes fully autotrophic (Johnson and Lakso, 1986). Early season increases in photosynthesis are, therefore, clearly advantageous particularly for such deciduous species.

In support of this hypothesis, seasonal changes in grapevines, notably for cv. Chardonnay and cv. Merlot (Greer, 2017a), indicated the highest rates of light saturated (800–900 μmol (photons) $\text{m}^{-2} \text{s}^{-1}$)

photosynthesis indeed occurred in early spring, with rates of 8.2 and 10.3 $\mu\text{mol} \text{m}^{-2} \text{s}^{-1}$ and these rates declined continuously throughout the growing season. Similar results occurred cv. Kékfrankos (Zsófi et al., 2009) whereas for cv. Riesling vines, rates were lowest about 30 days after budbreak (DAB) and highest at harvest (Downton and Grant, 1992), although the measurement light intensity was not provided. However, light saturated (1000 μmol (photons) $\text{m}^{-2} \text{s}^{-1}$) photosynthesis of cv. Braeburn trees peaked in about mid-season, although rates shortly after full bloom averaged about 13.5 $\mu\text{mol} \text{m}^{-2} \text{s}^{-1}$ (Palmer et al., 2002). Similarly, peak light saturated (1800 μmol (photons) $\text{m}^{-2} \text{s}^{-1}$) photosynthesis of sweet cherry (*Prunus avium*) appeared to occur several months after full bloom (Quentin et al., 2013). Thus, there is some consensus that deciduous species mostly have the seasonally highest rates of photosynthesis soon after budbreak.

The corollary of this hypothesis was that evergreen trees should not require high rates of photosynthesis in spring. Indeed, this was shown for two Mediterranean species, *Quercus ilex* and *Phillyrea latifolia*, where photosynthetic rates in spring averaged about 4 $\mu\text{mol} \text{m}^{-2} \text{s}^{-1}$ whereas in summer and autumn, the rates averaged 6–7 $\mu\text{mol} \text{m}^{-2} \text{s}^{-1}$ (Ogaya and Peñuelas, 2003). Similarly, for the evergreen shrub *Pistacia*

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lentiscus, for a coastal population, light saturated rates did not vary between winter and spring at about $8 \mu\text{mol m}^{-2} \text{s}^{-1}$ but the rates increased in early summer to $13 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Flexas et al., 2001). For sweet orange (*Citrus sinensis*) across the year from May until April, light saturated rates were generally constant in the range from 6 to $12 \mu\text{mol m}^{-2} \text{s}^{-1}$, although there was a sustained light and temperature-driven decrease in rates in midwinter, but otherwise, there was no spring induced increase in photosynthesis (Nebauer et al., 2013). Thus, there is a general consensus that the photosynthetic rates of evergreen shrubs and trees do not increase specifically in the spring.

While photosynthesis over the growing season is largely driven by photon flux densities and temperatures that prevail during the season, there are a number of other factors that can influence the rates. The diffusion of the substrate CO_2 into the chloroplast is strongly regulated by the mesophyll and stomatal conductances, as indicated by the linear to curvilinear relationships between stomatal conductance and light saturated photosynthesis (Soar et al., 2009). Similarly, photosynthesis can be limited by the activity of Rubisco in fixing the CO_2 by the carboxylation of ribulose 1,5-bisphosphate (RuBP) but also by the electron transport rate which drives the regeneration of RuBP to enable the CO_2 fixation process to continue (von Caemmerer, 2013). There is increasing acceptance that at temperatures below about 30°C , photosynthesis at ambient CO_2 concentrations is limited by the maximum rates of RuBP regeneration while for temperatures above 30°C , photosynthesis is limited by the maximum rates of RuBP carboxylation (Greer and Weedon, 2012; Hikosaka et al., 1999; Yamori et al., 2010) although leaf nitrogen, growth conditions and genotype can influence the temperature-dependency (Yamori et al., 2011). However, Silva-Pérez et al. (2017) have suggested for wheat that carboxylation limited CO_2 assimilation occurred below 30°C and RuBP regeneration became the limitation at temperatures above. In addition, at very high temperatures, as occurs at the present location, Rubisco and/or Rubisco activase can be inactivated by heat stress (Salvucci and Crafts-Brandner, 2004), further reducing the photosynthetic capacity. Thus, there is a need when assessing the changes in seasonal photosynthesis to also examine the various stomatal and non-stomatal limitations to the process to fully comprehend the changes across the season.

Vitis vinifera cv. Shiraz is the most dominant grapevine cultivar grown across Australia, with about 39,000 ha and nearly 400,000 tonnes of Shiraz grapes produced (ABS, 2015). Despite this, relatively little is known about the photosynthetic capacity in relation to the seasonal climate. Rogiers et al. (2009), in a comparison of 10 common grapevine cultivars, on one occasion during the season determined that cv. Shiraz vines at $10.4 \mu\text{mol m}^{-2} \text{s}^{-1}$, had the lowest photosynthetic rate among the cultivars. Furthermore, photosynthetic rates of potted Shiraz vines, averaged between budbreak and fruit-set, gave a mean rate of $9 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Rogiers and Clarke, 2013). By contrast, light saturated rates of field-grown cv. Shiraz vines across the growing season, from about 60 days after anthesis, averaged $12 \mu\text{mol m}^{-2} \text{s}^{-1}$ but rates declined into late summer to about $3 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Caravia et al., 2016). Thereafter, the rates increased in early autumn. However, these apparent seasonal reductions in photosynthesis were largely attributable to high temperatures, as the measurements were conducted at $39.6\text{--}42.9^\circ\text{C}$, and, therefore, nothing could be inferred of the seasonal photosynthetic capacity. Over a restricted part of the growing season (fruit set to veraison), Soar et al. (2009) indicated that field grown vines had about constant rates during this period, at $15 \mu\text{mol m}^{-2} \text{s}^{-1}$. Similar rates also occurred for controlled environment-grown Shiraz vines (Hochberg et al., 2015). Thus, there were some indications of photosynthesis across the growing season of the cv. Shiraz grapevines but a comprehensive evaluation in this economically important grapevine cultivar is apparently missing.

Accordingly, the objective of the present study was to examine the seasonal changes in the photosynthetic response to CO_2 as a function of leaf temperature of cv. Shiraz vine leaves growing in out-door conditions. A second objective was to evaluate and model the stomatal and

non-stomatal limitations to the seasonality of photosynthesis.

2. Materials and methods

2.1. Plant material and growth conditions

This study was carried out at the National Wine and Grape Industry Centre plant growth facilities at Charles Sturt University (latitude 35.05°S and longitude 147.35°E , 212 m above sea level) in the Riverina, New South Wales, Australia over the 2017/18 growing season. Own-rooted 10 year-old *Vitis vinifera* cv. Shiraz vines were grown in 52 L pots in a commercial bulk composted potting mix and fertilised with liquid fertiliser (Megamix Plus, RUTEC, Tamworth, Australia). Additional applications of nitrogen (sulphate of ammonia, Richgro Garden Products, Jandakot, WA, Australia) were made at a rate of 100 g per vine at flowering time. The vines were grown in rows with 3 m spacing between rows and 1 m spacing between vines. There were 4 rows, each with 5 vines per row, and were all trained on a single wire cordon (east – west orientation) at a height of 0.8 m. A second foliage wire was set at 1.2 m above the ground. The shoots were not otherwise restrained and allowed to sprawl on either side of the cordon. The vines were grown in a wire framed bird exclusion cage but were otherwise exposed to the natural ambient conditions. The vines were watered four times daily at 10 min duration with an automatic irrigation system and water generally ran to runoff on each occasion. Bud break occurred in late September, flowering in mid November and the vines were harvested in early March (158 days after budbreak (DAB)). At harvest, there was average of 25 bunches vine⁻¹ with an average dry weight of $195 \pm 18 \text{ g vine}^{-1}$.

Screened air temperatures and humidities (Humitter, Vaisala, Helsinki, Finland) were measured at a height of 1.2 m above the ground and within 2 m of the grapevines rows. The data were logged at hourly intervals on a CR1000 data logger (Campbell Scientific, Logan, UT, USA) for each day of the growing season. Atmospheric vapour pressure deficits were determined from the measured air temperatures to calculate saturated vapour pressure using the Magnus formula (Junzeng et al., 2012) and from the measured relative humidities, the atmospheric vapour pressures and hence the vapour pressure deficits (VPD) were determined. Photon flux densities (PFDs) were also measured simultaneously using a quantum sensor (QSO, Apogee, Logan, UT, USA) located above the temperature screen.

2.2. Photosynthetic CO_2 responses

Gas exchange in response to internal CO_2 concentrations (A/c_i responses) at constant leaf temperatures ranging from 15 to 45°C were measured on leaves of randomly chosen vines on ten occasions across the growing season, starting in mid-October and finishing in late-February. The LI-6400 gas exchange system (Li-Cor Biosciences, Lincoln, NE, USA) fitted with the LI6400-40 leaf chamber fluorometer attached to the cuvette system was used for the measurements. On each occasion, fully expanded leaves were used, initially those near the inflorescence (nodes 4–5) and subsequently at higher node positions to ensure the measured leaves remained the youngest fully expanded leaves. For each CO_2 response, the PFD was set at $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ (known to be saturating for grapevines grown here (Greer, 2017b) and at the ambient CO_2 concentration ($400 \mu\text{mol mol}^{-1}$) and when rates were steady, the CO_2 was decreased progressively in selected steps of $50\text{--}100 \mu\text{mol mol}^{-1}$ to a CO_2 concentration of $50 \mu\text{mol mol}^{-1}$ before being increased back to $400 \mu\text{mol mol}^{-1}$ to ensure rates had fully recovered. Then the CO_2 concentration was increased in selected steps of $100\text{--}200 \mu\text{mol mol}^{-1}$ to $1600 \mu\text{mol mol}^{-1}$. The vapour pressure in the leaf chamber was maintained through a simple air humidifying system (for further details see Greer (2018b)). The procedure was repeated 2–3 times at each leaf temperature, with a new leaf for each CO_2 response. A total of 22–24 leaves were measured each day and these were selected

from 3 to 4 vines on each occasion. The measurements started with the lowest temperature at about 8 a.m. and finishing with the highest temperature at about 5 p.m., thus following the diurnal increase in air temperature as light was saturating at all times. Not all temperatures could be achieved on each occasion, especially at the lowest range, because of the prevailing ambient conditions. All responses were repeated over two consecutive days on each measurement occasion.

2.3. Data analysis

All data were analysed using a general linear model (GLM) approach using SAS 9.3 (SAS Institute Inc., Cary, NC, USA) and least squares means and standard errors determined. A fully randomised experimental design was used.

The fitting of the Farquhar et al. (1980) C_3 model of photosynthesis followed the procedure of Greer and Weedon (2012). The internal CO_2 concentrations (c_i) were first converted to chloroplast concentrations (c_c) using the electron transport rate determined by the simultaneous chlorophyll fluorescence and gas exchange measurements to estimate mesophyll conductance (g_m) according to Flexas et al. (2007). Subsequently, the maximum rate of Ribulose 1,5-Bisphosphate (RuBP) carboxylation (V_{cmax}) and the maximum rate of Ribulose 1,5-Bisphosphate regeneration (J_{max}) were calculated with SAS using the temperature-dependent coefficients for the Michaelis constants of CO_2 (K_c), oxygen (K_o) and the CO_2 compensation point in the absence of mitochondrial respiration (Γ^*) from Sharkey et al. (2007).

2.4. Model description

To model the photosynthetic responses to CO_2 across the different temperatures, the approach developed by Farquhar et al. (1980) and adopted by Greer and Weedon (2012) was used. In all cases, nonlinear regression analysis was used with SAS in an iterative process to calculate the maximum capacity of Rubisco, V_{cmax} , when CO_2 was limiting ($c_c < 200 \mu\text{mol mol}^{-1}$) and the maximum capacity of the electron transport rate, J_{max} , when CO_2 was saturating for each A/c_i curve, taking into account the temperature dependency of kinetic parameters of Rubisco according to Sharkey et al. (2007). Net assimilation rates, A are given as

$$A = \min(A_c, A_j) - R_d \quad (1)$$

where

$$A_c = \frac{V_{cmax}(C_i - \Gamma^*)}{C_i + K_c \left(1 + \frac{O_i}{K_o}\right)} \quad (2)$$

and

$$A_j = \left(\frac{J}{4}\right) \times \frac{(C_c - \Gamma^*)}{(C_c + 2\Gamma^*)} \quad (3)$$

Where A_c and A_j are the processes of photosynthesis limited by carboxylation and RuBP regeneration, respectively, K_c and K_o are the Michaelis-Menten constants for carboxylation and oxygenation, respectively, c_i and o_i are the intercellular concentrations of CO_2 and O_2 , respectively, and Γ^* is the CO_2 compensation point in the absence of mitochondrial respiration.

Non-linear regression analysis was also used to fit the following equation to the A/c_i curves to estimate mesophyll conductance, g_m according to the methods of Flexas et al. (2007) as;

$$g_m = A / (c_i - (\Gamma^*(J_{fl} + 8(A + R_d)) / (J_{fl} + 4(A + R_d)))) \quad (4)$$

Where A and c_i were taken from the A/c_i curves, Γ^* and R_d were estimated during the analysis of V_{cmax} and J_{max} and J_{fl} was measured with the concurrent fluorescence measurements. The estimated values of g_m were then used to convert all A/c_i curves to the chloroplast CO_2 concentration c_c , using the following equation;

$$c_c = c_i - (A / g_m) \quad (5)$$

The fitting of the C_3 model to the A/c_c data was carried out for all measurement occasions using non-linear regression of SAS following Greer and Weedon (2012). Non-linear regression was used in an iterative process to calculate the apparent maximum carboxylation capacity (V_{cmax}) and the rate of respiration in the light (R_d) when CO_2 was assumed to be limiting ($c_c < 200 \mu\text{mol mol}^{-1}$) and then the apparent maximum light saturated rate of RuBP regeneration (J_{max}) when CO_2 was assumed to be saturating ($c_c > 200 \mu\text{mol mol}^{-1}$) following Salim et al. (2010).

The chloroplast CO_2 concentrations ($C_{transition}$) at which the transition from carboxylation limited assimilation to RuBP regeneration limited assimilation occurred at each leaf temperature and on each occasion were determined according to Yamori et al. (2010). In addition, the chloroplast CO_2 concentration ($C_{ambient}$) was estimated for assimilation at the ambient concentrations ($400 \mu\text{mol mol}^{-1}$) for each temperature and on each occasion from the A/c_c data.

Additional modelling was carried out using the GLM procedure with SAS to explore relationships between the various photosynthetic attributes and the seasonal climate, including day and night temperature, vapour pressure deficits and daily maximum photon flux densities. PROC GLM enables specification any degree of interaction (crossed effects) and nested effects. It also provides for polynomial, continuous-by-class, and continuous-nesting-class effects. Through the concept of estimability, the GLM procedure can provide tests of hypotheses for the effects of a linear model regardless of the number of missing cells or the extent of confounding. PROC GLM displays the sum of squares (SS) associated with each hypothesis tested and, upon request, the form of the estimable functions employed in the test. PROC GLM can produce the general form of all estimable functions (SAS Institute Inc. 2008. SAS/STAT® 9.2 User's Guide. Cary, NC: SAS Institute Inc.).

3. Results

3.1. Climate over the growing season

The mean air temperatures during each day of the growing season (Fig. 1A) were initially in the mid 20–25 °C range but increased progressively to above 30 °C and remained at these high temperatures for most of the season. There were several occasions when the temperatures decreased to as low as 15 °C but these were relatively transient. Furthermore, maximum daily air temperatures exceeded 35 °C on about 20 days in mid-summer and were greater than 40 °C on 4–5 days (not shown). The night temperatures over the growing season were initially as low as 15 °C but also increased through the season and reached as high as 30 °C on the hotter days but were typically less than 25 °C for the most part.

Mean daily maximum photon flux densities, (Fig. 1B), averaged between 2 and 4 p.m. each day, were typically above about $1600 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the start of the season and gradually increased to about $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the mid-season (90 DAB) but thereafter declined, such that at harvest, were typically below about $1600 \mu\text{mol m}^{-2} \text{s}^{-1}$.

The mean atmospheric vapour pressure deficits during each day of the growing season (not shown) mostly ranged between 1 and 3 kPa but were as high as 4 kPa on the hotter days. However, towards the end of the growing season, the VPDs ranged between 2 and 3 kPa in concert with air temperature becoming steadier.

3.2. Response of gas exchange to leaf temperature

The light and CO_2 saturated rates (A_{max}) of photosynthesis (averaged over the whole growing season) as a function of leaf temperature (Fig. 2A) increased strongly from 15 °C, with the lowest rate at $16.6 \pm 1.0 \mu\text{mol m}^{-2} \text{s}^{-1}$, in a curvilinear pattern with a 1.5-fold

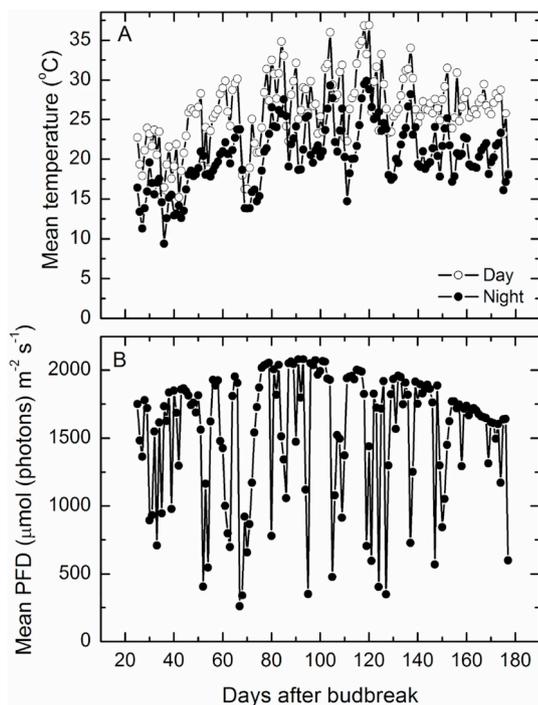


Fig. 1. Climatic data across the growing season. Seasonal changes in the (A) mean day and night temperatures as indicated and (B) daily maximum photon flux densities (PFD) measured in close proximity to the grapevines and all values are averages of hourly measurements. The PFDs were determined as the means between 2 and 4 p.m. each day.

higher rate at 30 °C. There was a broad optimal rate between 30 and 40 °C at $24.2 \pm 0.5 \mu\text{mol m}^{-2} \text{s}^{-1}$. There was a 30% decrease in the saturated photosynthetic rates at 45 °C below the maximum rate but not notably to a comparable rate to that at 20 °C, thus over the whole range, the lowest rates occurred at 15 °C.

The light-saturated photosynthetic rates at the ambient CO₂ concentration (A_{400}) were markedly lower than the CO₂ saturated rates, averaging between 5 and $11.7 \pm 0.2 \mu\text{mol m}^{-2} \text{s}^{-1}$, again with a broad optimum, but at 25–35 °C. It was notable that the lowest rates occurred at 45 °C, with rates more than 50% lower than at 30 °C. Although there were also lower rates at 15 °C, these were about 25% lower compared to those at 30 °C. Thus at ambient CO₂, light saturated photosynthetic rates were lowest at the high temperature range.

Mean rates of respiration in the light (Fig. 2B) as a function of leaf temperature increased in a curvilinear pattern, with only small differences between 15 and 25 °C ($0.72\text{--}0.93 \pm 0.08 \mu\text{mol m}^{-2} \text{s}^{-1}$) and a further 2.4-fold increase in rates between 25 and 45 °C to the highest rate at $2.29 \pm 0.07 \mu\text{mol m}^{-2} \text{s}^{-1}$.

By contrast, mean stomatal conductances (Fig. 2C) declined with increasing temperature, with the largest decrease between 15 and 20 °C. Thereafter, the conductances ranged between 150 and $175 \text{mmol m}^{-2} \text{s}^{-1}$ as the leaf temperature increased, but with only a slight (20%) decline overall. Also shown (Fig. 2C inset) are the mean leaf-to-air VPDs measured at each leaf temperature and these showed an exponential rise occurred with increasing leaf temperature, increasing from 15 to 45 °C by more than 8-fold. This was a much greater change than occurred with the stomatal conductance and it would seem unlikely that the decrease in conductance was attributable to the leaf-to-air VPD and more likely a response to leaf temperature.

However, in accordance with the exponential rise in leaf-to-air VPD, the mean rates of transpiration (Fig. 2D) also increased in an

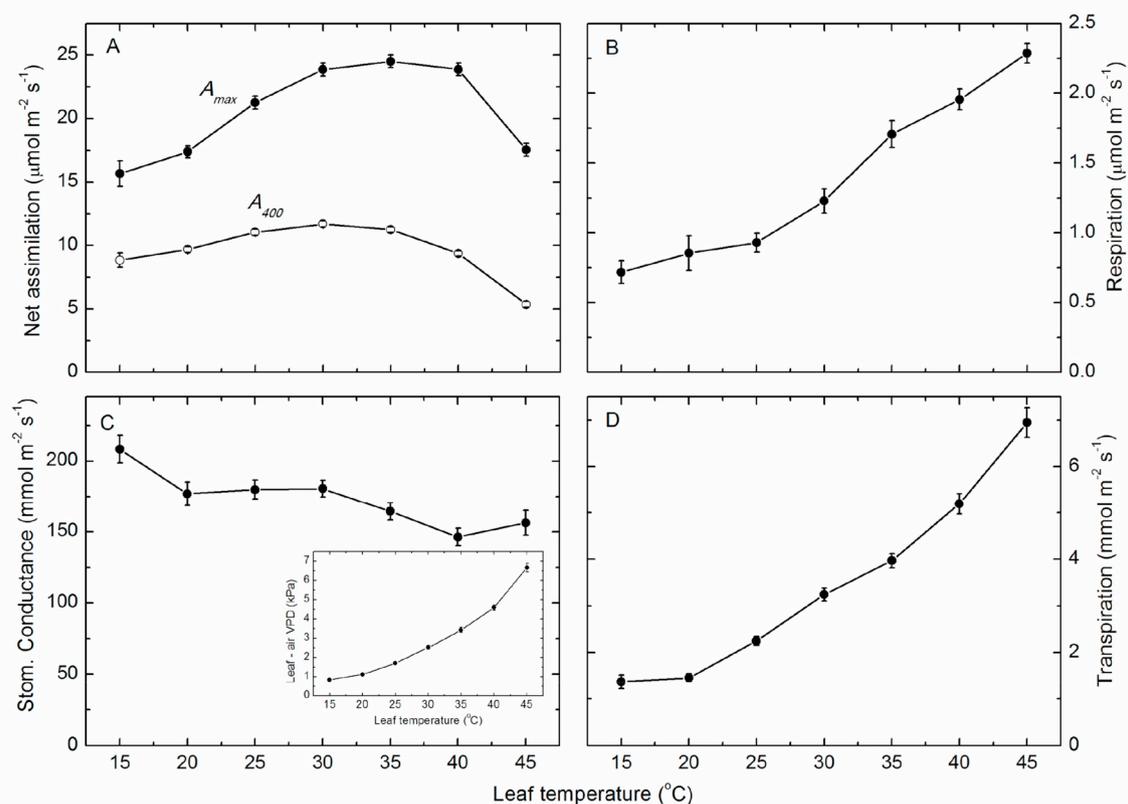


Fig. 2. Gas exchange responses to leaf temperature. Responses of the (A) rates of light saturated photosynthesis at ambient CO₂ (A_{400}) and at saturating CO₂ (A_{max}) concentrations, (B) rates of respiration in the light, (C) stomatal conductance and (D) transpiration as a function of leaf temperature. The inset in C is the mean leaf to air vapour pressure deficit (VPD) as function of leaf temperature. In all cases, these data are averaged over all days of measurement and are means \pm SE ($n = 30$).

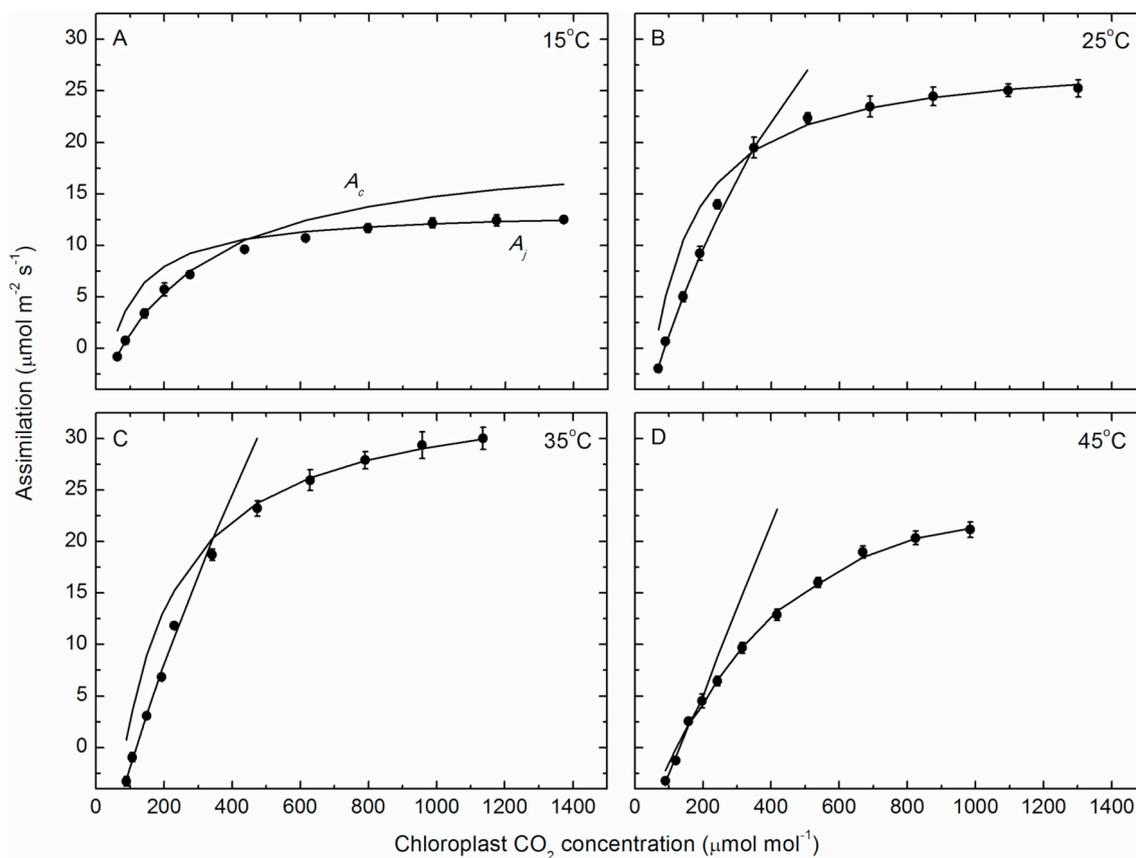


Fig. 3. Assimilation responses to CO₂ concentration. Examples of assimilation as a function of chloroplast CO₂ concentration (closed symbols) at selected leaf temperatures (mean \pm SE, $n = 6$) as indicated. Also included are the fits (solid lines) to the C₃ photosynthesis model (Farquhar et al., 1980), including the apparent rates of RuBP carboxylation (A_c) and RuBP regeneration (A_r) as function of the CO₂ concentration. Rates of RuBP carboxylation were determined at $c_c < 200 \mu\text{mol mol}^{-1}$ CO₂ and rates of RuBP regeneration were determined at CO₂ concentrations above.

exponential pattern but this also reflected the fact that the stomatal conductance was relatively constant over the range of temperatures. Between 15 and 45 °C, rates of transpiration increased from $1.37 \pm 0.15 \text{ mmol m}^{-2} \text{ s}^{-1}$ to $6.95 \pm 0.32 \text{ mmol m}^{-2} \text{ s}^{-1}$, therefore, a 5-fold increase in rates.

3.3. A/c_c responses to leaf temperature

Examples of the photosynthetic response to the chloroplast CO₂ concentration at selected leaf temperatures, along with the fits to the C₃ photosynthetic model, are shown in Fig. 3. In all cases, the model fitted the data extremely well ($P < 0.001$, $r^2 = 0.97\text{--}0.99$). In accordance with Fig. 2, the rates were lowest at 15 °C and highest at 35 °C. At temperatures at and below 35 °C, there were clear distinctions in the carboxylation limitation at chloroplast concentrations below about $300 \mu\text{mol mol}^{-1}$ and an RuBP regeneration limitation at concentrations above this, whereas at 45 °C, both limitations were apparent at the low CO₂ range. Notably, this did not occur at 40 °C (not shown). It was also apparent that the CO₂ saturated photosynthetic rates did not decline at the high CO₂ concentrations and, thus, a triose phosphate limitation was not invoked in this modelling approach.

3.4. Seasonal changes in A/c_c temperature responses

There were changes in the temperature-dependency of light and CO₂ saturated photosynthetic rates as well as shifts in the optimum temperature at the selected occasions as the growing season progressed (Fig. 4). The A_{max} rates were overall highest at the beginning of the growing season, ranging from 18.9 ± 1.05 to $28.8 \pm 1.22 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and there was a marked bias in the response toward the higher

temperatures.

In addition, there was a clear optimum at 30 °C. At the next occasion (72 DAB), the optimum for A_{max} remained at 30 °C and the maximum rates were largely unchanged, especially at the higher temperatures. However, a difference occurred at the low temperature range, where the A_{max} rates were from 17 to 35% lower than earlier in spring. Notably, the difference was extended as the temperature declined, suggesting that the vines were less tolerant of the low temperatures at the stage of development (berry growth) compared to when inflorescences were developing.

The A_{max} rates continued to decline later into the growing season (mid-summer, 101 DAB) across all temperatures, but the change was greatest at both 30 and 45 °C. In part, this occurred because there was a shift in the optimum to 35 °C and A_{max} averaged $24.8 \pm 0.7 \mu\text{mol m}^{-2} \text{ s}^{-1}$, equivalent to a 14% decrease in the maximum rates since spring (29 DAB). This trend continued through to just before harvest time (158 DAB), where the A_{max} rates had universally decreased at all leaf temperatures and the optimum had shifted to 40 °C, although the response to temperature was flatter than at any other stage of the growing season. The maximum rates were reduced to $18.1 \pm 0.6 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and nearly 30% below those rates at 101 DAB. It was also notable that the differences between the last two measurement occasions were most evident at 30 °C and least apparent at 20 and 45 °C.

By contrast, the photosynthetic rates at ambient conditions (Fig. 4B) changed relatively little between spring and midsummer and the pattern of response to temperature was generally similar. However, there were slight changes in the optimum temperature, which shifted from 25 °C at 29 DAB to 30 °C at 72 DAB, but remained at 30 °C for the rest of the growing season, although the optimum was relatively broad. There was, however, a marked depreciation in photosynthetic rates just before

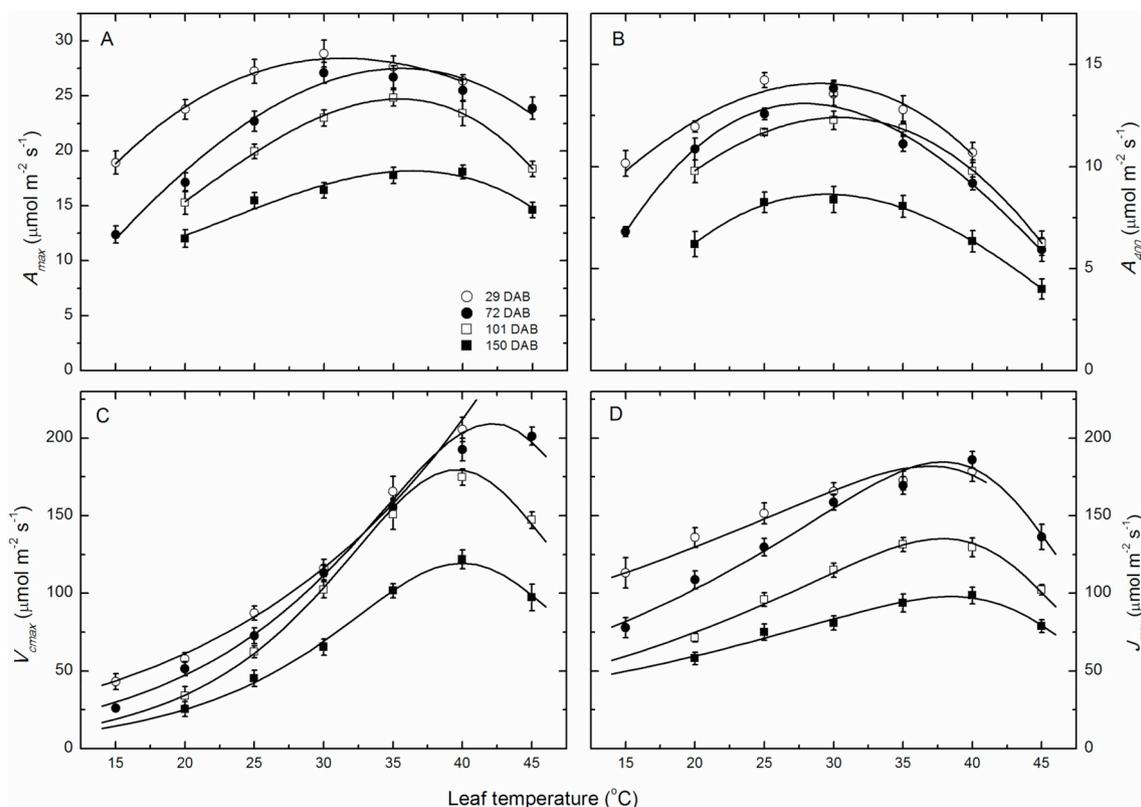


Fig. 4. Assimilation responses to leaf temperature. Changes in the responses of (A) the rates of light saturated photosynthesis at saturating CO_2 (A_{max}) concentrations and (B) at ambient CO_2 (A_{400}) as a function of leaf temperature at selected days of the growing season, as indicated. Included were (C), the apparent maximum rates of ribulose 1,5-bisphosphate (RuBP) carboxylation (V_{cmax}) and (D), the apparent maximum rates of electron transport driving RuBP regeneration (J_{max}) as a function of leaf temperature. For the A_{max} and A_{400} data, the lines are the best fit to a cubic polynomial ($r^2 = 0.87\text{--}0.99$; $P < 0.01$) whereas for V_{cmax} and J_{max} , the lines are a fit to the Arrhenius function according to (Medlyn et al., 2002a) with a term for deactivation at high temperatures included. All data are means \pm SE, ($n = 12$). Note, not all temperatures could be measured at all times.

harvest time, averaging a 33% decrease across all leaf temperatures compared to the rates in mid-summer, in accord with the CO_2 saturated rates.

Apparent maximum rates of RuBP carboxylation (Fig. 4C) followed an exponential pattern as a function of leaf temperature on all occasions. However, in spring and early summer, there were no marked differences in rates at any temperature and the maximum V_{cmax} rates of $200 \pm 6 \mu\text{mol m}^{-2} \text{s}^{-1}$ occurred at least as high as 45°C , although an optimum was apparent at 42°C in early summer. By contrast, in mid-summer, although the V_{cmax} rates at most temperatures were generally lower than those occurring earlier in the season, the exception was at 35°C where the rates were all similar while at 40 and 45°C the rates had decreased, most evidently at 45°C , by 27%. Thus, there was a clear optimum at about 40°C at this time. This trend of decreasing rates was extended to all temperatures at about harvest time, where the V_{cmax} rates declined increasingly more as the temperature increased compared to the rates at mid-summer. Thus at the optimum at 40°C , the rates declined from $174.9 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$ to $121.8 \pm 6 \mu\text{mol m}^{-2} \text{s}^{-1}$, a 30% decrease. These changes in V_{cmax} rates and the temperature response were well in accord with the changes occurring with assimilation late in the season.

The Arrhenius function fitted to these data indicated that the apparent maximum rate of RuBP carboxylation at 25°C , k_{25} (Table 1) decreased significantly throughout the growing season while the activation energy, H_a , was initially stable but increased significantly in mid-summer and remained higher thereafter. There were only slight changes in the deactivation energy, H_d .

The response of the maximum rates of RuBP regeneration to leaf temperature (Fig. 4D) differed markedly compared with that for carboxylation. In spring, the J_{max} rates followed a more curvilinear

response to temperature, most responsive at the lower temperature range and least responsive at the upper range. The maximum J_{max} rates of $178.6 \pm 6.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ appeared to occur at about 37°C . The pattern of response of J_{max} to temperature in early summer was exponential but there was a marked decrease in rates across all temperature below 30°C . For example at 25°C , the J_{max} rates declined from 157.5 ± 7 to $129.7 \pm 6 \mu\text{mol m}^{-2} \text{s}^{-1}$. Most notable, however, was the rates at the higher temperatures were comparable between the two occasions. This trend of decreasing rates continued to mid-summer, when there was a universal and marked decrease in J_{max} rates across all leaf temperatures, from 22 to 37% and again near to harvest, when the rates declined again by a further 19–29%. Thus, from the peak at summer to the minimum at harvest, the maximum J_{max} rates declined from $186.1 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$ to $98.6 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$, a 47% decrease. However, the optimum temperature for the maximum rates of RuBP regeneration remained at about 37°C throughout. These late seasonal induced changes in J_{max} were in accord with the reduction in assimilation rates.

The Arrhenius function fitted to these J_{max} data indicated that the maximum rate of RuBP regeneration at 25°C , k_{25} (Table 1) decreased significantly throughout the growing season while the activation energy, H_a , initially increased significantly from spring to early summer but thereafter remained steady. There were only slight changes in the deactivation energy, H_d .

3.5. Changes in assimilation across the whole season

Despite shifting progressively along the shoots to measure the youngest fully expanded leaves, there were sustained decreases in light and CO_2 saturated photosynthesis (A_{max}) across the growing season at

Table 1

Attributes (mean \pm SE, $n = 6$) of fitting the Arrhenius function to the apparent rates of ribulose 1,5-bisphosphate (RuBP) carboxylation (V_{cmax}) and RuBP regeneration (J_{max}) as a function of leaf temperature and determined on selected days of the growing season (cf. Fig. 4). The Arrhenius attributes include the maximum rate at 25 °C (k_{25}), the activation energy (H_a) and the deactivation energy (H_d) see Medlyn et al. (2002a). Also included is the probability (P) that the differences across the season were significant from the GLM fitting to all data over the growing season.

DAB	V_{cmax}			J_{max}		
	k_{25} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	H_a (kJ mol^{-1})	H_d (kJ mol^{-1})	k_{25} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	H_a (kJ mol^{-1})	H_d (kJ mol^{-1})
29	83.2 \pm 3.4	64.4 \pm 5.7	205.5 \pm 0.9	148.0 \pm 4.8	19.4 \pm 4.2	207.3 \pm 1.7
72	73.6 \pm 4.3	64.5 \pm 5.7	206.9 \pm 0.7	127.4 \pm 4.6	31.9 \pm 4.1	206.5 \pm 0.7
101	61.3 \pm 2.5	85.1 \pm 5.5	204.0 \pm 0.4	95.9 \pm 1.9	32.3 \pm 2.6	206.4 \pm 0.3
150	42.6 \pm 1.9	77.6 \pm 5.36	204.7 \pm 0.5	71.0 \pm 1.7	25.6 \pm 2.8	207.6 \pm 0.5
P	< 0.05	< 0.05	< 0.01	< 0.01	< 0.05	< 0.01

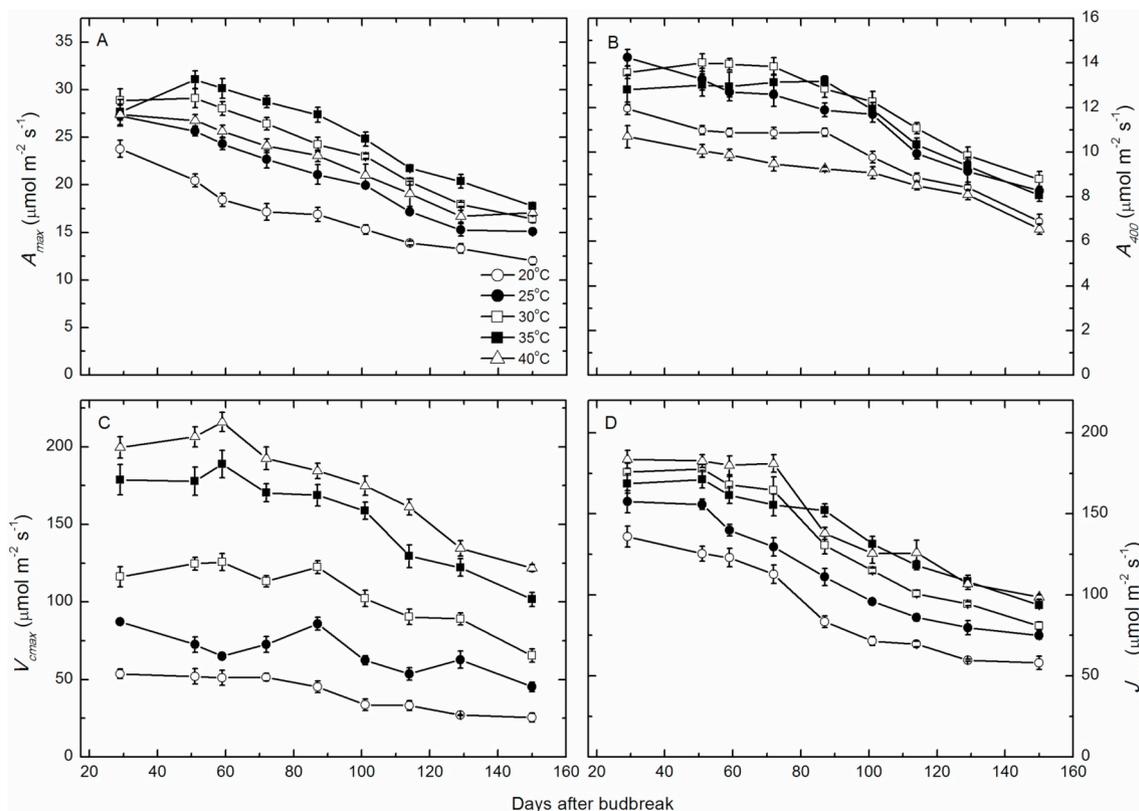


Fig. 5. Seasonal changes in assimilation. Changes in (A) the rates of light saturated photosynthesis at saturating CO_2 (A_{max}) concentrations and (B) at ambient CO_2 (A_{400}) across the growing season at selected leaf temperatures, as indicated. Also included are changes in (C) estimated maximum rates of ribulose 1,5-bisphosphate (RuBP) carboxylation (V_{cmax}) and (D) estimated rates of RuBP regeneration (J_{max}) across the growing season at selected leaf temperatures. All data are means \pm SE, ($n = 12$). For comparison, A_{max} at 20 °C, a linear regression fitted to the change between 51 and 151 DAB had a slope of $-0.079 \pm 0.0076 \mu\text{mol m}^{-2} \text{s}^{-1} \text{day}^{-1}$ ($r^2 = 0.938$, $P < 0.001$) while at 35 °C, the slope was $-0.131 \pm 0.0071 \mu\text{mol m}^{-2} \text{s}^{-1} \text{day}^{-1}$ ($r^2 = 0.979$, $P < 0.001$) and similarly for A_{400} , the respective slopes were $-0.059 \pm 0.0056 \mu\text{mol m}^{-2} \text{s}^{-1} \text{day}^{-1}$ ($r^2 = 0.964$, $P < 0.01$) and (for 30 °C) $-0.067 \pm 0.0042 \mu\text{mol m}^{-2} \text{s}^{-1} \text{day}^{-1}$ ($r^2 = 0.984$, $P < 0.001$), confirming the higher rates of decrease in photosynthesis at the higher temperatures.

all leaf temperatures (Fig. 5A). Also apparent was the decrease in temperature sensitivity across the season, for example at 51 DAB, rates declined from $31.1 \pm 0.9 \mu\text{mol m}^{-2} \text{s}^{-1}$ at 35 °C to $20.5 \pm 0.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ at 20 °C, a rate of decrease of $0.7 \mu\text{mol m}^{-2} \text{s}^{-1} \text{ } ^\circ\text{C}^{-1}$. For the same temperature range at 150 DAB, the A_{max} rates declined from $17.8 \pm 0.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ to $12.0 \pm 0.4 \mu\text{mol m}^{-2} \text{s}^{-1}$, a rate of decrease of $0.39 \mu\text{mol m}^{-2} \text{s}^{-1} \text{ } ^\circ\text{C}^{-1}$, almost half the sensitivity that occurred in spring. Modelling of these data using the GLM procedure indicated that 90% of the variance could be explained by a day effect and the measurement temperature ($P < 0.001$) but no seasonal temperature, PFD or stomatal conductance effects were significant.

There was a similar seasonal trend with photosynthesis at ambient CO_2 concentrations (Fig. 5B), except that the A_{400} rates were relatively

stable through to early summer (~ 90 DAB) and declined markedly after that, again at all leaf temperatures. In addition, there was the same decrease in temperature sensitivity, from $0.31 \mu\text{mol m}^{-2} \text{s}^{-1} \text{ } ^\circ\text{C}^{-1}$ at 51 DAB to $0.19 \mu\text{mol m}^{-2} \text{s}^{-1} \text{ } ^\circ\text{C}^{-1}$ at 150 DAB. Although the rates were consistently lowest at 40 °C throughout the season, the temperature for the maximum rates varied somewhat between 25 and 35 °C but most commonly at 30 °C. GLM modelling of these data indicated that 93% of the variance was explained by the day and stomatal conductance ($P < 0.001$) but no other seasonal effect or the measurement temperature had significant effects.

There were clear and marked seasonal differences in maximum rates of RuBP carboxylation at the different temperatures, with the lowest rates at 20 °C and the highest rates at 40 °C. Although there were decreases in the rates across the growing season and evidence of a

reduction in temperature sensitivity, the seasonal decrease was somewhat temperature-dependent. For example, at 20 °C the maximum rates declined by 47% between 51 and 150 DAB while at 40 °C, the rates declined by 58%. However, the temperature sensitivity at 51 DAB averaged $7.7 \mu\text{mol m}^{-2} \text{s}^{-1} \text{ } ^\circ\text{C}^{-1}$ while at 150 DAB, the rate of decrease averaged $4.8 \mu\text{mol m}^{-2} \text{s}^{-1} \text{ } ^\circ\text{C}^{-1}$, an almost 40% decrease. There were no correlations between V_{cmax} and the seasonal climate, however, 91% of the variance could be explained by the day and measurement temperature ($P < 0.001$).

Although the maximum rates of RuBP regeneration declined across the season (Fig. 5D), the temperature sensitivity was less affected in comparison with that for V_{cmax} . For example at 51 DAB, the rate of decrease in J_{max} between 20 and 40 °C averaged $2.9 \mu\text{mol m}^{-2} \text{s}^{-1} \text{ } ^\circ\text{C}^{-1}$ whereas at 150 DAB, the comparable rate was $2.0 \mu\text{mol m}^{-2} \text{s}^{-1} \text{ } ^\circ\text{C}^{-1}$, a 30% decrease. However, the changes in J_{max} across the season were comparable with that for V_{cmax} with a 46% decrease at 20 °C and a 53% decrease at 40 °C. It was notable that across the season, the highest J_{max} rates occurred at 40 °C early on but later varied between 35 and 40 °C. GLM modelling the seasonal changes in J_{max} for all data indicated no seasonal climate effect was significant but there was a highly significant effect of the day and measurement temperature ($P < 0.001$), which accounted for 77% of the variance.

Similar modelling of maximum light and CO₂-saturated assimilation across the growing season indicated that the day, stomatal conductance, maximum rates of RuBP carboxylation and the maximum rate of RuBP regeneration could significantly ($P < 0.001$) account for 97.6% of the A_{max} variance and notably, the measurement temperature was not significant in the model.

3.6. Seasonal shifts in transition temperature between RuBP carboxylation and regeneration

The shift from carboxylation-limited (A_c) assimilation to RuBP regeneration-limited assimilation (A_j) with increasing chloroplast CO₂ concentration (cf. Fig. 3) and the effect of leaf temperature on this transition is shown in Fig. 6.

Also included is the chloroplast CO₂ concentration for assimilation measured at ambient ($400 \mu\text{mol mol}^{-1}$) CO₂ concentrations and high light intensities for each temperature on selected days of the growing season. A_j limited assimilation when $C_{ambient}$ was greater than $C_{transition}$ and A_c limited assimilation when $C_{transition}$ was greater than $C_{ambient}$ (cf. Yamori et al., 2010). Thus on each occasion, between 15 and about 30 °C, $C_{ambient}$ was higher than $C_{transition}$ and suggested assimilation was strongly limited by RuBP regeneration at the lower temperatures whereas above about 30 °C, $C_{transition}$ was increasingly higher than $C_{ambient}$ and assimilation was strongly limited by RuBP carboxylation at the higher temperatures. Early in the growing season, this transition temperature actually occurred between 27.5 and 28 °C but later in the growing season (101 DAB), the transition temperature increased to 34 °C but decreased to 30 °C just before harvest. This change in the transition temperature across the whole growing season is shown more generally in Fig. 7.

This indicated that the temperature switch from regeneration-limited to carboxylation limited-assimilation occurred initially at relatively low temperatures (23–27 °C) in spring but increased over midsummer (87–130 DAB) to between 34 and 35 °C and then declined again to 30 °C in late summer. This pattern of changes in the transition temperature generally reflected the change in mean air temperatures that occurred across the growing season (cf. Fig. 1).

4. Discussion

4.1. Photosynthetic response to leaf temperature

The climate across the growing season for the *Vitis vinifera* cv. Shiraz vines was typical for the region, where air temperatures commonly

exceeded 30 °C and mean day temperatures frequently exceeded 35 °C and over 40 °C on several occasions. These temperatures were consistent with the long-term mean maximum temperatures of the summer months (Dec–Feb) at 29.4, 31.7 and 30.8 °C (Greer, 2015). The site was also characterised by relatively dry atmospheric conditions, with mean vapour pressure deficits typically above 3 kPa and as high as 4 kPa on occasions. Similar high temperatures and VPDs have been shown for other vineyards (Zsófi et al., 2009) and these conditions were known to impact on grapevine performance. For example, leaf and shoot growth rates, and reproductive development were impacted on by these high temperatures (Greer and Weedon, 2016) and generally have a greater depreciation in rates towards lower temperatures than towards high temperatures (Kriedemann, 1968), thus, indicated these species were generally adapted to these recurrent high temperatures.

For the Shiraz vines in the present study, the photosynthetic response to temperature was entirely typical for the hot climate, in that the optimum temperature at ambient growth conditions ranged between 30 and 35 °C. This compared with cool grown Riesling vines, where the optimum temperature for light saturated photosynthesis was about 27 °C and the rates declined by 17 and 35% at 32 and 35 °C, respectively, (Schultz, 2003). For the cv. Shiraz vines, however, there appeared to be greater depreciation in rates of photosynthesis at high (54% reduction at 45 °C) compared to low temperatures (24% reduction at 15 °C). A similar effect occurred with cv. Chardonnay and cv. Merlot vines (Greer, 2017b). Although also grown in a hot climate, cv. Sultana leaves had a 12% reduction in photosynthetic rates at 15 °C but 37 and 77% reductions at 40 and 45 °C, respectively (Kriedemann, 1968) and Higgins et al. (1992) reported 100% inhibition for cv. Thompson Seedless vines. It was possible that genotypic effects might have explained the greater high temperature inhibition of photosynthesis in cv. Sultana and cv. Thompson Seedless leaves in comparison to some other grape cultivars. However, these effects on photosynthesis at high temperatures most likely occurred because of increased respiration (see Fig. 2) as well as from increased photorespiration (Osmond, 1981). Further support for this conclusion came from the photosynthetic response to temperature when CO₂ was saturated, that is when photorespiration would be suppressed, in that the A_{max} rates increased over all temperatures, but increasingly so with higher temperatures. In addition, the optimum temperature shifted upwards to 35–40 °C and the depreciation at 45 °C was reduced compared to the reduction at 15 °C. Similar shifts in both the optimum temperature and the increased photosynthetic rates occurred with cv. Semillon (Greer and Weedon, 2012) and cv. Chardonnay and cv. Merlot (Greer, 2017b), when measured at elevated CO₂. It was clear, therefore, that the photosynthetic temperature responses of these grapevine cultivars at the ambient growth conditions were strongly CO₂ limited, certainly stomatal and mesophyll conductances contributed, and this appeared to restrict the acclimation potential in contrast to when CO₂ was saturating.

However, it was apparent that the temperature-dependency of photosynthesis of the Shiraz vines varied across the growing season, both in rates and patterns of response. Early in the season, the rates measured at ambient conditions were maximal at 25 °C and rates were reduced about equally (25–29%) as leaf temperatures went up and down. In early summer, the photosynthetic response was optimal at 30 °C, but the overall response was steeper, with the rates reduced markedly at the low and high temperatures compared to the response in spring. In mid-summer, the photosynthetic response to temperature was generally similar, with little change in rates. At harvest, the photosynthetic temperature response was reduced overall and much flatter, with a generally broad optimum at 30 °C and the rates were generally lower. As part of this change in response to temperature, the reduction in photosynthesis from the maximum rate at 30 °C to that at 20 °C, increased from 16% in spring to 26% at harvest while at 40 °C, the comparable changes were 25 and 24%, consistent with a shift to higher rates at the higher temperatures. It is known that the growth temperature can have a marked effect on the temperature-dependency of

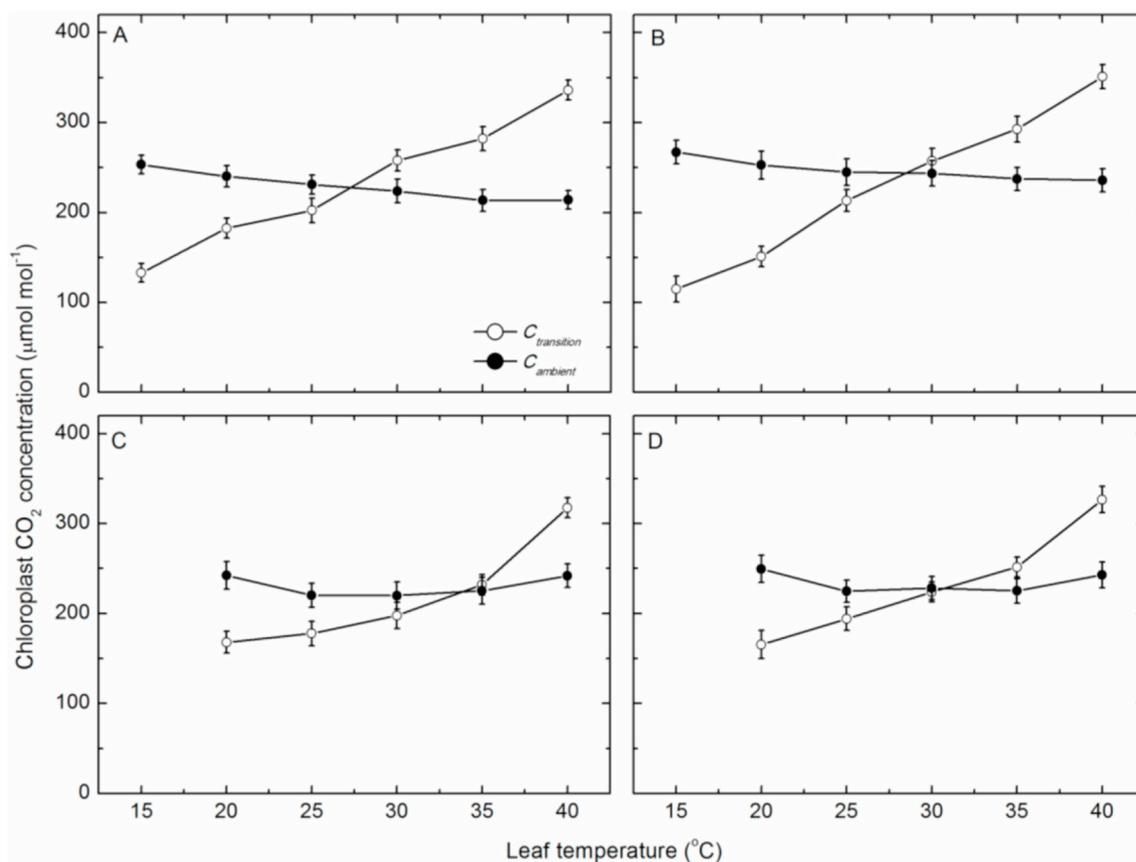


Fig. 6. Transition response of assimilation to temperature. Changes in the chloroplast CO₂ concentration at ambient (400 μmol mol⁻¹) CO₂ concentrations (closed symbols) and the chloroplast CO₂ concentration at which the transition from ribulose 1,5-bisphosphate (RuBP) regeneration-to RuBP carboxylation-limited assimilation occurred as function of leaf temperature and at selected stages of the growing season, A, 29 DAB; B, 72 DAB; C, 87 DAB and D, 150 DAB. The transition temperature was determined as to where these two lines intersected.

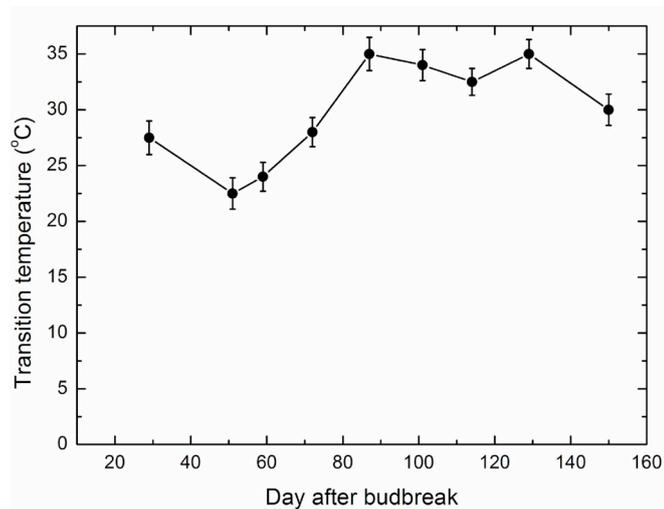


Fig. 7. Seasonal changes in transition temperature. Seasonal changes in the transition temperature (mean ± SE, n = 18) at which ribulose 1,5-bisphosphate (RuBP) regeneration-assimilation shifted to RuBP carboxylation-limited assimilation. These data were averaged over all A/c_c curves for each day. Note these data were correlated with the mean day temperature (see Fig. 1) determined on each day of measurement ($r^2 = 0.58$, $P < 0.01$).

photosynthesis, with the optimum temperature increasing with growth conditions (Slatyer, 1977). It was probable, therefore, that the shift in temperature sensitivity of photosynthesis and the change in optima were part of an acclimation response to the seasonal temperatures.

These changes in the cv. Shiraz photosynthetic response to temperature were much more pronounced with the CO₂ saturated rates. The maximum rates in spring initially occurred at 30 °C, but the response was steeper towards the low temperatures (34% reduction at 15 °C) compared with the high temperatures (8% at 40 °C). In early summer, the response shifted to an optimum at 35 °C, but with increased sensitivity at the low to moderate temperatures (53% reduction in rates at 15 °C) compared to the high temperatures. In midsummer, the photosynthetic temperature response was generally unchanged except for an overall reduction in rates. However by harvest, the response had become much flatter, with a 33% reduction in rates at the low temperatures and 19% reduction at high temperatures, with the optimum temperature increasing to 40 °C. Across the season, therefore, the temperature response was generally biased towards the higher temperatures, with much greater decreases from the maximum rates at the lower temperatures. For two other grapevine cultivars, cv. Chardonnay and cv. Merlot, similar changes occurred (Greer, 2017b). There were clearly genotypic effects on the seasonal photosynthetic responses of these cultivars to temperature but there was still general support for there being apparent acclimation responses to the climatic conditions.

It was notable that despite the marked changes in maximum assimilation rates, the response of RuBP carboxylation to leaf temperature did not vary over the spring to early summer period, as indicated by the similar activation energies (Table 1) and generally comparable maximum rates. However, by mid-summer the activation energy had increased significantly, the maximum rates of carboxylation declined at both low and high temperatures, but especially at 45 °C and this was in accord with rates of assimilation. This high temperature decrease may

have been a consequence of Rubisco or Rubisco activase becoming inactivated by heat stress (Salvucci and Crafts-Brandner, 2004) or by impaired ATP hydrolysis (Spreitzer and Salvucci, 2002). The temperature response for RuBP carboxylation at harvest was overall much flatter than occurred earlier in the season, the maximum rates declined progressively more as leaf temperature increased. Again, this reduction in rates was very much in accord with the change in assimilation rates and also indicative potentially of inactivation of Rubisco. At this time, the activation energy was again significantly higher than occurred early in the growing season and evidence of a reduced sensitivity to temperature. By contrast, for both cv. Chardonnay and cv. Merlot, the activation energy for the RuBP carboxylation response to temperature progressively declined over the growing season, consistent with an overall flattening out of the temperature response of RuBP carboxylation as well as decreased rates from spring to harvest (Greer, 2017a). An increase in activation energy for RuBP carboxylation from spring to midsummer also occurred for the maritime pine, *Pinus pinaster* (Medlyn et al., 2002b). It would appear, therefore, that the temperature-dependency of RuBP carboxylation can vary over the growing season in many species.

The initial dependence of RuBP regeneration of the Shiraz leaves on leaf temperature was much less responsive (increasing 1.5-fold) than that for RuBP carboxylation (about 4-fold) but rates were optimal at about 36 °C. The temperature sensitivity of RuBP regeneration changed markedly in early summer compared with that in spring, with a steeper response at the low to moderate temperatures, although the rates were similar at the high temperatures and optimal at about 38 °C. This was in accord with the rates of assimilation, especially the A_{max} rates at the low temperatures. The J_{max} activation energy (Table 1) increased significantly between spring and early summer and indicative of the increased sensitivity of RuBP regeneration to temperature. There were no further changes in temperature-sensitivity for RuBP regeneration by mid-summer, however, there was a wholesale reduction in the maximum regeneration rates across all leaf temperatures. Again this was in accord with the CO₂ saturated assimilation rates and contrasted with the temperature dependency of RuBP carboxylation in mid-summer. At harvest, there was flattening out of the RuBP regeneration response to temperature in comparison to that in mid-summer, with greater reductions in rates of RuBP regeneration with increasing temperatures, at least up to 40 °C and consistent with a reduced temperature sensitivity. A significantly lower activation energy also conformed to this conclusion and may have occurred because of impaired ATP hydrolysis, this limiting RuBP regeneration (Spreitzer and Salvucci, 2002). A similar seasonal change in the temperature-dependency of RuBP regeneration also occurred in cv. Chardonnay and cv. Merlot vines, with a steep response in spring and a gradual flattening out of the response as the season progressed, especially with marked reductions in RuBP regeneration rates at the higher temperatures (Greer, 2017a). For a maritime pine, there were also seasonal changes in the activation energy for RuBP carboxylation, remaining high at about 45 kJ mol⁻¹ in summer but declining to 31 kJ mol⁻¹ in winter (Medlyn et al., 2002b) and hence a change in temperature sensitivity. Note that the temperature sensitivity for the cv. Shiraz vines was comparable with that for the maritime pine in winter (Table 1). Therefore across many species, there is some evidence that the temperature-sensitivity of RuBP regeneration can vary across the season, although the extent appeared to be genotypic and growth condition-dependent.

4.2. Seasonal changes in photosynthesis

Across the whole growing season, the CO₂-limited rates of photosynthesis (A_{400}) were initially relatively stable, up to about 87 DAB and then declined. A similar seasonal decline occurred with cv. Kékfrankos (Zsófi et al., 2009). For the most part, the cv. Shiraz rates were optimal at 30 °C, although vying with 35 °C on occasion, but consistently the lowest rates were at 40 °C (too few data at 15 and 45 °C to evaluate).

Thereafter, the A_{400} rates declined in a linear pattern at each temperature (see Fig. 5) but the sensitivity to temperature remained about constant (0.15 μmol m⁻² s⁻¹ °C⁻¹ at 87 DAB; 0.18 μmol m⁻² s⁻¹ °C⁻¹ at 151 DAB). By contrast, the CO₂-saturated photosynthetic rates (A_{max}) declined more or less continuously at all leaf temperatures, at least up until harvest. These rates remained optimal at 35 °C for almost the whole season and the lowest rates occurred at 20 °C. Again the decline in photosynthesis was linear, but clearly at different rates between the temperatures (see Fig. 5), because the temperature-sensitivity declined, for example from 0.46 μmol m⁻² s⁻¹ °C⁻¹ at 87 DAB to 0.08 μmol m⁻² s⁻¹ °C⁻¹ at 151 DAB, nearly a six-fold decrease in sensitivity. A similar change in A_{max} rates also occurred with cv. Chardonnay and cv. Merlot, (Greer, 2017a). This decrease in both A_{400} and A_{max} rates were not correlated with seasonal temperatures, as these peaked at about 100–120 DAB while the photosynthetic rates decreased from early in the season when air temperatures increased. It was also apparent that the day effect was a strong driver of the Shiraz vines A_{400} and A_{max} rates, accounting for 93 and 85% of the mean square, respectively, the stomatal conductance accounted for 5% of the A_{400} rates and measurement temperature accounted for 15% of the A_{max} rates. However, no specific effect of the seasonal climate could be identified that might explain the day effect in either case. Furthermore, the reduction in photosynthetic rates across all temperatures that occurred at 150 DAB, both with the CO₂-limited and CO₂-saturated responses was not a response to the removal of the fruit sink, as this occurred after measurements were completed.

As with assimilation, the maximum rates of RuBP carboxylation rates were initially relatively stable up to about 80 DAB but, thereafter, the rates declined across the growing season, although the extent was temperature-dependent. Although the temperature response was distinctive throughout, rates increased progressively from 20 to 40 °C at all times, the sensitivity to temperature declined, from 6.9 μmol m⁻² s⁻¹ °C⁻¹ at 87 DAB to 4.8 μmol m⁻² s⁻¹ °C⁻¹ at 151 DAB, for example. At most temperatures, there were comparable and steady decreases in rates of RuBP carboxylation of cv. Chardonnay and cv. Merlot vines across the growing season (Greer, 2017a). For an equivalent grape cultivar cv. Syrah (aka Shiraz), rates of RuBP carboxylation declined progressively from flowering to berry maturity and the rates measured at 27 °C varied from 89 to 50 μmol m⁻² s⁻¹ (Prieto et al., 2012) and, therefore, comparable with those for the present study (Table 1, k_{25}). As for all woody species and many others, there was a marked and strong dependency of the maximum rates of RuBP carboxylation on leaf temperature (Greer and Weedon, 2012; Medlyn et al., 2002a). Therefore, it was reasonable to assume that the seasonal changes in carboxylation were largely attributable to the seasonal changes in temperature (see Medlyn et al., 2002b). However, no direct evidence was found in the present study, where the day effect accounted for 20% of the variance and the seasonal variation in V_{cmax} was most correlated with the measurement temperature, which accounted for 80%.

A similar seasonal pattern occurred with the maximum rates of RuBP regeneration of the Shiraz vines in that the rates were initially stable but then declined progressively until about harvest. The effect of temperature on the rates across the season was mostly distinct, but the differences in rates across the temperature range were markedly smaller than occurred with the rates of RuBP carboxylation. Rates of RuBP regeneration were lowest at 20 °C throughout the season but at the higher range, the optimal temperature varied somewhat erratically from 35 to 40 °C. There was, however, a decrease in temperature sensitivity, such that at 87 DAB, the dependency was 4.59 μmol m⁻² s⁻¹ °C⁻¹ while at 151 DAB, the dependency was 2.37 μmol m⁻² s⁻¹ °C⁻¹, thus about a two-fold decrease in temperature sensitivity, in keeping with that for both assimilation and rates of carboxylation. Similar results occurred with cv. Chardonnay and cv. Merlot vines (Greer, 2017a). Seasonal changes in rates of RuBP regeneration with cv. Syrah also occurred although somewhat erratically, but were lowest at harvest (Prieto et al., 2012), in keeping with the present study. Although

the seasonal changes in RuBP regeneration of the Shiraz vines were not correlated with the seasonal climate, the day effect, nevertheless accounted for 86% of the variance while the measurement temperature only accounted for 14%.

Because the RuBP regeneration process has a low sensitivity to temperature and the affinity of Rubisco for CO₂ declines, then at high temperatures, RuBP carboxylation becomes limiting (Silim et al., 2010; Yamori et al., 2010). The transition temperature at which this shift to a carboxylation limitation for the cv. Shiraz vines occurred was about 27 °C at the start of the growing season and by early summer was slightly higher but it was in mid-summer that a marked increase in temperature occurred, with the transition at 34 °C. However, at harvest the transition had declined to about 30 °C. Other estimates include 22 °C for *Quercus myrsinaefolia* seedlings (Hikosaka et al., 1999) and 30 °C for cv. Semillon vines (Greer and Weedon, 2012). Notably, however, for *Quercus myrsinaefolia*, when grown at low temperatures, assimilation was only limited by RuBP carboxylation, (Hikosaka et al., 1999), suggestive of growth effects on the transition temperature. As shown in Fig. 7, the transition temperature indeed varied across the growing season. Initially, the transition declined to about 23 °C but then increased in midsummer to about 35 °C. The temperature remained there until late summer, when the transition declined strongly to 30 °C by the last measurement. This pattern was significantly correlated with the seasonal temperatures (cf. Fig. 1), specifically the mean day temperature, which accounted for 58% of the variance in the transition temperature. This strongly affirmed that the transition temperature was dependent on the seasonal growth conditions.

5. Conclusions

The seasonal changes in cv. Shiraz photosynthesis across all measurement temperatures and ambient (A_{400}) and saturated CO₂ concentrations (A_{max}), declined from high rates in spring to low rates at harvest. The high rates in spring were consistent with the deciduous habit of the grapevines and the need to reduce the reliance on stored carbohydrate reserves to meet the growth demands. While it was apparent that some decreases in the rates of photosynthesis were in concert with the seasonal climate, this could only be determined by an unknown effect exemplified by the day of measurement. However, over 97% of the variance in A_{max} could be accounted for by the day effect and by the changes in maximum rates of RuBP carboxylation and RuBP regeneration. The transition temperature at which a shift from a RuBP regeneration limitation at temperatures below to a RuBP carboxylation limitation of assimilation above, varied across the growing season and this was driven by the seasonal day temperatures. Thus, early in the season, at temperatures above about 25 °C, carboxylation limited assimilation, but this moved upwards to temperatures above 35 °C in midsummer and back down to below 30 °C by harvest. This demonstrated that the assimilation process in these grapevines was highly dynamic to the seasonal climate and under strong attendant control by the RuBP carboxylation and regeneration processes, in keeping with the Farquhar et al. (1980) model.

Contribution

Dennis Greer; Designed the trial, Conducted all the research, Analysed all data, Wrote the paper.

Declarations of interest

None.

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