

Nocturnal and daytime stomatal conductance respond to root-zone temperature in ‘Shiraz’ grapevines

Suzy Y. Rogiers^{1,2,*} and Simon J. Clarke^{1,3}

¹National Wine and Grape Industry Centre, Wagga Wagga, NSW, Australia, ²NSW Department of Primary Industries, Wagga Wagga, NSW, Australia and ³School of Agriculture and Wine Science, Faculty of Science, Charles Sturt University, Wagga Wagga, NSW, Australia

*For correspondence. E-mail suzy.rogiers@industry.nsw.gov.au

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- **Background and Aims** Daytime root-zone temperature may be a significant factor regulating water flux through plants. Water flux can also occur during the night but nocturnal stomatal response to environmental drivers such as root-zone temperature remains largely unknown.
- **Methods** Here nocturnal and daytime leaf gas exchange was quantified in ‘Shiraz’ grapevines (*Vitis vinifera*) exposed to three root-zone temperatures from budburst to fruit-set, for a total of 8 weeks in spring.
- **Key Results** Despite lower stomatal density, night-time stomatal conductance and transpiration rates were greater for plants grown in warm root-zones. Elevated root-zone temperature resulted in higher daytime stomatal conductance, transpiration and net assimilation rates across a range of leaf-to-air vapour pressure deficits, air temperatures and light levels. Intrinsic water-use efficiency was, however, lowest in those plants with warm root-zones. CO₂ response curves of foliar gas exchange indicated that the maximum rate of electron transport and the maximum rate of Rubisco activity did not differ between the root-zone treatments, and therefore it was likely that the lower photosynthesis in cool root-zones was predominantly the result of a stomatal limitation. One week after discontinuation of the temperature treatments, gas exchange was similar between the plants, indicating a reversible physiological response to soil temperature.
- **Conclusions** In this anisohydric grapevine variety both night-time and daytime stomatal conductance were responsive to root-zone temperature. Because nocturnal transpiration has implications for overall plant water status, predictive climate change models using stomatal conductance will need to factor in this root-zone variable.

Key words: Water-use efficiency, leaf gas exchange, CO₂ assimilation, grapevine, *Vitis vinifera*, stomatal conductance, root-zone temperature, ‘Shiraz’, grapevine, soil temperature.

INTRODUCTION

Rising global temperatures can be accompanied by smaller, but significant, changes in soil surface temperatures (van Gestel *et al.*, 2011) and this has the potential to affect root physiology and root activity. In particular, a gradual rise in night-time air temperature over the past few decades (Vose *et al.*, 2005) has probably resulted in an increase in soil temperature during the night and early morning, depending on soil depth. Root growth (Larson, 1970; Kasper and Bland, 1992), water uptake (Wan *et al.*, 1999), nutrient uptake and availability (MacDonald *et al.*, 1995; BassiriRad, 2000; Melillo *et al.*, 2002; Pregitzer and King, 2005) and signal transduction (Zhang *et al.*, 2008; Field *et al.*, 2009) are all influenced by the temperature of the soil. These processes affect above-ground growth (Lopushinsky and Kaufmann, 1984; Dawes *et al.*, 2011; Rogiers *et al.*, 2011b) and productivity (He *et al.*, 2001). As a result, analogous to air temperature, soil temperature can limit the geographical distribution of plants and crops (Fosaa *et al.*, 2004).

Leaf gas exchange is particularly responsive to the root-zone environment. While there are many studies

characterizing the consequences of soil moisture on leaf gas exchange, soil temperature has also been found to affect stomatal conductance and photosynthesis in perennials (Cai and Dang, 2002; Wan *et al.*, 2004; Wu *et al.*, 2012) and annuals (He and Lee, 2001; He *et al.*, 2001; Zhang *et al.*, 2008). Root-zone temperature can restrict photosynthesis through alterations in photosynthetic reactions (Cai and Dang, 2002; Zhou *et al.*, 2004; Erice *et al.*, 2006) or changes in stomatal conductance (Wan *et al.*, 2004). For instance, boreal and alpine plants are often exposed to low soil temperature stress and in conifer species the maximum rate of carboxylation ($V_{c,max}$) and the maximum rate of electron transport (J_{max}) increased with soil temperature up to an optimum and then declined (Cai and Dang, 2002). Conversely, it was found that when *Populus tremuloides* was exposed to low temperatures soil net assimilation was limited by stomatal conductance (Wan *et al.*, 1999). As a consequence, it was suggested that similar root-to-shoot signalling pathways may operate in plants subjected to drought and low root temperature (Wan *et al.*, 2004). As such, it was found that roots of Cucurbitaceae species can regulate stomatal behaviour in response to root-zone temperature by

modifying delivery of abscisic acid (ABA) to shoots and leaves (Zhang *et al.*, 2008).

Those environmental parameters regulating stomatal conductance (g) and transpiration (E) during the day may also exert some control during the night. Stomata can remain partially open during the night and, although the function of night-time transpiration is yet to be resolved, this can result in substantial night-time transpiration if the vapour pressure difference between the leaf and air (VPD) is high. In some species and varieties, nocturnal transpiration can even cause disequilibrium in plant water status (Caird *et al.*, 2007; Dawson *et al.*, 2007; Fisher *et al.*, 2007; Kavanagh *et al.*, 2007). For instance, *Vitis vinifera* ‘Grenache’, an isohydric grapevine variety, has low g both during the day and night. This is in contrast to ‘Semillon’, an anisohydric variety, with comparatively high g throughout the diurnal cycle and as a result incomplete plant rehydration prior to dawn especially during warm, windy nights (Rogiers *et al.*, 2009). Aside from inherent genetic factors, nocturnal g appears to be influenced by abiotic factors such as VPD, wind, atmospheric CO₂ concentration and soil moisture (Ludwig *et al.*, 2006; Howard and Donovan, 2007; Zeppel *et al.*, 2010, 2012). Another key environmental factor which may regulate nocturnal g is root-zone temperature. To date, the consequences of root-zone temperature on nocturnal stomatal conductance, to our knowledge, are unknown. It is therefore difficult to incorporate soil temperature into crop production models because there is little information available on how soil temperature affects daytime and especially night-time foliar gas exchange.

We assessed the foliar gas exchange response of ‘Shiraz’, an anisohydric grapevine variety (Schultz, 2003) originating from temperate Bordeaux, France, and grown widely in warm climates across southern Australia and Argentina. ‘Shiraz’ is grown across a broad range of latitudes, elevations and topographic conditions that expose this variety to a wide range in root-zone temperatures. Slope orientation and the extent of ground cover in the inter-row area by cover crops or weeds will also affect the temperature of the soil. Other factors such as rain patterns, irrigation and soil type influence rooting depth, and therefore root-zone temperature, and this will also affect plant response. The objective of this work was to clarify the role of root-zone temperature on both nocturnal and daytime g in ‘Shiraz’. Using well-watered grapevines grown in three different root-zone temperatures for 2 months we assessed the response of g to this below-ground parameter during both the day and the night. The three root-zone temperatures consisted of ambient, 5 °C below or 5 °C above ambient levels to encompass the natural variation that can occur across a vineyard site in spring.

MATERIALS AND METHODS

Plant material

Six-year-old ‘Shiraz’ grapevines ($n = 60$) on their own roots (*Vitis vinifera* L., clone 1654) were re-potted during dormancy into 40-litre pots containing a well-draining potting mix. The plants were placed outdoors in a bird-proof enclosure in a randomized block design surrounded on all sides by buffer plants. The root-zone temperature treatments were administered by circulating

water at controlled temperatures through a 1.1-m long coil of 13-mm polyethylene irrigation tubing inserted within the pot and embedded through the root-zone to a depth of 30 cm. This tubing was attached to 50-mm DWV pipe connected to a recirculating water system (UPS 32-80 N 180; Grundfos, Bjerringbro, Denmark) and one of three temperature-controlled 1200-litre water tanks. The sides and bottom of the pots were insulated with blue 10-mm dense foam to minimize heat exchange between the soil and air or ground. At the time of re-potting, a soil temperature probe (DS18B20; Maxim Integrated Products, Sunnyvale, CA, USA) was inserted into the centre of the root mass of every vine. A TDR soil moisture probe (Trace; Soil Moisture Equipment Corp., Santa Barbara, CA, USA) was also inserted in the same location in nine pots per treatment. Both these probes were logged at 30-min intervals (Mini Trase data logger; Soil Moisture Equipment Corp.). A weather station, equipped with an air temperature probe (Intercap HMP50; Vaisala, Hawthorn, VIC, Australia) and a quantum sensor (Apogee, SQ-110; Logan UT, USA) logging at half hourly intervals, was placed at canopy height within the trial. Ten weeks prior to bud-burst, the plants were pruned to two spurs, each carrying two buds. After bud-burst the plants were irrigated daily and excess water was allowed to drain from the bottom of the pot. Beginning 25 d after bud-burst plants were fertilized with 200 mL tap water containing 1 mL nutrient concentrate (Megamix Plus, equivalent to N/P/K 130 : 100 : 150 mg per plant) every 5 d until veraison, then every 10 d thereafter.

Root-zone temperature treatments

Three root-zone temperature treatments were initiated at the onset of budburst (E-L Stage 4) and terminated at fruit-set (E-L stages 27–29). The root-zone temperatures were allowed to fluctuate diurnally, with the ambient treatment mimicking the nearby vineyard under-vine soil at a depth of 15 cm. Maximum root-zone temperatures over the 63-d treatment period averaged 16.9 ± 0.3 °C for the cool, 22.6 ± 0.5 °C for the ambient and 24.8 ± 0.4 °C for the warm treatment, while minimum root-zone temperatures averaged 13.3 ± 0.3 , 17.8 ± 0.5 °C and 22.6 ± 0.2 °C, respectively. Air temperature at the canopy level (approx. 1 m above the soil surface) was not altered by the treatments and ranged between an average minimum of 10.3 °C and maximum of 28.5 °C. VPD ranged between 1.97 and 0.17 kPa while daily maximum photosynthetically active radiation (PAR) averaged $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$. Soil moisture over the 63-d treatment period averaged 21.9 ± 1.3 % for the cool, 21.5 ± 1.4 % for the ambient and 21.1 ± 1.4 % for the warm treatment.

Plant measurements

Instantaneous net assimilation, stomatal conductance, transpiration and leaf intercellular CO₂ concentrations were measured using a portable photosynthesis system (LI-6400; LI-COR Biosciences Inc., Lincoln NE, USA). Midday measurements were carried out weekly from bud-burst to fruit-set. An artificial red–blue light source attached to the chamber head was used to illuminate the leaf at $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR. The CO₂ concentration of the incoming air was maintained at $400 \mu\text{mol mol}^{-1}$ and the block temperature was set

at 25 °C. On each shoot (18 plants per treatment with four shoots per plant), the leaf opposite the basal inflorescence (most often located at the fourth or fifth node from the base) was measured. Nocturnal measurements (nine plants per treatment) were carried out every 2 weeks on the same leaves prior to dawn with the chamber light turned off, incoming CO₂ concentration set at 400 μmol mol⁻¹ and the block temperature set to 18 °C. Time-integrated values of transpiration were calculated based on a normal distribution of leaf stomatal transpiration (Rogiers *et al.*, 2009), over a 15-h light period and constant *E* during the 9-h dark period. Light, temperature and VPD response curves were carried out at midday, 43–50 d after the onset of the root-zone temperature treatments, on four to six plants per treatment. VPD was not controlled in the temperature or light response curves.

Leaf chlorophyll was assessed non-destructively with a chlorophyll meter (SPAD-502; Minolta Co., Ltd, Tokyo, Japan) twice weekly from 30 d after bud-burst to fruit-set. The meter readings were converted to chlorophyll content using a calibration curve derived from paired meter and spectrophotometer readings. The spectrophotometer readings were converted to an estimate of chlorophyll content following the method of Steele *et al.* (2008). Measurements were carried out on the leaf opposite the basal inflorescence and all leaves opposite the basal tendril in the three-node repeating metameter pattern that occurs along *V. vinifera* shoots. Stomatal density was assessed on these same leaves on six plants per treatment according to the method of Rogiers *et al.* (2011a).

A/C_i responses to root-zone temperatures

Measurements of photosynthetic responses to cool and warm root-zone temperatures were carried out 50 d after onset of treatments on four plants per treatment, targeting fully expanded leaves opposite the basal inflorescence. The leaf respiration/internal CO₂ concentration (*A/C_i*) response was measured according to Ainsworth *et al.* (2002). Photosynthesis was initially measured at 400 μmol mol⁻¹ CO₂ under saturating light (1500 μmol mol⁻¹ s⁻¹) and a block temperature of 25 °C, until *A* was steady-state. The CO₂ concentration surrounding the leaf (*C_a*) was then reduced to 50 μmol mol⁻¹ over nine steps with *A* and *C_i* recorded as soon as *C_a* was stable. *C_a* was then returned to 400 μmol mol⁻¹ and increased over eight steps to 2000 μmol mol⁻¹. Each *A/C_i* curve was processed to obtain

J_{max}, the maximum rate of electron transport, *V_{c,max}*, the *in vivo* maximum rates of Rubisco activity, and *R_d*, the day respiration, according to the method of Greer and Weedon (2012) using SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

Statistical analysis

SigmaPlot graphing and statistics software (version 12.3; SPSS Inc., Chicago, IL, USA) was used to fit linear or non-linear regressions of gas exchange parameters with soil temperature, air temperature, VPD and PAR. The three-dimensional mesh plots of nocturnal leaf stomatal conductance (*g_n*) and nocturnal leaf stomatal conductance (*E_n*) with soil moisture and root-zone temperature were created with smoothed data calculated from running averages using SigmaPlot. An analysis of variance (GenStat release 15.0; VSN, Hertfordshire, UK) was used for comparison of treatment effects during the root-zone temperature treatments and least significant differences were calculated at 5 % significance. Results of the statistical analyses are presented in the tables and figures. Values presented in the text are means ± s.e.

RESULTS

Nocturnal gas exchange

Nocturnal leaf stomatal conductance, *g_n*, (*P* < 0.001) and transpiration, *E_n*, (*P* < 0.001) responded to root-zone temperature (Table 1). In the cool root-zone treatment, *g_n* was 14.5 % lower than in the ambient treatment and 20.7 % less than in the warm treatment. Similarly, compared with the ambient and warm root-zone treatments, *E_n* was 13.8 and 19.8 % lower, respectively, in the cool treatment. This decline was apparent without any treatment differences in soil moisture (*P* = 0.105) which averaged at 20.9 % at the time of measurement. A linear regression of *g_n* with soil moisture at the time of the gas exchange measurements (*P* < 0.01) explained only 4 % of the variance in the data while a regression of this parameter with root-zone temperature (*P* < 0.002) accounted for 3 % of the variance. Further detailed examination of these two environmental parameters, however, showed that vines exposed to high root-zone temperatures combined with high soil moistures had highest *g_n* and *E_n* values while those exposed to low temperatures and low moisture responded with low *g_n* and *E_n* (Fig. 1). The combined factors of leaf age, leaf temperature, soil moisture and soil temperature accounted

TABLE 1. Nocturnal leaf *A*, *g_n*, *E_n* and *C_i* of grapevines under cool, ambient or warm root-zone conditions

Treatment	Root-zone temperature (°C)	Soil moisture (%)	<i>A</i> (μmol CO ₂ m ⁻² s ⁻¹)	<i>g_n</i> (mol H ₂ O m ⁻² s ⁻¹)	<i>E_n</i> (mmol H ₂ O m ⁻² s ⁻¹)	<i>C_i</i> (μmol mol ⁻¹)
Cool	14.8 ^a	21.2 ^a	-0.53 ^a	0.0314 ^a	0.275 ^a	455.2 ^a
Ambient	20.8 ^b	20.9 ^a	-0.45 ^a	0.0367 ^b	0.319 ^b	437.8 ^b
Warm	24.2 ^c	19.7 ^a	-0.48 ^a	0.0396 ^b	0.343 ^b	435.5 ^b
LSD	0.2	1.8	0.11	0.0034	0.025	11
<i>F</i> -test, <i>P</i> <	0.001	0.179	0.199	0.001	0.001	0.001

Values are means of three sampling dates over a 60-d treatment period (*n* = 9 on each sampling date). Root-zone temperatures and soil moistures represent the means at the time the gas exchange measurements were made. Means followed by different letters are significantly different at *P* < 0.05.

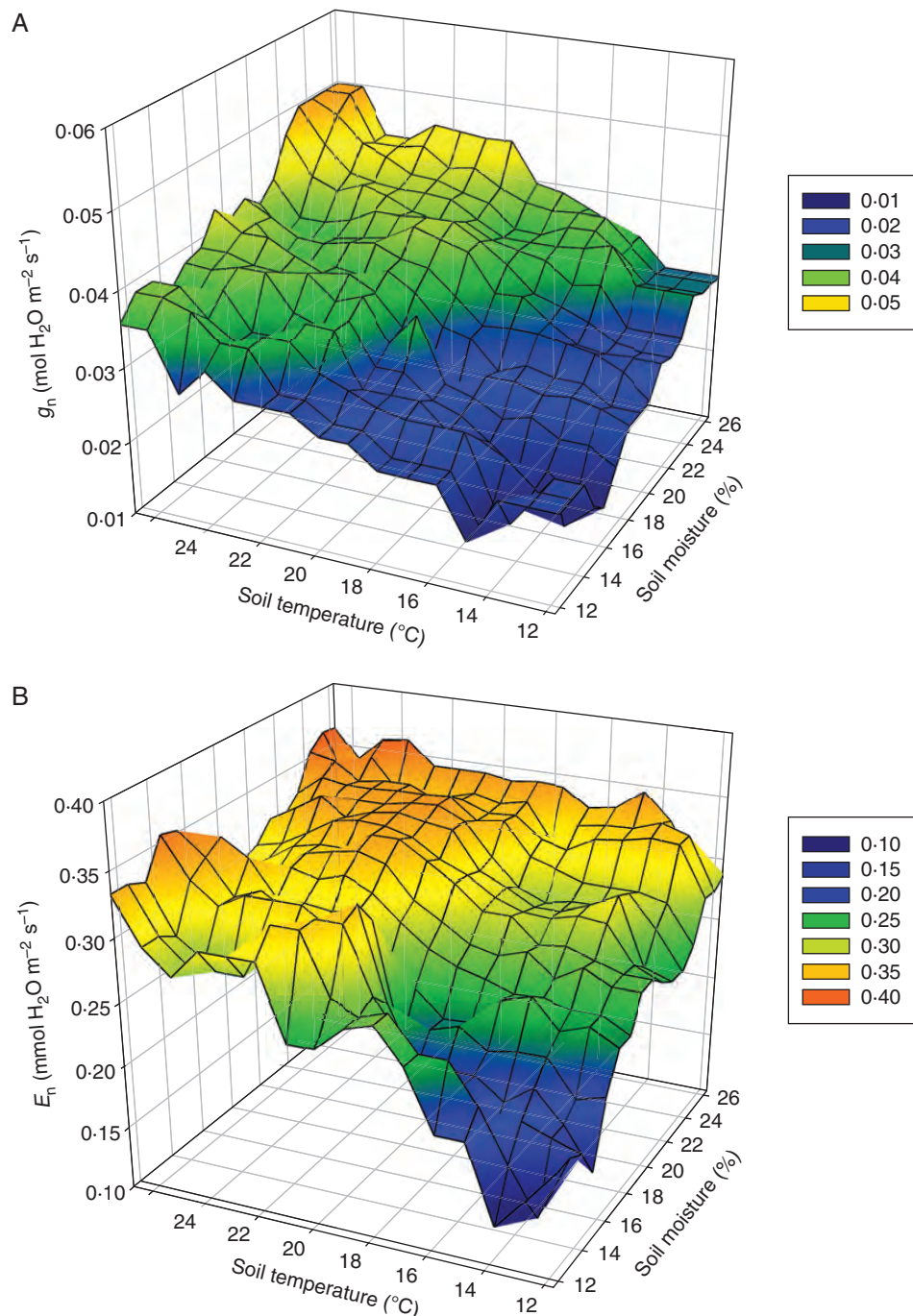


FIG. 1. Nocturnal leaf (A) g_n and (B) E_n as a function of root-zone temperature and soil moisture. 'Shiraz' plants were treated with cool, ambient or warm root-zone conditions over a 60-d period from bud-burst to fruit-set. Smoothed data represent three sampling dates ($n = 9$ on each sampling date).

for 31 % of the variance in g_n ($P < 0.001$) and 58 % of the variance in E_n ($P < 0.002$).

Leaf respiration ($-A$) was not affected ($P = 0.20$) by root-zone temperatures and averaged $0.49 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (Table 1). Internal CO_2 concentrations (C_i), were higher by about $20 \mu\text{mol mol}^{-1}$ in cool root-zone temperatures compared with the other treatments ($P < 0.001$), although this was apparently not linked to g_n because a linear regression of C_i with g_n was not significant ($P = 0.15$).

Midday gas exchange

As leaves matured over a 20-d period of rapid shoot growth, midday stomatal conductance (g_d) increased ($P < 0.001$) and had doubled by the time full lamina expansion had taken place. This occurred in all three treatments within 1 month after bud-burst (Fig. 2). On each measuring date, g_d was substantially less in the cool root-zone as compared with the two other treatments. As the season progressed, air temperatures

rose, the soil temperatures of the ambient and the warm treatments converged, and so did leaf g_d of plants grown in those treatments.

Rates of CO_2 assimilation (A) were highest in the warm and lowest in the cool root-zone temperatures ($P < 0.001$), each diverging by about 5% from the ambient treatment (Table 2).

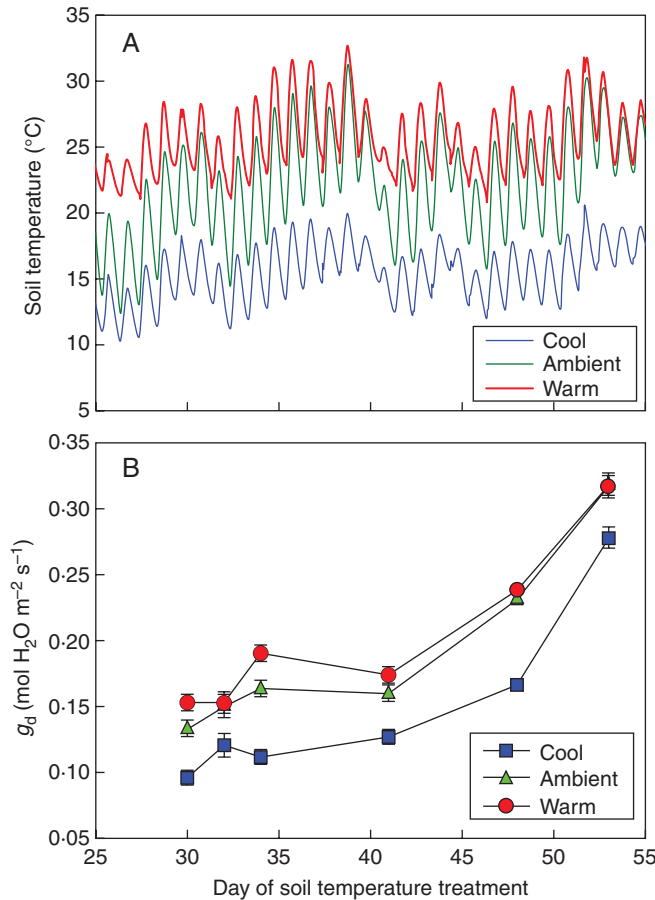


FIG. 2. (A) Average root-zone temperature of the cool, ambient and warm treatments ($n = 18$), and (B) midday leaf stomatal conductance (g_d) in response to cool, ambient and warm root-zone temperatures applied over a 60-d period from bud-burst to fruit-set in ‘Shiraz’ vines. The first measurements were made when leaves opposite the basal inflorescence had obtained an approximate area of 10 cm^2 .

This was not the result of earlier bud-burst in the warm or later bud-burst in the cool treatment because bud-burst occurred at roughly the same time for all the treatments ($P = 0.37$). Leaf development after bud-burst may have proceeded at a greater rate in the warm root-zone treatment, but even though leaves of plants grown in this treatment were larger (Table 4), lamina length and A were not correlated ($P = 0.822$). Higher A was also not due to higher chlorophyll levels in warm plants because chlorophyll levels in the leaves from nodes 2 to 6 over this period were not different from each other in the cool and warm treatments (Table 3). Soil moisture can also not explain these differences because, at the time gas exchange was measured, this parameter varied only slightly between the treatments with lower levels in the warm treatment ($22.8 \pm 0.5\%$ for cool, $22.6 \pm 0.5\%$ for ambient and $20.6 \pm 0.5\%$ for warm) which would result in stomatal closure and decline in A rather than an increase.

Despite no differences in soil moisture between the cool and the ambient treatments, midday g_d ($P < 0.001$) and E_d ($P < 0.001$) were lower by 22 and 17%, respectively, in the cool treatment (Table 2). While there were no differences in g_d or E_d between the ambient and warm treatments, regressions of g_d and E_d with root-zone temperature were highly ($P < 0.001$) significant (Fig. 3). During a 9-h period of darkness, total E_n amounted to $160 \text{ mL H}_2\text{O m}^{-2}$ leaf area in the cool, $186 \text{ mL H}_2\text{O m}^{-2}$ in the ambient and $200 \text{ mL H}_2\text{O m}^{-2}$ in the warm treatment. During the 15-h light period, total E_d amounted to $1312 \text{ mL H}_2\text{O m}^{-2}$ in the cool, $1584 \text{ mL H}_2\text{O m}^{-2}$ in the ambient and $1580 \text{ mL H}_2\text{O m}^{-2}$ in the warm treatment. Daily E_n as a proportion of E_d varied by only 1% between the treatments (12.2% for the cool treatment, 11.7% for the ambient treatment and 12.7% for the warm treatment).

Plants grown with cool root-zones had 15% greater transpiration efficiency (A/E) ($P < 0.001$) and 23% greater intrinsic transpiration efficiency (A/g) ($P < 0.001$) than plants grown in ambient soil (Table 2). Those grown in the warm soil also had somewhat higher A/g (6%) but this may have been an artefact of the slightly reduced soil moistures in this treatment. While there were no differences in A/E between the ambient and warm treatments (Table 2), the regression of A/E with soil temperature was significant due to the strong cool treatment effect (Fig. 3). Similar to g_d , midday leaf C_i was somewhat less (7%) in the cool treatment, indicating depleted CO_2 concentrations and a stomatal limitation to photosynthesis.

TABLE 2. Midday leaf A , g_d , E_d , A/g , A/E and C_i of grapevines under cool, ambient or warm root-zone conditions

Treatment	Root-zone temperature ($^{\circ}\text{C}$)	A ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	g_d ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	E_d ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	A/g ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$)	A/E ($\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$)	C_i ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$)
Cool	16.7 ^a	8.83 ^a	0.155 ^a	2.70 ^a	62.0 ^a	3.74 ^a	262.3 ^a
Ambient	23.1 ^b	9.17 ^b	0.198 ^b	3.26 ^b	50.5 ^b	3.24 ^b	280.0 ^b
Warm	26.5 ^c	9.60 ^c	0.201 ^b	3.25 ^b	53.8 ^c	3.35 ^b	275.4 ^b
LSD	0.3	0.30	0.006	0.09	2.7	0.13	5.0
F -test, $P <$	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Values are means of seven sampling dates over a 60-d treatment period ($n = 18$ on each sampling date). Root-zone temperatures represent the means at the time the gas exchange measurements were made. Means followed by different letters are significantly different at $P < 0.05$.

TABLE 3. Leaf chlorophyll concentrations and leaf photosynthetic characteristics derived from intercellular CO_2 response curves of mature leaves that emerged during the cool or warm rootzone treatments ($n = 4$)

Treatment	Chlorophyll (mg m^{-2})	J_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	$V_{\text{c,max}}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	R_{d} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)
Cool	424.4 ^a	91.0 ^a	34.8 ^a	0.29 ^a
Warm	424.9 ^a	97.1 ^a	33.0 ^b	0.58 ^a
LSD	2.56	17.4	9.15	0.45
F -test, $P <$	0.756	0.442	0.655	0.18

J_{max} is the maximum rate of electron transport, $V_{\text{c,max}}$ is the *in vivo* maximum rates of Rubisco activity and R_{d} is the daytime respiration rate. Chlorophyll concentrations represent averages of nodes 2–6 of weekly measurements during the temperature treatments ($n = 18$ plants, four shoots per plant). Means followed by different letters are significantly different at $P < 0.05$.

Light, temperature and VPD response curves

Air temperature, light and VPD response curves (Figs 4–6) provide more extensive data of how leaf gas exchange was affected by root-zone temperatures. In both cool and warm root-zones, stomata were progressively more closed across an air temperature range of 18–38 °C (Fig. 4). VPD was not controlled over this temperature range and E_{d} increased linearly ($P < 0.001$) with air temperature in both root-zone temperature treatments. However, A did not change with this environmental parameter in either the cool ($P = 0.38$) or the warm ($P = 0.73$) root-zones. C_i followed similar trends to that of g_{d} with lower values in the cool root-zones.

There were similar trends in g_{d} and E_{d} with increasing VPD (Fig. 5). While g_{d} declined, E_{d} increased linearly across the VPD range of 1–4 kPa. Increases in E_{d} in the warm root-zones were double (slope $1.9 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1} \text{ kPa}^{-1}$) that in the cool root-zones (slope $0.86 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1} \text{ kPa}^{-1}$) ($P < 0.001$). Despite stomatal closure, A increased slightly with VPD in both root-zone treatments. C_i was lower in the cool root-zones and responded in a curvilinear manner to VPD, unlike in the warm root-zone treatment.

As with the air temperature and VPD curves, light response curves showed good separation between the root-zone treatments with higher values in the warm treatment as compared with the cool treatment (Fig. 6). Both g_{d} and E_{d} increases were curvilinear and did not plateau until the highest PAR value tested ($2000 \mu\text{mol m}^{-2} \text{ s}^{-1}$). A increased steeply in response to rising PAR and leaves from the warm root-zone reached a 15% higher plateau at $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ than in the cool root-zone. Conversely, C_i values declined with increasing PAR and above $1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ C_i settled at $300 \mu\text{mol mol}^{-1}$ in the warm and $260 \mu\text{mol mol}^{-1}$ in the cool root-zone treatment.

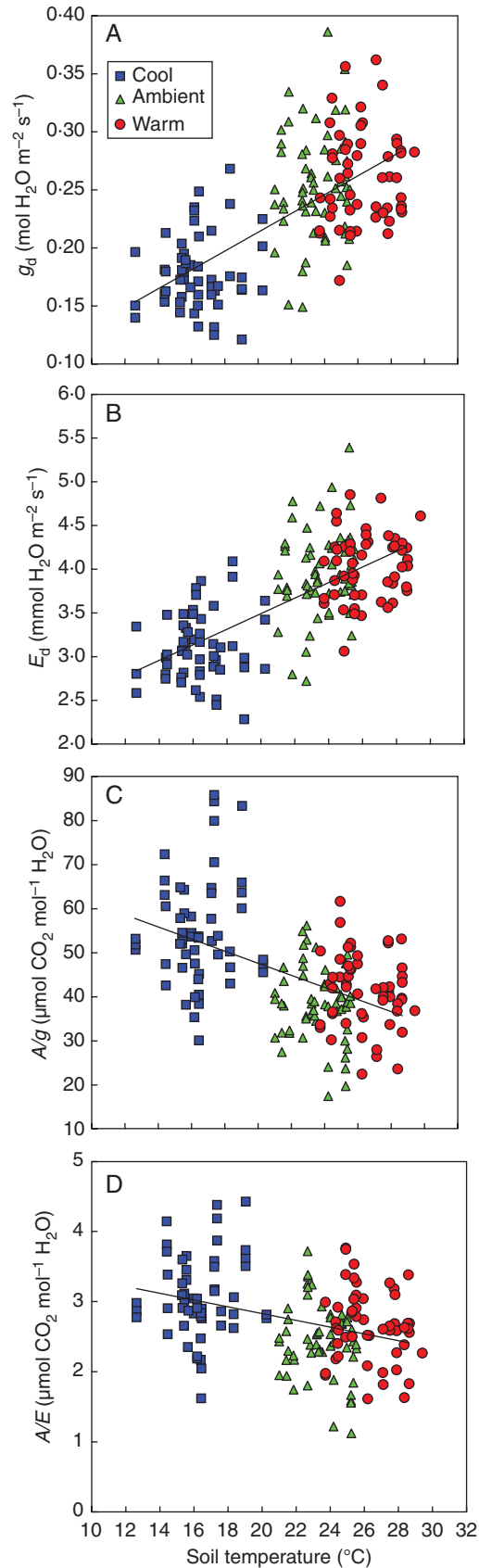


FIG. 3. Midday leaf g_{d} , E_{d} , A/g and A/E as a function of root-zone temperature 48 d after the onset of treatments. Soil moisture was not different between the treatments (F -test, $P = 0.72$) and averaged $22.6 \pm 1.4\%$ at the time of measurement. F -test of linear regression for $g = 0.001$, $E = 0.001$, $A/g = 0.001$ and $A/E = 0.001$; variance accounted for in $g = 0.47$, $E = 0.49$, $A/g = 0.34$ and $A/E = 0.19$.

Leaf and photosynthetic characteristics

A/C_i curves in response to root-zone temperatures are presented in Fig. 7. Further analyses of these curves indicate there were no differences in J_{\max} ($P = 0.44$) or $V_{c,\max}$ ($P = 0.65$) between the treatments and they averaged 94.1 ± 7.5 and $33.0 \pm 4.0 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively (Table 3). Daytime dark respiration was also not different between the treatments ($P = 0.18$) and averaged $0.4 \pm 0.2 \mu\text{mol m}^{-2} \text{s}^{-1}$. Furthermore, leaf chlorophyll concentrations did not differ ($P = 0.75$) and averaged $424.6 \pm 1.3 \text{ mg m}^{-2}$.

One leaf characteristic that did differ between the treatments was stomatal density. Of those leaves that emerged during the treatments, stomatal density was 10% ($P < 0.05$) higher in the cool treatment as compared with the warm treatment (Table 4). Despite 8% larger leaves in the warm treatment as compared with the cool treatment (Table 4), there was no significant relationship between stomatal density and leaf area (F -test, $P = 0.10$ for a data set with combined treatments), and therefore other factors aside from leaf area appear to be involved. There were no differences in stomatal density of those leaves that emerged after the discontinuation of the treatments and they averaged 125.6 ± 5 stomata mm^{-2} . Note, however, that stomatal density of newly emerging leaves of plants grown in the warm treatment increased by 11% after that treatment had been removed. As averaged over 60 d following the termination of the treatments, leaf length remained 6% larger in the warm treatment than the cool treatment ($P < 0.001$).

Gas exchange after termination of the treatments

After discontinuation of the treatments the midday root-zone temperatures averaged at $20.6 \pm 0.3^\circ\text{C}$ and varied by less than 4% (Table 5). Despite equal amounts of water applied, soil moisture was slightly (3%) higher in those plants that received the cool treatment as compared with the warm treatment. This is probably because the cool plants had less total leaf area (Table 4) and therefore less whole plant transpiration and lower rates of soil water utilization. Leaf A , g_d and E_d were no longer different between plants that had previously received the three treatments.

DISCUSSION

Nocturnal stomatal conductance

Our study confirms that daytime stomatal conductance of grapevines, like many other species, responds to root-zone temperature. Our present findings extend this observation to nocturnal stomatal conductance. There is very little experimental work on the interaction of root-zone temperatures with nocturnal gas exchange and we believe this is one of the first reports to characterize this. Cool root-zones resulted in a sustained suppression in g and E during both the day and the night and leaves transpired nearly 20% less than those grown in warm root-zones. Aside from the stomatal limitation evident in our data, others have shown that low E can be the result of reductions in both soil and plant hydraulic conductance (Kramer and Boyer, 1995; Fennel and Markhart, 1998; Berndt *et al.*, 1999) possibly through an increase in water viscosity (Muhsin and Zwiazek, 2002). After

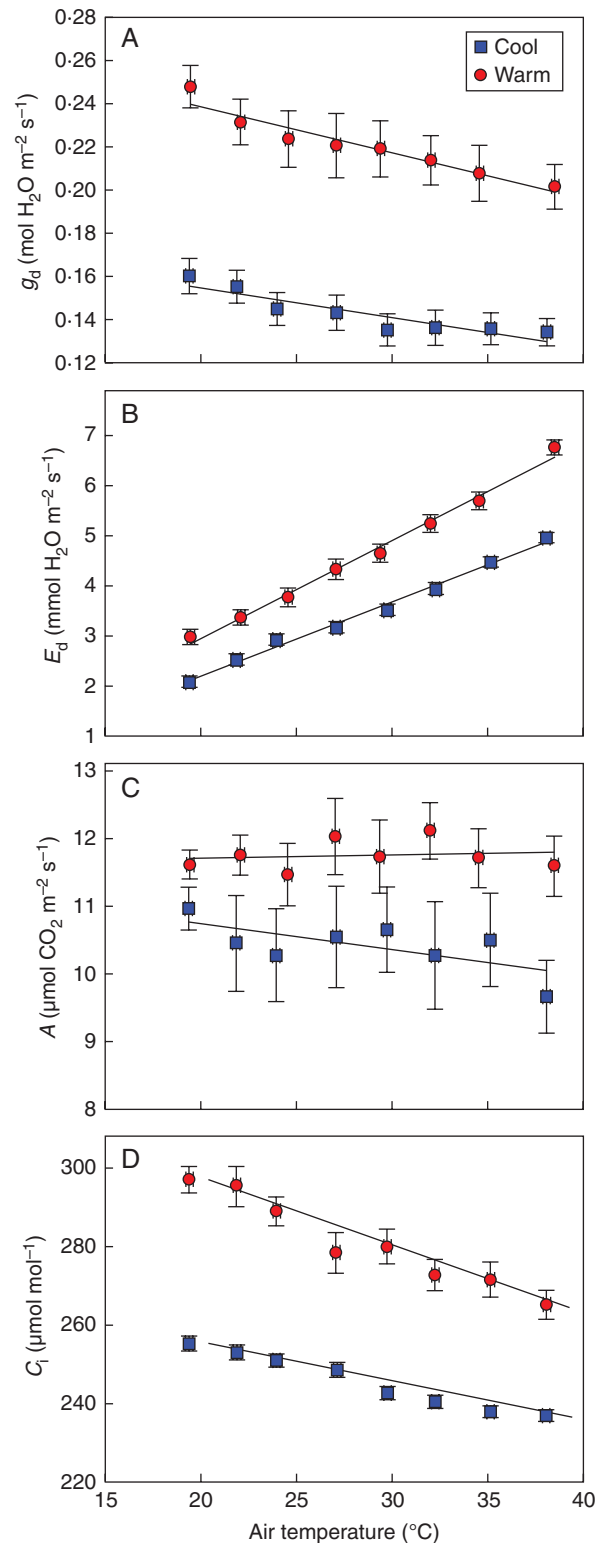
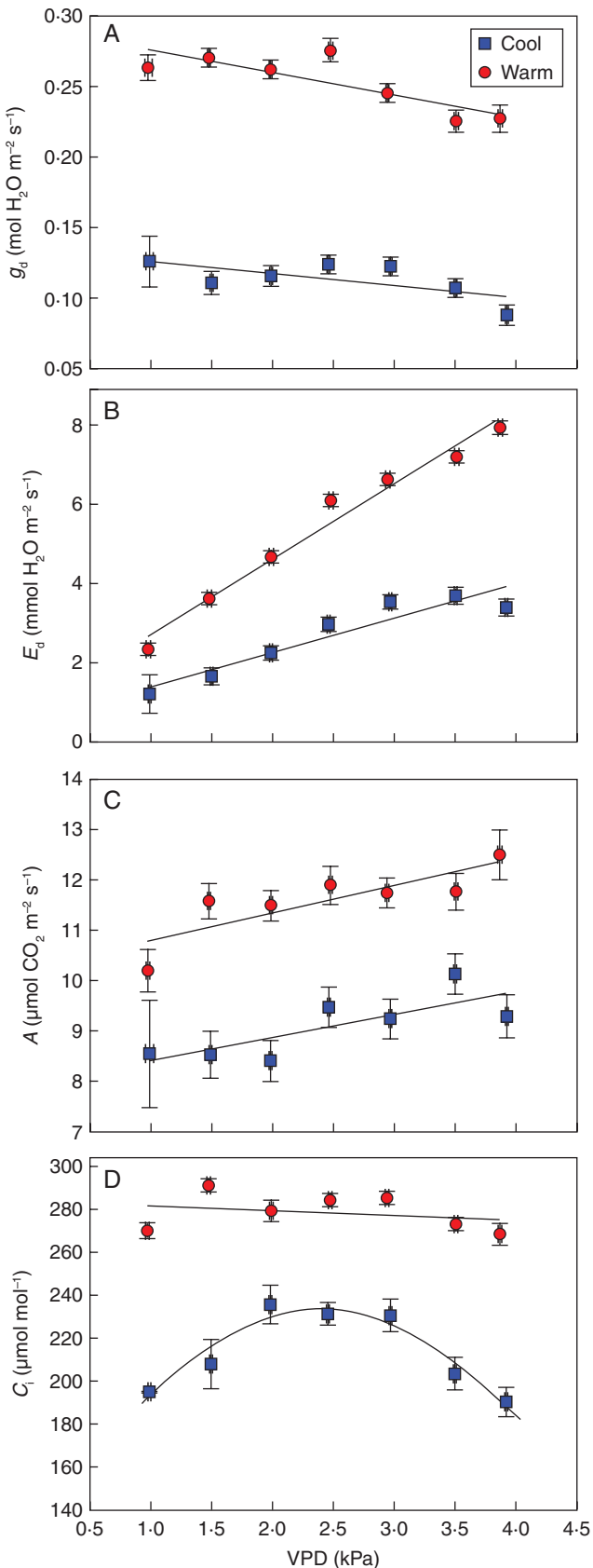


FIG. 4. Midday leaf g_d , E_d , A and C_i in response to air temperature ($n = 4$) of vines grown in either cool or warm soil. At the time of measurement, root-zone temperature of the warm treatment was $26.9 \pm 1.3^\circ\text{C}$ and of the cool treatment was $15.0 \pm 1.0^\circ\text{C}$ ($P < 0.001$). Soil moisture was not different between the treatments (F -test, $P = 0.72$) and averaged $17.6 \pm 0.6\%$. The intercepts and slopes of the regressions for the cool and warm cohorts are different from each other for g_d ($P < 0.001$), E_d ($P < 0.001$), A ($P < 0.001$) and C_i ($P < 0.001$).



discontinuation of the treatments, differences in leaf g_d and E_d were no longer apparent. This signifies that the root-zone temperature effect on gas exchange was not due to a permanent anatomical change but rather a transient physiological effect, perhaps relayed through signals targeted at the stomata. Another study on aspen has demonstrated that low root-zone temperatures can reduce g through pH, ion and ABA signals transported in the xylem sap (Wan *et al.*, 2004). It would therefore be expected that these signals persist throughout the diurnal cycle, including the night. The role of such signals was not addressed in this study, however, and further work is required to verify their contribution to g_d and g_n in grapevines.

The resultant reduction in g_n of plants growing in cool root-zones may thus be an after-effect of daytime changes, or it may offer a physiological advantage such as hastened plant rehydration through concomitant decreases in E_n . Nocturnal transpiration, assessed in this study under mild spring-time conditions, was generally about 12% of E_d . This is similar to other species grown in wetter environments (Caird *et al.*, 2007; Dawson *et al.*, 2007) and less than the 40–75% found in desert species (Ogle *et al.*, 2012). Substantial night-time transpiration as a consequence of elevated g_n in warm night-time root-zones may prevent complete plant rehydration prior to dawn (Kavanagh *et al.*, 2007), resulting in greater water stress during the day as evaporative demand increases. Because our plants were well watered in all treatments with soil moistures not dropping below 20%, soil moisture was not a stressor. In grapevines, wilting tendrils at the shoot tips are the first signs of water stress (Winkler *et al.*, 1974) and we did not observe this in any of our plants. In a field situation, however, under the absence of irrigation or under reduced water allocations, plants may experience daytime water stress due to elevated night-time soil temperatures, especially in warm, dry climates. It follows then that daily minimum soil temperature may be just as critical as daily maximum or average soil temperature in determining plant water relations.

Absence of acclimation

One of most significant findings of this study was that stomatal conductance remained low in the cool root-zones throughout the 2-month treatment period, with apparently no temperature acclimation. Sustained reduction in g may be required over weeks if plants are to prevent dehydration, especially with rising midday and nocturnal VPD driving E as the season progresses.

FIG. 5. Midday leaf g_d , E_d , A and C_i in response to VPD ($n = 4$) of vines grown in either cool or warm soil. At the time of measurement, root-zone temperature of the warm treatment was 20.3 ± 0.9 °C and of the cool treatment was 13.3 ± 0.7 °C ($P < 0.001$). Soil moisture was not different between the treatments (F -test, $P = 0.50$) and averaged 16.8 ± 0.5 %. The intercepts and slopes of the regressions for the cool and warm cohorts are different from each other for E_d ($P < 0.001$), but only the intercept for g_d ($P < 0.001$) and A ($P < 0.001$). A non-linear curve was fitted to the C_i data of the cool root-zone temperature treatment ($P < 0.001$).

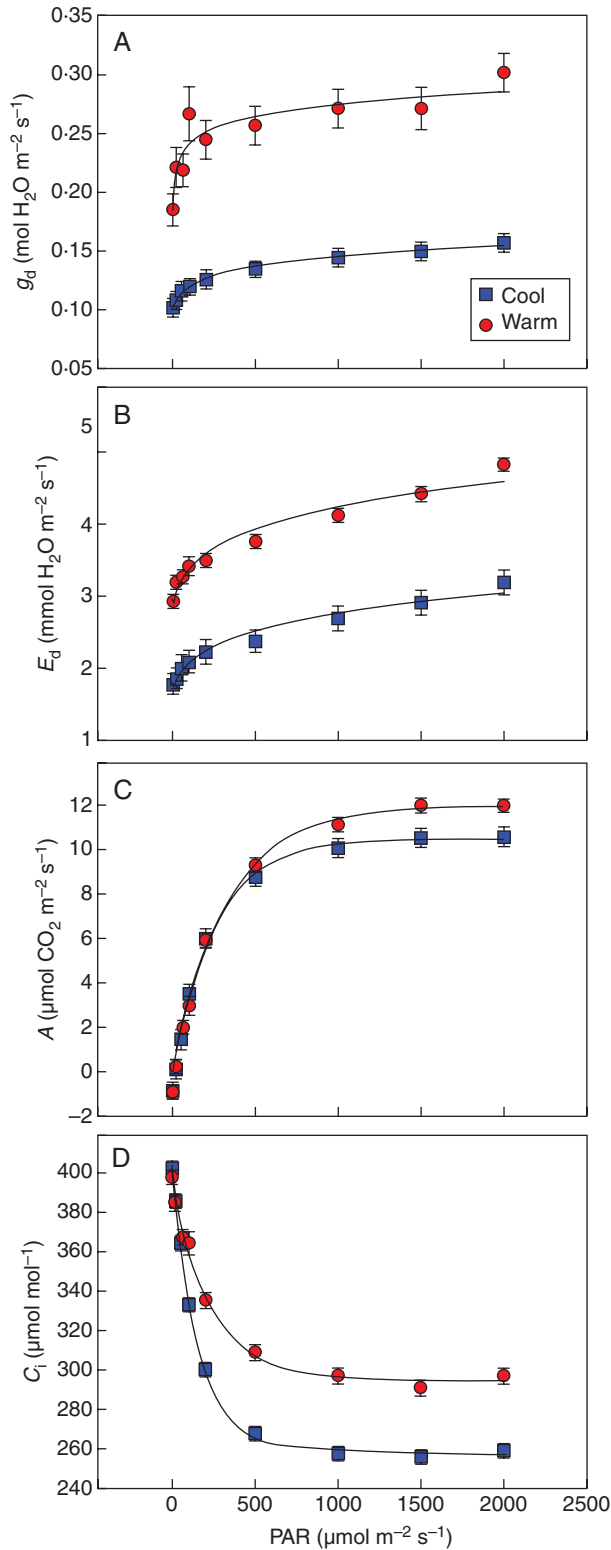


FIG. 6. Midday leaf g_d , E_d , A and C_i in response to PAR ($n = 6$) of vines grown in either cool or warm soil. At the time of measurement, root-zone temperature of the warm treatment was $24.2 \pm 1.1^\circ\text{C}$ and of the cool treatment was $13.3 \pm 0.7^\circ\text{C}$ ($P < 0.001$). Soil moisture was not different between the treatments (F -test, $P = 0.63$) and averaged $17.6 \pm 0.9\%$.

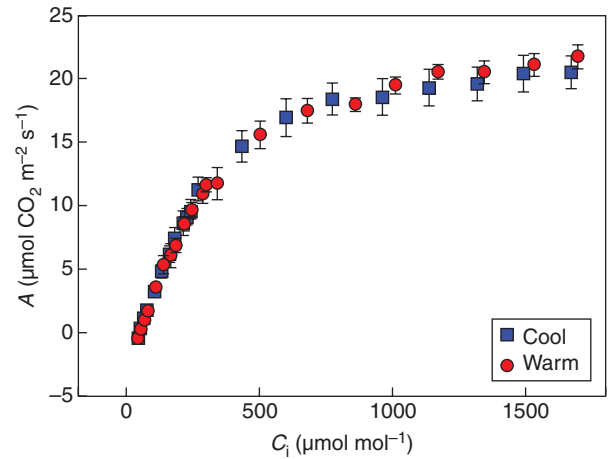


FIG. 7. A/C_i curves of grapevines grown in warm or cool soil ($n = 4$). At the time of measurement, root-zone temperature of the warm treatment was $25.9 \pm 0.8^\circ\text{C}$ and of the cool treatment was $15.1 \pm 1.3^\circ\text{C}$ ($P < 0.001$). Soil moisture was not different between the treatments (F -test, $P = 0.12$) and averaged $18.0 \pm 0.5\%$.

TABLE 4. Stomatal density of leaves that emerged during the root-zone temperature treatments and of those leaves that emerged after the cessation of the treatments

Treatment	Stomatal density (stomata mm^{-2})		Lamina length (mm)	
	During treatments	Following treatments	During treatments	Following treatments
Cool	127.93 ^a	127.04 ^a	83.6 ^a	77.2 ^a
Ambient	123.15 ^{ab}	120.08 ^a	87.0 ^b	79.5 ^b
Warm	116.67 ^b	129.70 ^a	90.6 ^c	81.8 ^c
LSD, $P <$	8.26	9.76	3.0	1.4
F -test, $P <$	0.018	0.127	0.001	0.001

Data during the treatments are the means of leaves up to and including node 6, where gas exchange measurements were made ($n = 5$ plants, four shoots per plant). Data of leaves that emerged after the treatments were discontinued up to node 27. Means followed by different letters are significantly different at $P < 0.05$.

Other factors controlling nocturnal stomatal conductance

Because nocturnal gas exchange has not yet received the same depth of attention as daytime gas exchange, far less is known about the plasticity of g_n to environmental factors. We found that aside from root-zone temperature, soil moisture was another variable that impinged on nocturnal stomatal conductance. Nocturnal stomatal conductance was found to decline with drying soil (Cavender-Bares *et al.*, 2007; Howard and Donovan, 2007) and increasing VPD (Barbour and Buckley, 2007) in other studies. VPD did not explain any of the variance in g_n in our ‘Shiraz’ plants but this may be because the leaf chamber temperature was held constant and the resultant VPD range was fairly narrow.

TABLE 5. Midday leaf A , g_d , E_d and C_i of grapevines after root-zone temperature treatments were discontinued

Treatment	Root-zone temperature (°C)	Soil moisture (%)	A ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	g_d ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	E_d ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	C_i ($\mu\text{mol mol}^{-1}$)
Cool	21.0 ^a	23.6 ^a	10.3 ^a	0.223 ^a	3.70 ^a	286.2 ^a
Ambient	20.2 ^b	20.1 ^b	10.2 ^a	0.225 ^a	3.74 ^a	288.9 ^{ab}
Warm	20.7 ^a	20.0 ^c	10.5 ^a	0.218 ^a	3.66 ^a	283.0 ^b
LSD	0.3	2.1	0.4	0.008	0.14	4.5
F -test, $P <$	0.007	0.001	0.314	0.703	0.573	0.038

Values are means of three sampling dates over a 54-d period after the discontinuation of the treatments ($n = 12$ on each sampling date). Root-zone temperatures and soil moistures represent the means at the time the gas exchange measurements were made. Means followed by different letters are significantly different at $P < 0.05$.

In the first few weeks after bud-burst g_n increased with leaf age, mimicking trends in g_d . Leaf maturity thus appears to be another variable controlling g_n . When leaves first emerge there is a progressive increase in g_d as stomata mature and the stomatal apertures expand (England and Attiwill, 2011) and we reason that a similar developmental effect persists on g_n during the night. We did not measure g_n during the latter half of the season but ageing and senescing leaves often have lower g_d and thus probably g_n as well, but this is yet to be verified. Other factors that might affect g_n and E_n include developmental stage of the plant and plant nutrient status (Ludwig *et al.*, 2006).

Root-zone temperature regulates photosynthesis

Rates of CO_2 assimilation were highest in the warm and lowest in the cool root-zones, and this was apparent across a wide range in PAR, VPD and air temperatures. The decline in C_i values under cool root-zone temperatures points to a stomatal limitation and therefore a curtailed CO_2 supply to photosynthesis under these conditions. There were no apparent limitations at the chloroplast level as chlorophyll content and both J_{max} and $V_{\text{c,max}}$ did not differ between the treatments. This indicates that the low photosynthesis of the cool root-zones was not due to Rubisco or RuBP limitations. Cai and Dang (2002) found that root-zone temperature had an effect on both J_{max} and $V_{\text{c,max}}$ in conifer species, although a much wider temperature range (5–35 °C) was employed. These parameters had a maximum at around 25 °C, declining at supra-optimal temperatures. Similar to our grapevines, dark respiration was not affected by root-zone temperature in any of the four species tested.

The key point is that soil temperature did influence A , and furthermore A was more sensitive to soil temperature than to an air temperature range of 20–35 °C. Similarly, photosynthesis of red spruce saplings grown in a cold region was limited more by minimum soil temperature than minimum air temperature, especially in spring (Schwarz *et al.*, 1997). If this is the case for grapevines grown in cool regions, it would be advantageous for the plant to direct root growth to a depth where diurnal temperature fluctuations are dampened. When field grapevines are drip irrigated, however, most of the roots can be found in the top layers (Stevens and Douglas, 1994) and therefore they are exposed to significant fluctuations in temperature. Natural daily cycling in temperature is often

overlooked in root-zone studies (Pregitzer *et al.*, 2000), but to mimic field conditions we allowed soil temperature to fluctuate diurnally by approximately 5 °C. Diurnal soil temperature fluctuations would also be relevant to newly established seedlings with small root systems as well as shallow-rooted species growing on impenetrable soils.

Transpiration efficiency responds to root-zone temperature

Despite greater A in warm root-zones, both A/g and A/E were inversely proportional to root-zone temperature. In other words, leaves of plants grown in the warmer root-zones lost greater amounts of water per unit of carbon gained. This is similar to the drop in transpiration efficiency of grapevine varieties grown in high VPD (Düring, 1987; Pou *et al.*, 2008) or ample soil moisture (Cuevas *et al.*, 2006). There is wide varietal diversity in transpiration efficiency of grapevines (Schultz, 1996; Bota *et al.*, 2001; Gibberd *et al.*, 2001; Rogiers *et al.*, 2009). While the adoption of irrigation technology that confers water savings has improved water-use efficiency at the vineyard level, further gains can be made by planting inherently efficient varieties (Condon *et al.*, 2004). Varietal selection therefore must not only take into consideration VPD and soil moisture responses but, as shown here, soil temperature as well.

Stomatal density responds to root-zone temperature

Stomatal density of concurrently formed leaves was lowest in the warm root-zone treatment and highest in the cool treatment. It would seem intuitive that the high stomatal densities of the cold treatment might lead to higher g_d and g_n , but as is apparent here, there was no such relationship. The low g characteristic of the cool root-zone treatment is therefore not due to low stomatal density and must be related to stomatal aperture only.

In a previous study on ‘Chardonnay’ grapevines it was found that root-zone temperature and atmospheric carbon dioxide both impinged on stomatal density (Rogiers *et al.*, 2011a). Because this leaf parameter was closely and inversely correlated with starch concentration in roots and trunks we suggested that the carbohydrate reserve status of the plant may be an important endogenous determinant of stomatal density. Depleted starch reserves, elicited by several weeks of high metabolism in the warm root-zones, would require

replenishment and indeed we find that, in the current study, stomatal density of newly emerging leaves of grapevines grown in warm soil increased after the treatment had been removed.

Summary

We report that E_n of anisohydric grapevines can be a small (12 %) but significant part of daily transpiration. Similar to g_d , g_n was responsive to root-zone temperature and thus those regulatory signals that are operative during the day also persist during the night. Removal of the cool or warm treatments brought g back to ambient levels, indicating the presence of a system that is able to react to environmental conditions. The limitation in photosynthesis induced by cool root-zones was the result of a stomatal limitation rather than limitations at the chloroplast level, and accompanying increases in stomatal density brought on by the cool root-zones were not able to offset this stomatal limitation. Intrinsic transpiration efficiency declined with increasing root-zone temperature. Therefore, it is likely that carbon assimilation will occur at the expense of a high water loss when anisohydric grapevines are grown in warm climates with warm soils.

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