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Original Article

Is stomatal conductance optimized over both time and space in plant crowns? A field test in grapevine (*Vitis vinifera*)

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ABSTRACT

Crown carbon gain is maximized for a given total water loss if stomatal conductance (g_s) varies such that the marginal carbon product of water ($\partial A/\partial E$) remains invariant both over time and among leaves in a plant crown, provided the curvature of assimilation rate (A) versus transpiration rate (E) is negative. We tested this prediction across distinct crown positions *in situ* for the first time by parameterizing a biophysical model across 14 positions in four grapevine crowns (*Vitis vinifera*), computing optimal patterns of g_s and E over a day and comparing these to the observed patterns. Observed water use was higher than optimal for leaves in the crown interior, but lower than optimal in most other positions. Crown carbon gain was 18% lower under measured g_s than under optimal g_s. Positive curvature occurred in 39.6% of cases due to low boundary layer conductance (g_{bw}), and optimal g_s was zero in 11% of cases because $\partial A/\partial E$ was below the target value at all g_s. Some conclusions changed if we assumed infinite g_{bw}, but optimal and measured E still diverged systematically in time and space. We conclude that the theory's spatial dimension and assumption of positive curvature require further experimental testing.

Key-words: boundary layer; carbon water balance; optimization; stomata; water-use efficiency.

INTRODUCTION

Water is a major factor limiting plant growth and carbon sequestration in both natural and agricultural systems. To predict and manage these systems and to direct basic research into the underlying biological controls, we need formal mathematical models that can both predict and explain how carbon and water exchange are coordinated and regulated by stomatal conductance (g_s). However, no process-based model of g_s that can achieve this has yet gained consensus, and phenomenological models merely reproduce observed patterns of g_s, so they have limited ability to explain stomatal behaviour (Damour *et al*. 2010; Buckley & Mott 2013). Another approach, optimization theory, attempts to deduce g_s from the hypothesis that stomatal behaviour tends to maximize carbon gain (net CO_2 assimilation rate, A) for a given water loss (transpiration rate, E) (Cowan & Farquhar 1977). The rationale for this hypothesis is that natural selection has presumably favoured genotypes with more nearly optimal use of limiting resources, including water (Cowan & Farquhar 1977; Cowan 2002; Mäkelä *et al*. 2002).

Formally, the optimization hypothesis states that, among all possible spatiotemporal distributions of g_s that yield the same total transpiration rate, total carbon gain will be greatest for the distribution in which the ratio of the marginal sensitivities of A and E to g_s [$(\partial A/\partial g_s)/(\partial E/\partial g_s)$, often abbreviated as $\partial A/\partial E$ and referred to in this study as the *marginal carbon product of water*] is invariant within the domain in which total transpiration rate can be considered constant (Cowan & Farquhar 1977). That domain is typically taken to be one day (at longer time scales, the total water supply available to the canopy, and with it the target value μ for $\partial A/\partial E$, may change). This result assumes that the A versus E curve generated by varying g_s has negative curvature; that is, $\partial A/\partial E$ always declines when E increases by stomatal opening ($\partial^2 A/\partial E^2 < 0$). Pioneering work by Farquhar (1973) and Cowan & Farquhar (1977) showed that the patterns of stomatal behaviour predicted by this hypothesis share important qualitative features with the observed behaviour, including reduced g_s under high evaporative demand or low light (photosynthetic photon flux density, PPFD).

The subsequent four decades have seen this theory tested many times – most commonly in relation to controlled variations in individual environmental variables such as evaporative demand, but also in relation to natural variation in environmental conditions *in situ* (e.g. Farquhar *et al*. 1980a; Meinzer 1982; Williams 1983; Ball & Farquhar 1984; Küppers 1984; Sandford & Jarvis 1986; Guehl & Aussenac 1987; Fites

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& Teskey 1988; Berninger *et al*. 1996; Hari *et al*. 1999; Thomas *et al*. 1999; Schymanski *et al*. 2008; Way *et al*. 2011). However, two critical elements of the original theory remain largely untested: neither its spatial dimension – that is, the prediction that $\partial A/\partial E$ should not vary among leaves at distinct crown positions within the same individual – nor the assumption that $\partial^2 A/\partial E^2 < 0$ have ever been tested in the field. The prediction that the target value of $\partial A/\partial E$ should be the same for all leaves in the canopy follows from the premise that the plant has a single total water supply, and the ability, in principle, to distribute water arbitrarily among leaves. The original Cowan–Farquhar theory does not distinguish temporal and spatial variations in $\partial A/\partial E$, either of which will reduce whole-canopy carbon gain (provided $\partial^2 A/\partial E^2 < 0$). Furthermore, few tests have accounted for variations in mesophyll and boundary layer conductances (g_m and g_{bc}, respectively), both of which restrict CO_2 diffusion and can strongly influence the predictions and assumptions of optimization theory (Buckley *et al*. 1999, 2013; Buckley & Warren 2014).

The objective of this study was to test the spatial dimension of the optimization hypothesis and its assumption of negative curvature in A versus E, while accounting for mesophyll and boundary layer conductances. We parameterized a biochemical gas exchange model (which included mesophyll conductance and its temperature response) for one leaf at each of 14 standardized positions in each of the four individual crowns of grapevine (*Vitis vinifera* L. var. Grenache) and then monitored *in situ* environmental conditions and stomatal conductance for each of those leaves over time across a single day. We used these data to test the theory's assumption that $\partial^2 A/\partial E^2 < 0$, to infer the optimal spatiotemporal distributions of g_s (and E) and to compare the inferred optimal patterns with the observed patterns.

MATERIALS AND METHODS

Study system

This study was conducted from 17 to 24 August 2012 in the experimental field of the University of Balearic Islands during summer 2012 on grapevines of Grenache varietal. Soil was a clay loam type 1.5 m deep. Plants were 3 years old grafted on rootstock Richter-110 and planted in rows (distance between rows was 2.5 m and between plants, 1 m). Plants were situated in a bilateral double cordon having between 10 and 12 canes per plant. Plants had been irrigated throughout the summer with 9.0 L per plant per day, an amount that had been established as adequate to sustain high plant water status in a previous experiment. Pre-dawn water potential of plants on the day of *in situ* gas exchange measurements (22 August 2012) was –0.24 ± 0.06 MPa.

Four plants and 14 crown positions of each plant were selected for gas exchange measurements. Four of these positions were on the east face of the crown (positions 1–4), two were on the top of the crown (positions 5 and 6), four were on the west face (positions 7–10) and four were located in the inner part of the crown (positions 11–14). These crown positions are illustrated in Fig. 1.

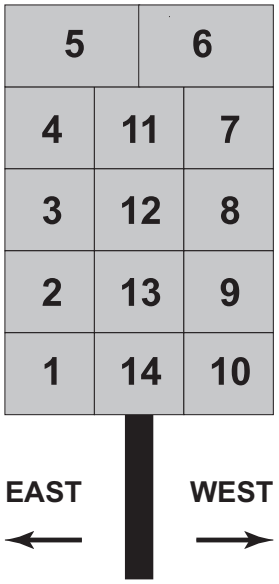

Figure 1. Diagram illustrating the 14 crown positions at which leaf gas exchange was measured in this study. The diagram represents a cross section of the grapevine crown, looking southward along the long axis of a planting row, with east (sunrise) to the left and west (sunset) to the right. Positions 1–10 are on the crown exterior, and positions 11–14 are in the crown interior.

Meteorological measurements

A meteorological station (Meteodata-3000) located in the experimental field with sensors of wind speed (Young 81000, R.M. Young Company, Traverse City, MI, USA) and air temperature and relative humidity (Young 41382, Young Company) was used. The height of the wind speed sensor was 2.7 m above the soil (approximately 0.5 m above the upper part of the canopy).

Biophysical gas exchange model

We used the photosynthesis model of Farquhar *et al*. (1980b) and the gas exchange equations of von Caemmerer & Farquhar (1981) to simulate CO_2 and H_2O exchange in grapevine. Briefly, the net CO_2 assimilation rate due to biochemical demand (A_d) is computed from RuBP-carboxylation-limited and RuBP-regeneration-limited rates (A_v and A_j) (a list of symbols is given in Table 1):

$$A_v = V_m \frac{c_c - \Gamma_*}{c_c + K_c(1 + O/K_o)} - R_d, \quad (1)$$

$$A_j = \frac{1}{4} J \frac{c_c - \Gamma_*}{c_c + 2\Gamma_*} - R_d, \quad (2)$$

where V_m is the carboxylation capacity, J is the potential electron transport rate, c_c is the chloroplastic CO_2 concentration, Γ_* is the photorespiratory CO_2 compensation point, K_c and K_o are the Michaelis constants for RuBP carboxylation and oxygenation, respectively, O is the oxygen concentration

Table 1. List of variables and parameters referred to in this study, including symbols, units and values where appropriate

Variable	Symbol	Units	Value
Net CO_2 assimilation rate	A	μmol m^{-2} s^{-1}	Varies
Leaf absorptance to photosynthetic photon flux	α	–	0.92
Demand or supply limited value of A	A_d, A_s	μmol m^{-2} s^{-1}	Varies
RuBP-carboxylation or regeneration-limited value of A_d	A_v, A_j	μmol m^{-2} s^{-1}	Varies
Ambient CO_2 mole fraction	c_a	μmol mol^{-1}	400
Intercellular or chloroplastic CO_2 mole fraction	c_i, c_c	μmol mol^{-1}	Varies
Molar heat capacity of air	c_p	J mol^{-1} K^{-1}	29.2
Curvature of A versus E relationship	$\partial^2 A/\partial E^2$	μmol m^2 s mmol^{-2}	Varies
Saturation vapour pressure deficit of air	D_a	Pa	Varies
Marginal carbon product of water	$\partial A/\partial E$	μmol mmol^{-1}	Varies
Leaf characteristic dimension	d_{leaf}	m	0.1
Effective leaf-air water vapour mole fraction gradient	Δw	mmol mol^{-1}	Varies
Leaf transpiration rate	E	mmol m^{-2} s^{-1}	Varies
Leaf emissivity to IR	ε_{leaf}	–	0.95
Fraction of absorbed photons that do not contribute to photochemistry	f	–	0.23
Absorbed shortwave radiation	Φ	J m^{-2} s^{-1}	Varies
Fraction of infrared radiation that comes from the sky	f_{ir}	–	Varies
Psychrometric constant	γ	Pa K^{-1}	66.0
Photorespiratory CO_2 compensation point (at 25 °C)	Γ_* (Γ_{*25})	μmol mol^{-1}	Varies (36.2)
Leaf boundary layer conductance to heat, water or CO_2	g_{bh}, g_{bw}, g_{bc}	mol m^{-2} s^{-1}	Varies
Mesophyll conductance to CO_2	g_m	mol m^{-2} s^{-1}	Varies
Radiation conductance	g_{Rn}	mol m^{-2} s^{-1}	Varies
Stomatal conductance to water or CO_2	g_s, g_{sc}	mol m^{-2} s^{-1}	Varies
Maximum stomatal conductance	g_{smax}	mol m^{-2} s^{-1}	Varies
Optimal stomatal conductance	g_{so}	mol m^{-2} s^{-1}	Varies
Total leaf conductance to water or CO_2	g_{tw}, g_{tc}	mol m^{-2} s^{-1}	Varies
Potential electron transport rate	J	μmol m^{-2} s^{-1}	Varies
Light-limited (capacity-saturated) value of J	J_i	μmol m^{-2} s^{-1}	Varies
Capacity-limited (light-saturated) value of J (at 25 °C)	J_m (J_{m25})	μmol m^{-2} s^{-1}	Varies
Michaelis constant for RuBP carboxylation or oxygenation	K_c, K_o	μmol mol^{-1}	Varies
Canopy extinction coefficient for diffuse irradiance	k_d	–	0.8
Cumulative leaf area index	L	m^2 m^{-2}	Varies
Target value for $\partial A/\partial E$	μ	μmol mmol^{-1}	1.28–1.59
Mole fraction of oxygen	O	μmol mol^{-1}	2.1×10^5
Atmospheric pressure	P_{atm}	Pa	1.0×10^5
Photosynthetic photon flux density	PPFD	μmol m^{-2} s^{-1}	Varies
Curvature parameter for relationship of A_d to A_v and A_j	θ_A	–	0.99
Curvature parameter for relationship of J to J_m and J_i	θ_J	–	0.90
Non-photorespiratory CO_2 release (at 25 °C)	R_d (R_{d25})	μmol m^{-2} s^{-1}	Varies
Net isothermal radiation	R_n^*	J m^{-2} s^{-1}	Varies
Stefan–Boltzmann constant	σ	J m^{-2} s^{-1} K^{-4}	5.67×10^{-8}
Air temperature (in Kelvin)	T_{air} ($T_{air,K}$)	°C (K)	Varies
Leaf temperature	T_{leaf} ($T_{leaf,K}$)	°C (K)	Varies
Carboxylation capacity (at 25 °C)	V_m (V_{m25})	μmol m^{-2} s^{-1}	Varies
Wind speed	v_{wind}	m s^{-1}	Varies
Water vapour mole fraction of intercellular spaces or air	w_i, w_a	mmol mol^{-1}	Varies

and R_d is the rate of non-photorespiratory CO_2 release. Actual assimilation rate is calculated as the hyperbolic minimum of A_v and A_j [the lesser root A_d of $\theta_A A_d^2 - A_d(A_v + A_j) + A_v A_j = 0$, where θ_A is a dimensionless curvature parameter less than unity]; this accounts for co-limitation by both carboxylation and regeneration near the transition between the two limitations, and it smoothes the transition, ensuring differentiability as required for continuous optimization. We calculated J as the hyperbolic minimum of light-limited and light-saturated rates, J_m and J_i [the lesser root J of $\theta_J J^2 - J(J_m + J_i) + J_m J_i = 0$; $J_i = 0.5\alpha(1 - f)\cdot$PPFD, α is the leaf absorptance to photosynthetic irradiance and f is the fraction of absorbed photons that do not contribute to photochemistry].

The supply of CO_2 by diffusion to the sites of carboxylation (A_s) was modelled as

$$A_s = g_{tc}(c_a - c_c), \tag{3}$$

where g_{tc} is total conductance to CO_2, given by

$$g_{tc} = \left(g_{sc}^{-1} + g_{bc}^{-1} + g_m^{-1}\right)^{-1}, \tag{4}$$

where g_{sc} is the stomatal conductance to CO_2 (g_s/1.6, where g_s is the stomatal conductance to H_2O), g_{bc} is the boundary layer

conductance to CO_2 and g_m is the mesophyll conductance to CO_2. At steady state, the supply and demand rates are equal ($A_d = A_s$), so the actual net CO_2 assimilation rate, A, is given by the intersection of A_d and A_s:

$$A = A_d \cap A_s. \tag{5}$$

This intersection leads to a quartic (fourth-order polynomial) expression for c_c, whose coefficients are functions of the parameters in Eqns 1–3, and which is readily solved for c_c (e.g. Abramowitz & Stegun 1972). Transpiration rate (E) is given by

$$E = g_{tw}\Delta w, \tag{6}$$

where

$$g_{tw} = \left(g_s^{-1} + g_{bw}^{-1}\right)^{-1}, \tag{7}$$

$$\Delta w = \frac{w_i - w_a}{1 - \frac{1}{2}\cdot 0.001\cdot(w_i + w_a)} \tag{8}$$

in which g_{bw} is the boundary layer conductance to H_2O and w_i and w_a are the water vapour mole fractions in the intercellular spaces and the ambient air, respectively. We assumed that the air spaces were saturated with water vapour, so that w_i was given by

$$w_i = 6.112\cdot\exp[17.62\cdot T_{leaf}/(243.13 + T_{leaf})]/P_{atm}, \tag{9}$$

where T_{leaf} is the leaf temperature in °C (World Meteorological Organization 2008). The expression in the numerator of Eqn 9 gives the saturation partial pressure of water, and P_{atm} is the total atmospheric pressure. We estimated *in situ* leaf temperature using the isothermal net radiation approximation as described by Leuning *et al.* (1995) and modified to molar units:

$$T_{leaf} = T_{air} + \frac{\gamma R_n^*/c_p - D_a g_{tw}}{s g_{tw} + \gamma(g_{bh} + g_{Rn})}, \tag{10}$$

where T_{air} is the air temperature, γ is the psychrometric constant, c_p is the molar heat capacity of air, D_a is the saturation vapour pressure deficit of air and s is the derivative of saturation vapour pressure with respect to temperature. g_{Rn} is the radiation conductance, given by

$$g_{Rn} = 4\varepsilon_{leaf} k_d f_{ir}\sigma T_{air}^3/c_p, \tag{11}$$

where ε_{leaf} is the leaf emissivity to longwave radiation, σ is the Stefan–Boltzmann constant, k_d is the canopy extinction coefficient for diffuse irradiance (0.8; Leuning *et al.* 1995) and f_{ir} is the fraction of the leaf's incoming infrared radiation that comes directly from the sky. In simulations on horizontally continuous canopies, f_{ir} is generally taken as $\exp(-k_d L)$, where L is the cumulative leaf area index (e.g. Leuning *et al.* 1995). We computed f_{ir} in this fashion for interior crown leaves (positions 11–14); for positions on the lateral crown exterior (positions 1–4 and 7–10), we computed f_{ir} as the fraction of each leaf's upwards sky view occupied by actual sky rather than by the adjacent canopy [β/180, where β (degrees) is the angle at which sky appears above the adjacent canopy, as viewed from the crown position in question]. We used $f_{ir} = 1.0$ for the two positions at the top of the crown (positions 5 and 6). R_n* is the isothermal net radiation, given by

$$R_n^* = \Phi - (1 - \varepsilon_{atm}) k_d f_{ir}\sigma T_{air,K}^4, \tag{12}$$

where Φ is the absorbed shortwave radiation, ε_{atm} is the atmospheric emissivity to longwave radiation, given by $0.642\cdot(0.001\cdot P_{atm}\cdot w_a/T_{air,K})^{1/7}$ for P_{atm} in Pa and w_a in mmol mol^{-1} (Leuning *et al.* 1995), and $T_{air,K}$ is T_{air} in Kelvin. Note that this assumes a canopy IR emissivity of unity. We calculated Φ by assuming that incident shortwave radiation was equal to 0.5666·PPFD (0.5666 is the ratio of total shortwave energy to photosynthetic photon flux in extraterrestrial solar radiation; de Pury & Farquhar 1997), and that this radiation was half visible and half near-infrared (Leuning *et al.* 1995), with leaf absorptances of 0.92 and 0.2, respectively (0.92 was the mean observed PAR absorptance of leaves in this study, and 0.2 is the complement of NIR reflection and transmission coefficients, both of which are approximately 0.4; Gates *et al.* 1965). This gives $\Phi = (0.5\cdot 0.92 + 0.5\cdot 0.2)\cdot 0.5666\cdot\text{PPFD} = 0.3173\cdot\text{PPFD}$.

Equation 10 requires a value for boundary layer conductances to heat (g_{bh}) and water [g_{bw}, which is embedded in g_{tw} (Eqn 7)], and Eqn 4 requires boundary layer conductance to CO_2 (g_{bc}). We assumed $g_{bc} = g_{bw}/1.37$ and $g_{bw} = 1.08\cdot g_{bh}$ and simulated g_{bh} using an expression based upon forced (wind-driven) convection (Leuning *et al.* 1995):

$$g_{bh} = 0.123(v_{wind}/d_{leaf})^{0.5}, \tag{13}$$

where v_{wind} is the wind speed and d_{leaf} is the leaf's characteristic dimension (approximately equivalent to its average downwind width; 0.1 m in this study). This ignores the possibility of free convection driven by buoyancy of air warmed by the leaf. However, most available data and theoretical studies suggest that free convection contributes only negligibly to heat exchange under natural conditions, even at very low wind speeds, and that modelling g_{bh} based upon forced convection alone provides accurate predictions (Leuning 1988; Brenner & Jarvis 1995; Grantz & Vaughn 1999; Roth-Nebelsick 2001). We simulated the attenuation of wind speed through the canopy profile by

$$v_{wind} = v_{wind(top)}\cdot\exp(-0.5L), \tag{14}$$

where L is the cumulative leaf area index (m^2 m^{-2}) and $v_{wind(top)}$ is the wind speed measured above the canopy. To calculate L for each canopy position, we summed the leaf area index of all canopy regions (as defined by Fig. 1) above that position. To measure those leaf area indices, we measured the total leaf area in each canopy region for each of six individuals and then divided these areas by the projected areas of each region to give the leaf area index contributed by

that region. The resulting values of L are as follows, for positions 1–14, respectively: 3.1, 2.6, 2.1, 1.8, 0, 0, 1.5, 1.8, 2.4, 2.7, 1.6, 2.1, 2.6 and 3.3.

Parameterizing the gas exchange model

We estimated photosynthetic parameters for each of 56 leaves (four individuals × 14 canopy positions) as follows. We measured the response of leaf net CO_2 assimilation rate (A) to intercellular CO_2 mole fraction (c_i) using an open flow gas exchange system (Li-6400; Li-Cor, Inc., Lincoln, NE, USA) equipped with an integrated leaf chamber fluorometer (Li-6400-40; Li-Cor). Curves were performed under saturating light (1500 μmol m^{-2} s^{-1}), with block temperature controlled at 30 °C. Ambient CO_2 (c_a) was set between 50 and 1600 μmol mol^{-1} and chamber humidity was set to track ambient conditions. After steady-state photosynthesis was reached, c_a was lowered stepwise from 400 to 50 μmol mol^{-1}, returned to 400 μmol mol^{-1} and increased stepwise to 1600 μmol mol^{-1}. A total of 16 points were recorded for each curve. We then estimated g_m, V_m and J_m by the curve fitting method proposed by Ethier & Livingston (2004). To simulate changes in these parameters with temperature, we corrected these values to 25 °C (as g_{m25}, V_{m25} and J_{m25}, respectively) using temperature responses measured on leaves of the same variety, grown in pots at the same site and transported to the laboratory to allow plants to acclimate to constant temperature and other atmospheric conditions. Temperature responses were measured by repeating CO_2 response curves at 15, 20, 25, 30, 35 and 40 °C, using the same protocol described above but with the expanded temperature control kit (Li-6400-88; Li-Cor) added to the gas exchange system. The temperature response data are shown in Fig. 2. Temperature response functions were as follows:

$$V_m(T_{leaf,K}) = V_{m25} \cdot \exp[a_v(T_{ref}^{-1} - T_{leaf,K}^{-1})], \quad (15)$$

$$J_m(T_{leaf,K}) = J_{m25} \cdot \exp[a_j(T_{ref}^{-1} - T_{leaf,K}^{-1})] \cdot \left\{\frac{1+\exp(b_j)}{1+\exp[b_j + c_j(T_{ref}^{-1} - T_{leaf,K}^{-1})]}\right\}, \quad (16)$$

$$g_m(T_{leaf}) = g_{m25} \cdot \exp\{-d[\ln(T_{leaf}/T_{opt})]^2\}, \quad (17)$$

where T_{ref} = 298.15 K, $T_{leaf,K}$ is the leaf temperature in Kelvin, and a_v, a_j, b_j, c_j, d and T_{opt} are empirical parameters: a_v = 7350.45 K, a_j = 6710.22 K, b_j = –2.15188 (unitless), c_j = 13 807.8 K, d = 0.71027 (unitless) and T_{opt} = 36.75 °C. Other parameters were taken from literature: 25 °C values and temperature responses for Rubisco kinetic parameters (K_c and K_o) and the photorespiratory CO_2 compensation point (Γ_*) were taken from Bernacchi *et al.* (2003). Non-photorespiratory CO_2 release in the light at 25 °C (R_{d25}) was estimated from photosynthetic response curves as $0.0089 \cdot V_{m25}$ according to de Pury & Farquhar (1997), and the temperature response of R_d was taken from Bernacchi *et al.* (2003).

Measuring leaf gas exchange *in situ*

At each of five times on a given day (approximately 0915, 1100, 1345, 1600 and 1830, CEDT), we used an open flow gas exchange system (Li-6400; Li-Cor) equipped with a clear chamber (Li-6400-08) to obtain a 30 s average measurement of stomatal conductance and incident PPFD on each of the leaves for which we had previously estimated photosynthetic parameters as described earlier. Prior to each measurement, we observed the leaf's orientation, and orientated the chamber such that the PPFD sensor surface was parallel to the original plane of the leaf; this ensured that the PPFD thus measured was very similar to the PPFD actually experienced by the leaf prior to measuring g_s. c_a was set at 400 μmol mol^{-1} and chamber air temperature and humidity were set to match ambient. Of the 280 expected measurements (5 times × 14 positions × 4 individuals), 10 were lost due to clerical errors, leaving 270 measurements.

Computing $\partial A/\partial E$

We calculated $\partial A/\partial E$ numerically, as follows. We computed A and E from the gas exchange model outlined earlier, added a very small increment (1.0×10^{-6} mol m^{-2} s^{-1}) to stomatal conductance and estimated $\partial A/\partial E$ as the ratio of the resulting increases in A and E. This ensured that changes in leaf temperature (T_{leaf}) resulting from the simulated increment in g_s, and the effects of those temperature changes on both A and E, would be calculated accurately (analytical description of the effects of changing T_{leaf} would be overly complex and prone to error, due to the many photosynthetic parameters

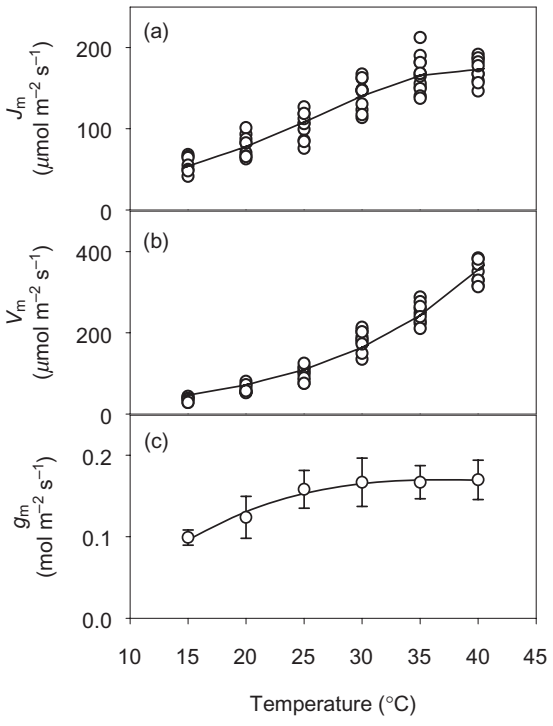

Figure 2. Temperature responses for photosynthetic parameters measured in grapevine for this study (symbols) and response curves fitted to these measurements (lines; Eqns 15–17 in the main text). (a) Electron transport capacity, J_m; (b) carboxylation capacity, V_m; and (c) mesophyll conductance to CO_2, g_m.

affected by T_{leaf}). We verified that this numerical approach did not suffer from discretization error by computing $\partial A/\partial E$ both numerically and analytically (using expressions given by Buckley *et al*. 2002) while holding leaf temperature constant; the two resulting values of $\partial A/\partial E$ were indistinguishable (not shown).

Computing optimal stomatal conductance

For each point in time at each crown position, we computed optimal stomatal conductance as follows. Firstly, we generated the theoretical A versus E relationship for that point by varying g_s from 2.0×10^{-4} to 2.0 mol m^{-2} s^{-1} in 10 000 steps. We then classified each point into one of three categories based upon the nature of the resulting A versus E relationship. In category I, $\partial A/\partial E$ declines monotonically as g_s increases (i.e. $\partial^2 A/\partial E^2 < 0$). In category II, $\partial A/\partial E$ increases at low g_s, reaches a maximum and then decreases at higher g_s (i.e. $\partial^2 A/\partial E^2 > 0$ at low g_s and $\partial^2 A/\partial E^2 < 0$ at high g_s). In category III, $\partial A/\partial E$ is below its crown-wide target value (μ, discussed below) for all positive g_s (typically because PPFD is quite low or Δw is quite high). Examples of relationships between g_s and $\partial A/\partial E$ for four randomly chosen instances of each category are shown in Fig. 3a.

Identification of optimal g_s (g_{so}) differs for each of these categories. For category III, g_{so} is zero. The category most clearly relevant to the original Cowan–Farquhar theory is category I; in this case, g_{so} is the value of g_s for which $\partial A/\partial E$ equals a target value, μ, that is invariant among leaves in the crown and over time (the choice of μ is discussed below). For category II, there exists a realistic positive g_s that maximizes instantaneous water-use efficiency, WUE = A/E; this occurs when $A/E = \partial A/\partial E$ (Buckley *et al*. 1999) (Fig. 3b). WUE is always greater at that value of g_s than for any other value, including any value (or values) for which $\partial A/\partial E = \mu$. However, although this value of g_s would maximize WUE for a category II leaf considered by itself, it is not optimal for the crown as a whole. For example, imagine a category I leaf and a category II leaf both initially at $\partial A/\partial E = \mu$ (Fig. 4a). Consider the effect of reducing E and g_s in the category II leaf in order to bring it to the point of maximum WUE, where $\partial A/\partial E = A/E$, and redistributing the water thus saved to the category I leaf (Fig. 4b). The total change in assimilation rate resulting from this redistribution is

$$\delta A_{total} = \int \left(\frac{\partial A}{\partial E_I} - \frac{\partial A}{\partial E_{II}} \right) dE, \quad (18)$$

where the subscripts I and II refer to variables in the category I and II leaves, respectively. Because $\partial A/\partial E$ is greater in the category II leaf than in the category I leaf across the range of g_s spanning this redistribution (Fig. 4b), the integrand in Eqn 18 is negative, so the net change in assimilation rate for both leaves combined is also negative (Fig. 4c). Thus, the optimal solution when some leaves are in category II is to increase transpiration in those leaves at the expense of other leaves until $\partial A/\partial E$ is invariant among all transpiring leaves.

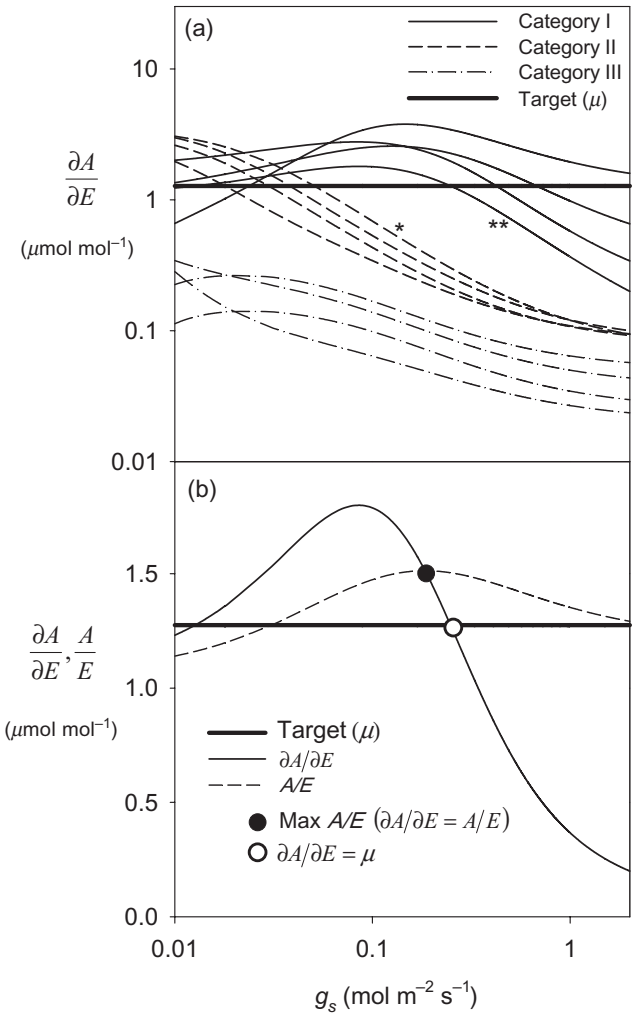

Figure 3. (a) Relationships between the marginal carbon product of water and g_s for four randomly chosen leaves in each of category I (for which $\partial^2 A/\partial E^2 < 0$; dashed lines) and category II (for which $\partial^2 A/\partial E^2 > 0$ at low g_s; solid lines); the thick black horizontal line represents the target value for $\partial A/\partial E$ (μ). (b) The relationships between g_s and $\partial A/\partial E$ (solid line) and instantaneous water-use efficiency, A/E (dashed line) for one leaf in category II, showing that A/E is maximized at a lower g_s (left-hand vertical grey line) than the g_s at which $\partial A/\partial E$ equals the crown-wide target value, μ (right-hand vertical grey line). μ is shown by the horizontal black line. [The curves marked with asterisks in (a) also appear in Fig. 4.]

We identified the optimal g_s in both category I and II leaves by searching the array of 10 000 g_s and $\partial A/\partial E$ values in reverse (i.e. beginning at high g_s and proceeding towards low g_s), finding the first point where $\partial A/\partial E > \mu$, and identifying optimal g_s as the average of the two values spanning the change in sign of $\partial A/\partial E$. In 21 instances of category II points (7.8% of all points), maximum WUE occurred at $g_s > 2.0$ mol m^{-2} s^{-1}; in these cases, we set g_{so} to 2.0 mol m^{-2} s^{-1} on the grounds that values greater than that are not physiologically realistic. We compared the resulting distributions of water loss with alternative simulations in which g_{so} was either capped at 1.0 mol m^{-2} s^{-1} or allowed to take on

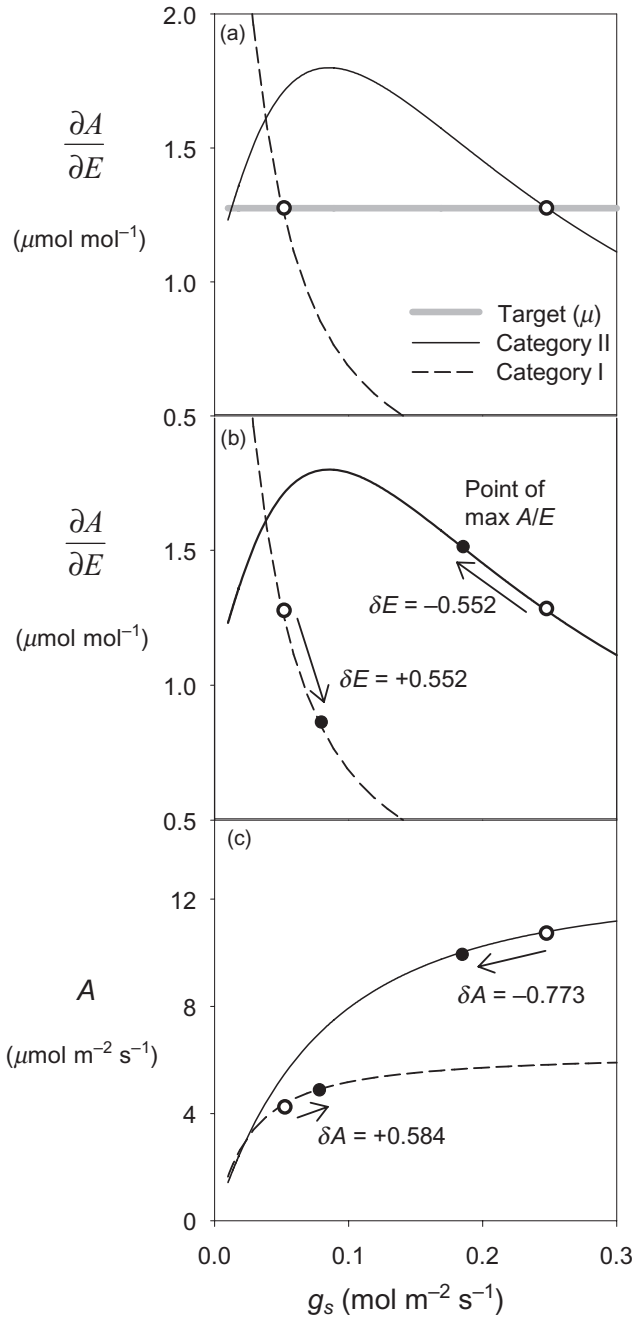

Figure 4. Illustration of the effect of redistributing water loss from a category II leaf (solid lines) to a category I leaf (dashed lines) in order to maximize water-use efficiency (A/E) in the former. (a, open symbols) Initial condition, in which $\partial A/\partial E$ equals the crown-wide target value, μ, for both leaves. (b, closed symbols) Condition after redistribution of water loss (δE = 0.552 mmol m^{-2} s^{-1}) from the category II leaf to the category I leaf. (c) Relationships between net CO_2 assimilation rate, A, and stomatal conductance, g_s, for both leaves, with symbols representing the initial and final conditions as in (a) and (b). The net change in A resulting from redistribution is negative. (Note that the category I and II leaves correspond to the curves marked with one and two asterisks, respectively, in Fig. 3a.)

arbitrarily high values, and the results were nearly identical (not shown); this is because boundary layer conductance (g_{bw}) was typically quite low in those instances, so that E was relatively insensitive to changes in g_s.

We identified the target value for $\partial A/\partial E$ (μ) separately for each individual by adjusting an initial estimate of μ repeatedly (re-optimizing g_s for all measurement points at each value of μ) until the whole-crown diurnal total water loss computed for the optimal pattern of g_s was as close as possible to the total water loss computed for the measured pattern of g_s. Because changes in μ sometimes caused one or more measurement points to change categories, the relationship between μ and total crown water loss was not smooth, so it was not possible to achieve arbitrarily precise agreement in crown total water use between optimal and measured g_s distributions. However, the two values agreed to within 1.53% in all cases and to within 0.21% when summed over all four crowns. To account for the effect of small remaining differences between measured and optimized crown water loss on comparisons of total carbon gain, we applied an approximate correction to total carbon gain: (Corrected optimal crown A) = (Computed optimal crown A) × (Measured crown E)/(Computed optimal crown E).

Numerical methods

All of the calculations described earlier were implemented in Microsoft Excel, in some cases using algorithms coded in VBA and in other cases using worksheet formulas. The Excel file containing the code is available from the authors upon request.

Statistical tests of the optimization hypothesis

We chose to compare transpiration rate, rather than stomatal conductance itself, between optimal and measured patterns, for two reasons. Firstly, mean optimal g_s was many times greater than mean measured g_s in some leaves due to low boundary layer conductance (when g_{bw} is low, E is nearly insensitive to g_s at high g_s), and this made direct comparisons between measured and optimal patterns of g_s somewhat uninformative. Secondly, because optimization theory is concerned with optimal allocation of finite resources, we felt it was more informative to compare distributions of the resource itself (water loss, E) rather than the biological parameter (g_s) that controls how that resource is distributed.

Residuals of E (optimal minus measured E) were distributed highly non-normally (as were the residuals of g_s), and normality could not be adequately improved by any transformation, so we used non-parametric tests (Kruskal–Wallis rank sum test) to assess the probability that observed systematic differences in residual E among crown positions, among times of day, and among times of day at each crown position, were due to chance alone. We also assessed variation in mesophyll conductance (g_{m25}) with crown position using the Kruskal–Wallis test. Variations in photosynthetic capacity (V_{m25} and J_{m25}) were distributed normally and were assessed

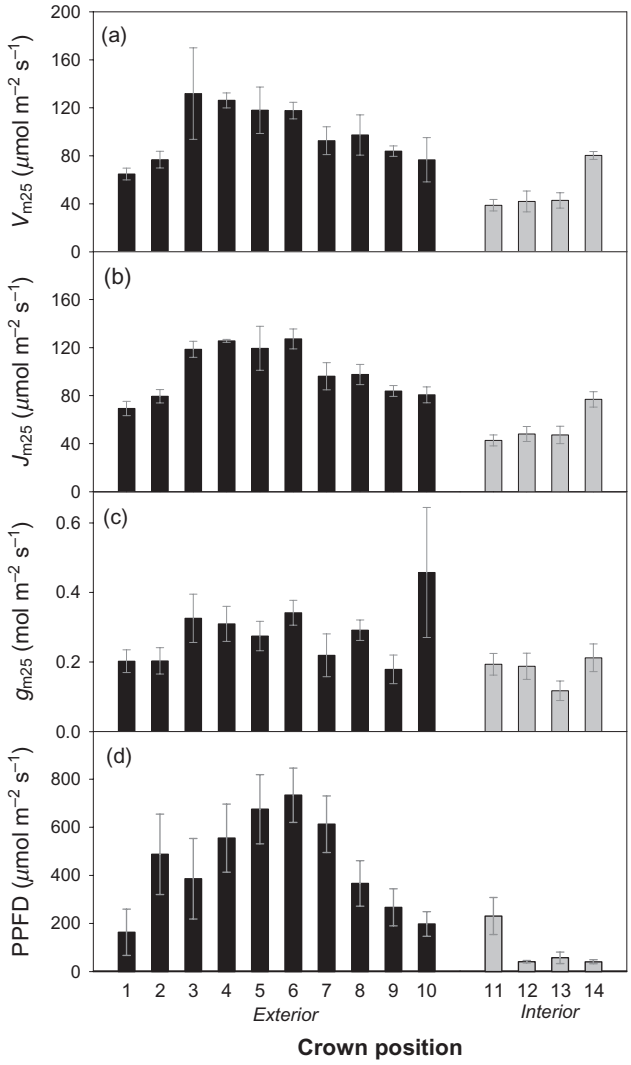

Figure 5. Gas exchange parameters (a, carboxylation capacity at 25 °C, V_{m25}; b, electron transport capacity at 25 °C, J_{m25}; c, mesophyll conductance at 25 °C, g_{m25}) and incident photosynthetic photon flux density on the day of *in situ* measurements (d, PPFD) at each of 14 crown positions (see diagram in Fig. 1). Black bars, exterior crown; grey bars, interior crown. Sample means ± SE.

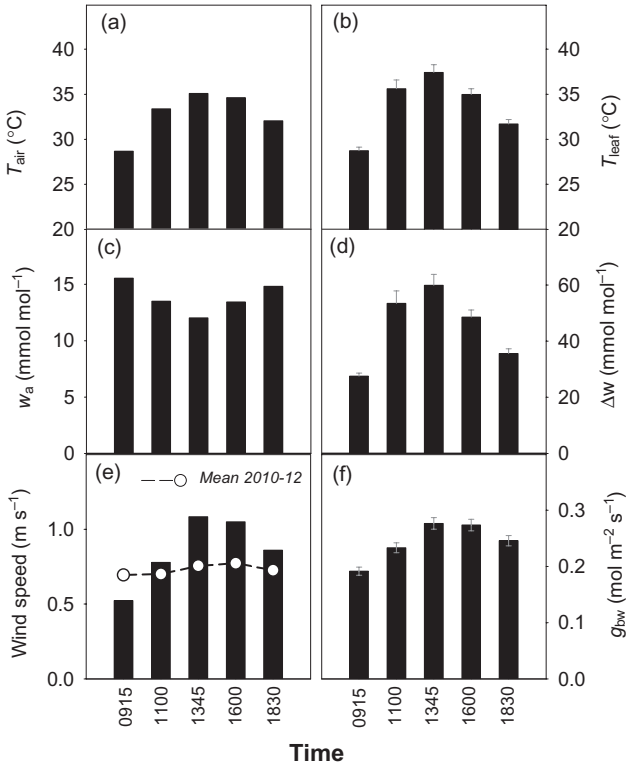

Figure 6. Environmental conditions measured at a meteorological station adjacent to the study site (a,c,e), and crown averages of associated leaf-level variables calculated from energy balance, based upon those environmental conditions (b, d, f). (a) Air temperature (T_{air}); (b) leaf temperature (T_{leaf}); (c) ambient water vapour mole fraction (w_a); (d) effective leaf-to-air water vapour mole fraction gradient (Δw); (e) wind speed (v_{wind}) measured on the day of the study (bars) and averaged over June–August in 2010–2012 (line and symbols); (f) boundary layer conductance to water (g_{bw}). For (b), (d) and (e), error bars are SEs among four individual crowns.

by traditional analysis of variance in linear models. All analyses were performed in base R (Team 2013).

RESULTS

Photosynthetic capacity and irradiance

Photosynthetic capacity estimated from CO_2 response curves (V_{m25} and J_{m25}) differed significantly among crown positions ($P < 0.0001$ for both variables) (Fig. 5a,b). Mesophyll conductance (g_{m25}; Fig. 5c) also differed among positions ($P = 0.013$). Each of these variables was generally greater in the upper crown (positions 4–7; Fig. 1). For comparison, mean PPFD measured *in situ* on the day of diurnal measurements (22 August 2012) was greatest at the top of the crown and decreased down the sides of the crown, and PPFD was very low at the three lower interior crown positions (12–14) (Fig. 5d).

Atmospheric conditions and associated leaf variables

Atmospheric conditions on 22 August 2012 were warm, calm and dry: air temperature ranged from 28.7 to 35.1 °C, ambient humidity ranged from 12.0 to 15.5 mmol mol^{-1} (16.5–25.4% relative humidity) and 1 h mean wind speed ranged from 0.5 to 1.1 m s^{-1} (Fig. 6). Based upon energy balance calculations, crown average leaf temperature (Fig. 6b) ranged from 28.7 to 37.4 °C, evaporative demand (Δw; Fig. 6d) ranged from 27.5 to 59.9 mmol mol^{-1} and boundary layer conductance ($g_{bw;}$ Fig. 6f) ranged from 0.19 to 0.28 mol m^{-2} s^{-1}, and each of these variables peaked in early afternoon (1345 h). Stomatal conductance and water use were moderate despite these conditions, with crown average g_s ranging from a minimum of 0.06 mol m^{-2} s^{-1} (at

1830 h) to a maximum of 0.13 (at 1100 h), and transpiration rate reaching a maximum of 4.4 mmol m^{-2} s^{-1} (at 1345 h) (Fig. 7).

Categorization of *A* versus *E* curves for each point

For each of 270 *in situ* measurement points, we calculated theoretical instantaneous relationships between A and E as described in the Materials and Methods section. Of these 270 points, 49.3% (133/270) were in category I, for which $\partial A/\partial E$ declines monotonically with increasing g_s. Due to the combination of high irradiance and evaporative demand and low boundary layer conductance, we observed positive curvature in the A versus E relationship ($\partial^2 A/\partial E^2 > 0$) in 39.6% (107/270) of A versus E curves. These points fall into category II, in which $\partial A/\partial E$ increases at low g_s and decreases at high g_s. Another 11.1% (30/270) were in category III (optimal g_s was zero because $\partial A/\partial E$ was below the target value, μ, for all positive g_s).

Optimal versus observed gas exchange patterns

The optimal values of g_s were generally quite high, yet this had a smaller effect on total conductance (g_{tw}) and hence transpiration rate (E) than one might expect, due to the low boundary layer conductances. As a consequence, mean g_s predicted by optimization greatly overestimated measured g_s in many cases, even though total crown water use was identical between the optimal and observed patterns of g_s. This is shown in panels A, C and E of Fig. 8, which present measured and predicted g_s in three ways: without any grouping (Fig. 8a), grouped by position and averaged over time (Fig. 8c), or grouped by time and averaged among positions (Fig. 8e).

Because the low boundary layer conductances led to such skewed differences between observed and optimal g_s, comparisons between observed and optimal transpiration rate (E) are more informative and are presented in panels B, D and F of Fig. 8. Optimal E was generally greater than measured E in cases where measured E itself was higher than the crown average (Fig. 8b,d). This pattern largely reflected a re-allocation of water loss from the interior crown (positions 11–14) to the upper and east-facing exterior crown (positions 1–6), as illustrated in Fig. 9a. Residuals of E (optimal minus measured E) differed significantly among positions ($P < 0.0001$). Optimal E was also lower than measured E in the morning and higher in the late afternoon (Fig. 10a) ($P < 0.05$). Additionally, the variation over time in residuals of E differed among crown positions (Fig. 11) (these changes were significant for positions 1, 2, 4, 6, 8 and 10; $P < 0.05$). The clearest pattern in this regard was for optimal E to be greater than measured E in the first half of the day on the eastern crown and in the second half of the day on the western crown (Fig. 11a,c). Thus, the spatial pattern of differences between optimal and measured E among exterior crown positions shown in Fig. 9 partly reflects a time-by-position interaction.

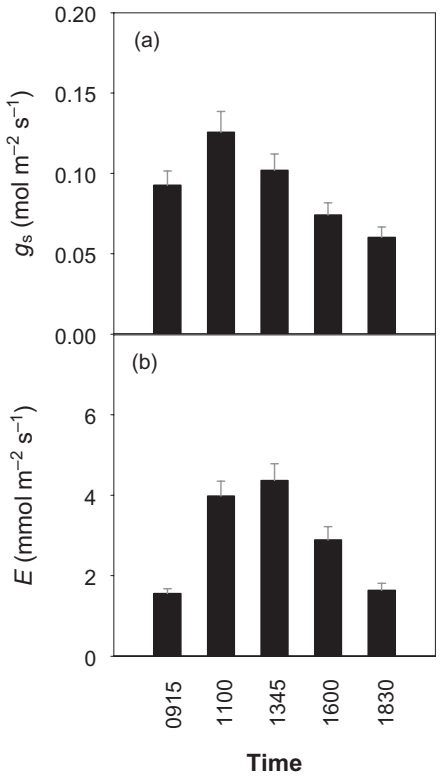

Figure 7. *In situ* measurements of stomatal conductance, g_s (a) and values of transpiration rate, E (b) calculated from measured g_s, averaged over 14 crown positions. Error bars are SEs among four individual crowns.

Effects of gas exchange distributions on total carbon gain

To assess how whole plant carbon/water balance would be impacted by these differences between measured and optimal patterns of water use, we computed total diurnal carbon gain for each crown in three ways: using either the measured or optimal spatiotemporal distributions of g_s or using a constant value of g_s, while controlling for total crown water loss in each case. We found that a constant g_s yielded 71.7 ± 0.6% of the total carbon gain achieved by the optimal g_s distribution, whereas the observed g_s distribution achieved 81.8 ± 0.3% of the optimum (Fig. 12).

Effects of aerodynamic coupling (boundary layer conductance)

Because boundary layer conductance impacts the validity of the assumption that $\partial^2 A/\partial E^2 < 0$, which underlies optimization theory, we repeated all calculations under an alternative scenario in which g_{bw} was imagined to be extremely large (which we simulated by setting wind speed to 3×10^8 m s^{-1}). The purpose of comparing the original results to this alternative scenario was to assess the sensitivity of inferred optima to assumptions about aerodynamic coupling between leaves and the air. Some conclusions were qualitatively

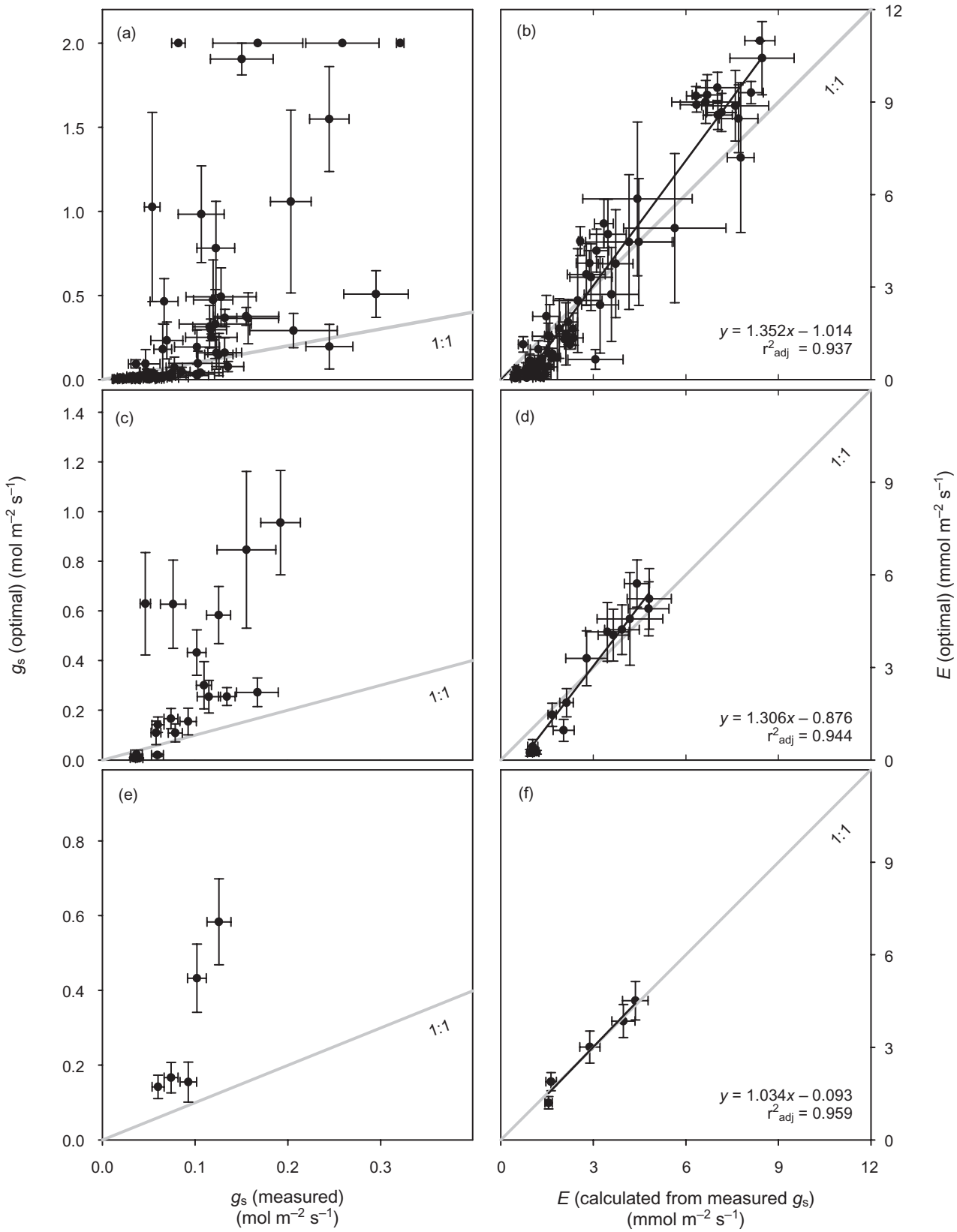

Figure 8. Measured versus optimal stomatal conductance to H_2O g_s (a, c, e) and transpiration rate, E (b, d, f). (a, b) Averages within each of 70 combinations of crown position and measurement time. (c, d) Averages over all measurement times within each of 14 crown positions. (e, f) Averages over all crown positions at each of five measurement times. Error bars are SEs among four individual crowns. Grey lines in (b), (d) and (f): one-to-one line. Note the *y*-axis scales differ in (a), (c) and (e).

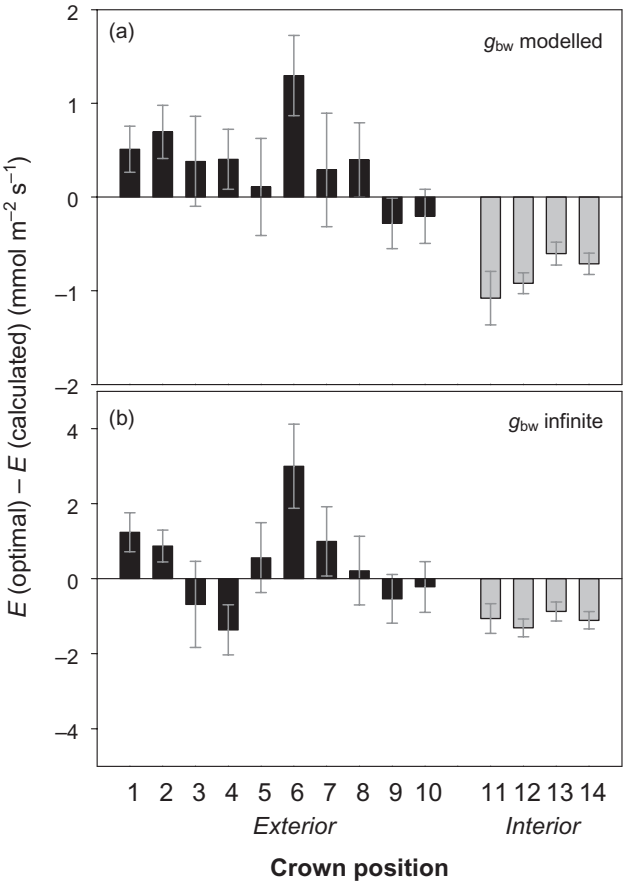

Figure 9. Residuals of transpiration rate (optimal minus measured E): diurnal means in relation to crown position. (a) Optimal E computed using boundary layer conductance, g_{bw}, modelled based upon measured wind speed. (b) Optimal E computed assuming negligible boundary layer resistance (infinite g_{bw}). Error bars are SEs among four individual crowns. Note the y-axis scales differ in (a) and (b).

similar between the 'decoupled' and 'coupled' scenarios: for example, in both scenarios, the optimal pattern shifted water use from the interior crown to the upper exterior crown (cf. Fig. 9a,b), and from early in the day to later in the day. However, some conclusions differed as well. For example, the optimal pattern shifted water use away from positions 3 and 4 on the east face (cf. Fig. 9a,b). The magnitude of redistribution of water loss required to achieve the optimum was also greater at many positions in the coupled scenario than in the decoupled scenario (e.g. position 6; cf. Fig. 9a,b), although the difference in total carbon gain between the observed g_s distribution and the theoretical optimum was smaller in the coupled scenario (11.6% versus 18.2%) (Fig. 12)

DISCUSSION

Our objective was to test two aspects of stomatal optimization theory that have largely been ignored by previous studies. Most work has focused upon the prediction that stomata should keep the marginal carbon product of water, $\partial A/\partial E$, invariant over time. However, the theory also predicts that stomata must hold $\partial A/\partial E$ invariant in space (i.e. among leaves in distinct environments within the same individual crown) and it assumes that water loss earns diminishing returns in terms of carbon gain (i.e. the curvature of A versus E is negative: $\partial^2 A/\partial E^2 < 0$) (Cowan & Farquhar 1977), yet these aspects of the theory remain largely untested. Our results suggest that neither the spatial aspect of the theory nor its assumption of negative curvature hold in grapevine canopies under the hot, dry, sunny and calm conditions typical of Mediterranean summer at our study site. We found that the measured spatial pattern of water use differed systematically from the optimal pattern, with some regions of the crown using more water than the optimum and other regions using less. We also found positive curvature in A versus E for 40% of leaf measurements, largely due to low boundary layer conductance. In addition, we found that if we had simply assumed negligible boundary layer resistance, as many applications of the theory have assumed, then the resulting predictions would have diverged substantially from the true optima, thereby altering some conclusions about the relationship between observed and optimal patterns.

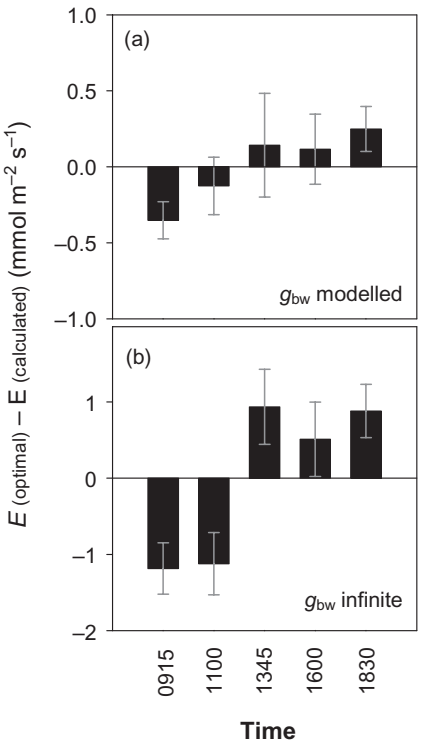

Figure 10. Residuals of transpiration rate (optimal minus measured E): averages over 14 crown positions, shown in relation to time. (a) Optimal E computed using boundary layer conductance, g_{bw}, modelled based upon measured wind speed. (b) Optimal E computed assuming negligible boundary layer resistance (infinite g_{bw}). Error bars are SEs among four individual crowns. Note the y-axis scales differ in (a) and (b).

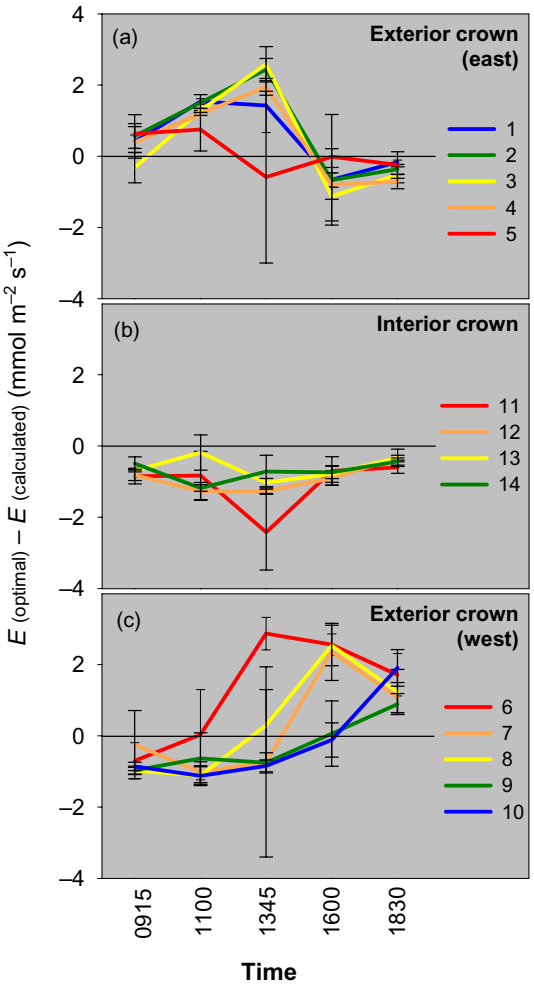

Figure 11. Residuals of transpiration rate (optimal minus measured E) over time for each of 14 crown positions: (a) positions 1–5 (the eastern side of the crown); (b) positions 11–14 (the interior crown); (c) positions 6–10 (the western side of the crown). Error bars are SEs among four individual crowns.

Positive curvature in *A* versus *E* and its implications

Water loss typically brings diminishing returns of carbon gain because stomatal opening tends to reduce the gradient for leaf CO_2 uptake more than that for H_2O loss. As g_s increases, intercellular CO_2 increases, this decreases the CO_2 gradient. Although a related effect occurs with transpiration – that is, increased E can decrease the evaporative gradient (Δw) by increasing ambient humidity – this effect is generally smaller than the CO_2 effect because the volume of air even in a dense canopy is vastly larger than the volume of the intercellular air spaces (Cowan 1977; Buckley *et al.* 1999). In this case, there is no instantaneous optimum for the trade-off between carbon gain and water loss: carbon gain per unit of water loss (instantaneous water-use efficiency, WUE = A/E) is greatest in the limit of zero g_s, which is a trivial solution. This is what led Cowan & Farquhar (1977) to ask what pattern of g_s maximizes total carbon gain for a given total water loss, which leads to the invariant-$\partial A/\partial E$ solution. However, increased g_s can strongly reduce Δw when boundary layer conductance (g_{bw}) is low. This is because low g_{bw} weakens convective heat transfer, increasing the scope of evaporative cooling to reduce leaf temperature and therefore Δw (Jones 1992). The resulting changes in Δw can lead to positive curvature in A versus E (Cowan 1977; Buckley *et al.* 1999). In such conditions, there is an instantaneous optimum for leaf-scale WUE, which occurs when $\partial A/\partial E = A/E$ (the point at which the tangent line to the A versus E curve goes through the origin) (Buckley *et al.* 1999). As a result, it is initially unclear whether the invariant-$\partial A/\partial E$ solution still applies in such conditions.

Buckley *et al.* (1999) suggested that if curvature is positive but a leaf cannot maintain E high enough to reach the maximum A/E, then the leaf should close some stomata entirely and open others more widely to achieve the optimum in the latter areas; that is, spatially heterogeneous g_s is beneficial in this case. A related argument can be made at the crown level. If some leaves have negative curvature and others have positive curvature, then water loss should be re-allocated from the former to the latter to allow the latter to maximize WUE. This will reduce E in the negative curvature leaves, thereby increasing $\partial A/\partial E$ and WUE in those leaves as well and ensuring that the re-allocation improves WUE throughout the crown. Furthermore, whole-crown WUE is maximized by increasing g_s even further in leaves with positive curvature – that is, beyond the point at which WUE is maximized for those individual leaves – as explained in the text surrounding Eqn 18 and illustrated in Fig. 4.

The Cowan & Farquhar (1977) solution therefore applies even if positive curvature occurs, provided curvature eventu-

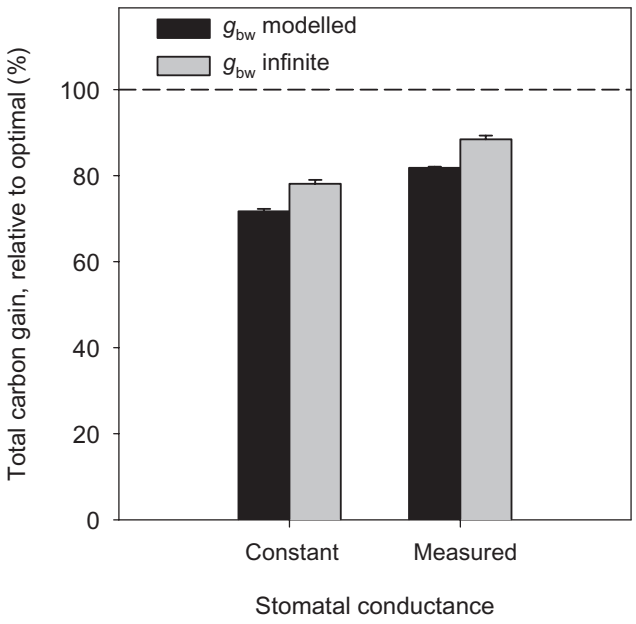

Figure 12. Total diurnal carbon gain calculated using either constant g_s, measured g_s or optimized g_s, expressed as a percentage of optimized values. Error bars are SEs among four individual crowns.

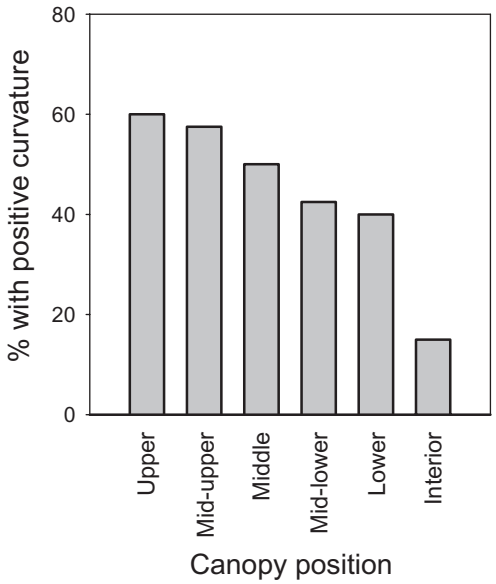

Figure 13. Proportion (as percent) of measurement points for which positive curvature in the relationship between assimilation rate and transpiration rate was observed. Position categories are as follows: upper (positions 5 and 6), mid-upper (positions 4 and 7), middle (positions 3 and 8), mid-lower (positions 2 and 9), lower (positions 1 and 10) and interior (positions 11–14). Position numbers are shown in Fig. 1.

ally becomes negative at higher g_s. There are two exceptions to this solution. Firstly, stomata should simply open as far as possible in leaves in which $\partial A/\partial E$ is always greater than the crown-wide target value (μ). This scenario applied in 7.8% of measured leaves in the present study. In these cases, boundary layer conductance was very low, so that changes in g_s had very little effect on $\partial A/\partial E$ at high g_s. Secondly, stomata should simply close in leaves for which the crown-wide target value of $\partial A/\partial E$ (μ) cannot be reached for any g_s ('category III' leaves in our terminology; Fig. 3a); this scenario applied in 11.1% of leaves in this study.

The implications of positive curvature will depend upon how often, in nature, boundary layer conductance is low enough to allow positive curvature to occur. Wind speed above the canopy ranged from 0.5 to 1.1 m s^{-1} in our study, and positive curvature occurred across this range. This range is low but not particularly unusual for our site: mean daytime summer wind speed was 0.69–0.77 m s^{-1} over 2010–2012 (Fig. 6e). Another study on grapevine (Daudet *et al*. 1998) found wind speed was below 1.0 m s^{-1} for 13% of a typical day, and Jones *et al*. (2002) found wind speed rarely exceeded 1.3 m s^{-1} during 2 of 4 days in a field study on grapevine. Similar ranges have been reported in other species (e.g. 1–2 m s^{-1}, cotton; Grantz & Vaughn 1999). Wind speed is much lower inside the crown because of wind attenuation by the canopy itself (e.g. Oliver 1971, Daudet *et al*. 1999, Grantz & Vaughn 1999). However, this was not a dominant factor in causing positive curvature in the present study, as the occurrence of positive curvature actually decreased with depth in the canopy (Fig. 13). We conclude that the occurrence of positive curvature in A versus E may not be as rare as previously thought, and that the matter requires further experimental study.

Why is the spatial distribution of water loss suboptimal?

We found that the observed distribution of water loss among leaves did not match the optimal pattern, that the residuals were systematically related to crown position and that these deviations reduced crown carbon gain by 18% compared to the optimum. It is helpful here to reiterate the rationale for this definition of 'optimal': total carbon gain will be greatest for a given total water loss if $\partial A/\partial E$ is invariant (provided $\partial^2 A/\partial E^2 > 0$). That statement is independent of spatial or temporal scale and is a generic mathematical result from the calculus of variations (Cowan & Farquhar 1977). It says that among all possible spatiotemporal distributions of g_s that give the same total crown water loss, carbon gain is greatest for the distribution in which $\partial A/\partial E$ is invariant. A separate question is, at what scale is it biologically meaningful to view total water loss as invariant (Cowan 1982, 1986; Mäkelä *et al*. 1996; Buckley & Schymanski 2014)? In the next section ('Is the optimization problem correctly posed?'), we discuss the possibility that it is not biologically appropriate to view total crown water loss as invariant, regardless of time scale. In this section, we discuss other possible explanations for the observed spatial deviations from optimality. One involves delays in stomatal opening. We found that optimal water loss typically exceeded observed water loss whenever the sun was orientated most directly towards a particular crown position (e.g. Fig. 11). It is possible that stomata in these positions could not respond quickly enough to the peak in PPFD to achieve optimal water loss. This effect would be exacerbated by low g_{bw}, which requires large changes in g_s to achieve a given change in water loss. Vico *et al*. (2011) suggested that delays in stomatal opening and closing in response to changes in PPFD create a quasi-optimal pattern of g_s, arguing that the costs of stomatal regulation itself must be subtracted from leaf net carbon gain in computing the optimum, so that the true optimum includes a finite time constant for stomatal adjustments to PPFD. This is unlikely to explain our results, given that the carbon cost of stomatal movements was on the order of 0.25% of net assimilation rate in the simulations presented by Vico *et al*. (2011) – far less than the potential improvement in carbon gain that could have been achieved by optimal stomatal control in our study.

Medlyn *et al*. (2011, 2013) suggested that stomata lack the physiological machinery to detect the shift between carboxylation- and regeneration-limited photosynthesis. Those authors noted that stomatal responses to short-term changes in atmospheric CO_2 were approximately optimal under regeneration-limited but not carboxylation-limited conditions, so they suggested that stomata were only capable of optimal behaviour under regeneration-limited conditions (i.e. under sub-saturating PPFD). Our results offer qualified support for that idea, as deviations from optimality at a given position tended to be greater when the sun was orientated

more directly towards that position, at which time PPFD would likely be saturating.

The spatial distribution of photosynthetic nitrogen may also have contributed to these deviations. The ratio of carboxylation capacity to PPFD was eight times greater in the interior crown (positions 11–14) than on the upper exterior crown (positions 4–7) (Fig. 5) – consistent with other reports that capacity is not proportional to local irradiance, contrary to the predictions of optimization theory for distribution of photosynthetic nitrogen (Evans 1993; Hirose & Werger 1994; Hollinger 1996; Makino *et al*. 1997; de Pury & Farquhar 1997; Bond *et al*. 1999; Friend 2001; Frak *et al*. 2002; Kull 2002; Lloyd *et al*. 2010; Buckley *et al*. 2013). It is well established that g_s is highly correlated with photosynthetic capacity (Wong *et al*. 1979). If this correlation represents a mechanistic constraint on stomatal regulation – that is, if the mechanisms that stomata have presumably evolved to optimize carbon/water balance include a physiological 'response' to photosynthetic capacity or some proxy thereof – then, such a response may present a physiological barrier to achieving optimal distributions of water loss in situations where photosynthetic capacity is suboptimally distributed. This highlights the important linkage between the economics of water loss and photosynthetic nitrogen use in plant crowns (Field 1983; Buckley *et al*. 2002, 2013; Farquhar *et al*. 2002; Peltoniemi *et al*. 2012; Buckley & Warren 2014; Palmroth *et al*. 2013).

Is the optimization problem correctly posed?

The requirement that $\partial A/\partial E$ be spatially invariant within the crown assumes that water loss can be arbitrarily allocated among leaves and over time within the crown. However, hydraulic constraints may make it impossible for leaves in some crown positions to achieve optimal water loss rates while also maintaining water potential above thresholds for catastrophic loss of hydraulic conductivity. Although this could be remedied by increasing hydraulic conductance to such leaves by re-allocating carbon, such re-allocation may itself be suboptimal, for two reasons. One is that stem carbon serves other functions, including mechanical support. Another is that hydraulic limitations to water loss may only manifest during brief periods in the growing season, in which case the large carbon investment needed to achieve optimal distribution of water loss may outweigh any resulting gains in crown WUE. Thus, each leaf may, in fact, require a different target value for $\partial A/\partial E$ to reflect the realities of its water supply constraints. A full exploration of this idea requires more intensive theoretical analysis.

Conclusions

We found systematic divergence between observed and optimal spatial patterns of water use, and evidence of widespread positive curvature ($\partial^2 A/\partial E^2 > 0$) in grapevine crowns under hot, dry and calm conditions. Positive curvature resulted from aerodynamic decoupling between the crown and atmosphere. Our results suggest that caution is warranted when using optimization theory to predict g_s at the crown scale, and that further study is required to assess the occurrence of conditions leading to positive curvature. We also suggest that it may be necessary to revise optimization theory to account for variations in hydraulic capacity within a crown.

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