

Modelling stomatal conductance of field-grown sunflower under varying soil water content and leaf environment: comparison of three models of stomatal response to leaf environment and coupling with an abscisic acid-based model of stomatal response to soil drying

V. P. GUTSCHICK¹ & T. SIMONNEAU²

¹Department of Biology, MSC 3AF, New Mexico State University, Las Cruces, NM 88003, USA and ²Laboratoire d'Ecophysiologie des Plantes sur Stress Environnementaux, Ecole Nationale Supérieure Agronomique – Montpellier, 2 place Viala, F-34000 Montpellier, France

ABSTRACT

Stomatal conductance, g_s , responds both to the immediate or local environment of the leaf, such as CO₂ partial pressure and irradiance, and to root-sourced signals of water stress, particularly abscisic acid (ABA). Two models for the combined control of g_s were formulated and tested in sunflower (*Helianthus annuus*). First, several empirical models were tested for the local control, demonstrating that the Ball–Berry model [Ball, Woodrow & Berry (in *Progress in Photosynthesis Research* Vol. 4, pp. 5 221–5.224; M. Nijhoff, Dordrecht, The Netherlands) 1987] is consistently among the most accurate. A problem of statistical non-independence in this model is shown to be minor. The model offers regularity of parameter values among most species and, despite an oversimplification in representing known humidity-response mechanisms, it incorporates other signalling loops from CO₂ and assimilation. In the first combined model, ABA as its concentration in xylem sap, [ABA]_{xy}, down-regulates the slope, m , in the Ball–Berry model by the factor $g_{\text{fac}} = \exp(-\beta[\text{ABA}]_{\text{xy}})$. The ABA-induced reduction in g_s decreases CO₂ assimilation and surface humidity, thus appearing to induce the local-control mechanism to amplify the ABA-induced stomatal closure. In the second combined model, g_s is estimated as the minimum of the local (Ball–Berry) response and the product $g_{\text{fac}} g_{s,\text{max}}$, with $g_{s,\text{max}}$ as a maximal unstressed conductance. Both models can predict g_s from the external environmental variables with good accuracy (r^2 near 0.8 over 20-fold variations in g_s). Further analyses show that g_s responds to humidity almost quadratically rather than linearly. It also responds to assimilation as a power law with an exponent that is significantly less than 1. These limitations, shared by other models, suggest more research into biochemical signalling.

Key-words: *Helianthus annuus*; abscisic acid; Ball–Berry model; modelling; stomatal conductance.

INTRODUCTION

Stomatal conductance responds to two distinct environments. The local or aerial environment of the leaf is defined by the irradiance, temperature, humidity, CO₂ content and boundary-layer condition. The distal environment, particularly that of the roots, commonly generates root-sourced signals of water stress or perhaps signals of hydraulic conductivity (Sperry *et al.* 1998). (It also contributes to determining leaf water potential, to which g_s responds in the short term (Zeiger, Farquhar & Cowan 1987; Assmann 1993). The rapid response is not modelled here.) Accurate models of both local and distal responses have been developed. Local responses are modelled with a variety of empirical models, such as those of Jarvis (1976) and, more recently, of Ball, Woodrow & Berry (1987), Leuning (1995), Jarvis & Davies (1998) and others. Responses to root-sourced signals of water stress have been described successfully with partly mechanistic models (Tardieu & Davies 1993; Tardieu, Zhang & Gowing 1993; Tardieu & Simonneau 1998). To date, the combined response to both environments has only been described empirically, as by Tenhunen *et al.* (1990, 1994). In the latter model, the effects are multiplicative,

$$g_s = g_{s,\text{BB}} \times g_{\text{fac}} + b \approx g_{s,\text{BB}} e^{a\psi} + b. \quad (1)$$

Here, the first or 'Ball–Berry' (BB) term $g_{s,\text{BB}}$ has the form mAh_s/C_s , with A as the rate of CO₂ assimilation and h_s , C_s as the relative humidity and CO₂ mixing ratio at the leaf surface, beneath the leaf boundary layer. We denote the form without the slope parameter m as the Ball–Berry index. The second term, g_{fac} , depends on the water potential P of leaf (ψ_L), root (ψ_R), or soil (ψ_S), variously chosen as deemed useful. When expressed as an exponential function

Correspondence: V. P. Gutschick. Fax: +1 505 6465665; e-mail: vince@nmsu.edu

of ψ_R or ψ_s , g_{fac} incorporates distal responses of g_s to root environment. The intercept, b , is typically small. This combination of responses is not fully satisfactory. In particular, it has been shown that the hormone, abscisic acid (ABA) is a major proximate control or messenger in stomatal response to water-stress (e.g. Cowan *et al.* 1982; Davies & Zhang 1991; Tardieu *et al.* 1991). The hormone IAA may also contribute (Dunleavy & Ladley 1995.) Further, in herbaceous species and tree seedlings, the synthesis of ABA is closely related to root water potential R_R (Simonneau, Barrieu & Tardieu 1998). The ultimate action of ABA may be amplified by R_L in some species (Tardieu & Davies 1993; Tardieu & Simonneau 1998). The relation of root water potential_r to that of soil depends upon water flux and soil hydraulic conductivity, a function of both soil texture and water potential. Formulated as a conductivity to root surfaces, it also depends upon root clumping (Tardieu, Bruckler & Lafolie 1992). Both a richness in interpreting the behaviour of field-cultivated plants and potential robust simplifications derive from these explicit linkages.

Herein we devised and tested a combined model similar to that in Eqn 1 but with the factor g_{fac} resolved as explicitly ABA-dependent. For anisohydric species such as sunflower, this is well approximated with the simple form $\exp(-\beta[\text{ABA}])$, with β as a parameter that is species-specific (at least) and $[\text{ABA}]$ as the concentration of ABA in the xylem sap delivered to the leaf. We analysed two data sets on sunflower (*Helianthus annuus* L); one data set was used to test the ABA model on sunflower as an anisohydric species (Tardieu, Lafarge & Simonneau 1996), and a new set was obtained in 1999 with closer attention to sampling and precise measurement of aerial conditions. In each data set, we measured both $[\text{ABA}]$ and the aerial environmental variables needed to compute the Ball–Berry behaviour. While testing this particular combination model, we also tested alternative models of aerial responses (Leuning 1995). These models reflect more directly (but not always with greater quantitative accuracy) the origin of leaf responsiveness to humidity as a response to epidermal transpiration rate (Dewar 1995; see ‘Discussion’). We also tested an alternative form in which aerial and ABA responses are not multiplicative; rather, g_s is the minimum of BB and ABA responses. The most accurate form is found to be $g_s = g_{s,\text{BB}} g_{\text{fac}}([\text{ABA}])$. This has implications for potential signalling mechanisms within the leaf. We discuss these, along with the more complicated signalling in woody plants and the possible sensing of plant hydraulic conductance in the control of stomatal conductance.

MATERIALS AND METHODS

Plants and growth conditions

Field experiments (1994 and 1999)

Sunflower plants (*Helianthus annuus*, hybrid Albena) were sown on 6 May 1994 and 17 May 1999 in a field with a deep clay–sandy–loam soil near Montpellier, France. Part of the field was irrigated with 18 mm of water every third day,

whereas the rest was not. Plants located at the boundary between irrigated and non-irrigated zones received intermediate irrigation depending on their distance from the sprinklers. Measurements were performed in July while the flowers were opening on plants of the three zones in 1994 and in the intermediate zone in 1999.

Glasshouse experiments (1994)

Plants of the same hybrid (Albena) as in the field experiments were sown in a glasshouse in Montpellier on 5 May and 15 June 1994, in 6.3 dm³ pots. Soil was maintained at retention capacity by daily irrigation (Hoagland N/10 nutrient solution) until experiments. Irrigation was then withheld and plants were sampled on the following days at various levels of soil dehydration. Measurements were performed in late June (for the first sowing date) and late July (second sowing date), when flower buds had appeared.

ABA feeding in the field and in the glasshouse (1994)

ABA solutions at concentrations ranging from 0.005 to 1 mol (+)-ABA m⁻³ were fed to well-watered plants from both the glasshouse and the field as previously described (Tardieu *et al.* 1996). Briefly, synthetic (\pm)-ABA (Lancaster synthesis) was dissolved in degassed artificial sap, poured into a funnel sealed around the basis of the stem, and allowed to enter the xylem stream via a 1 mm perforation drilled in the stem below the surface of the artificial sap.

Measurements of aerial environment and of plant variables

In the 1994 field experiment, air temperature, relative humidity and mean windspeed were measured every hour (CE 184, CE 190 and CE 155 sensors; Cimel, Paris, France) at a meteorological station located 500 m away from the field. In all other experiments, windspeed (A100R; Campbell Scientific Ltd, Shepshed, UK), air temperature and relative humidity (HMP35A; Vaisala Oy, Helsinki, Finland) were measured at 2 m above the soil surface every 20 s, averaged and stored in a data logger every 600 s.

Gas exchange of one of the four youngest fully developed leaves was measured at different times of the day using a ventilated closed cuvette coupled to a gas-exchange analyser (LI-6200; LI-COR, Lincoln, NE, USA; volume, 1 dm³). In the field, measured leaves were chosen either at the top of the canopy, or deeper in the canopy where they were shaded by upper leaves. In 1999, a shading shelter (3 m × 3 m) was installed at approximately 1.5 m above the top of the canopy, allowing for the upper leaves to be measured with a 30% reduction of photosynthetic photon flux density (PPFD). One-third of the measured plants were located below this shelter. Immediately after stomatal conductance to water vapour, g_s , was measured, the leaf was excised and enclosed in a plastic bag for measurement of leaf water potential using a pressure chamber. Then,

approximately 150 mm³ of sap were extracted from the same leaf by increasing the pressure at about 0.5 MPa above the balancing pressure. The sap was stored at -80 °C pending ABA analysis. ABA concentration was analysed in crude samples of xylem sap by radio-immunoassay (Quarrie *et al.* 1988) as previously described (Barrieu & Simonneau 2000). The specificity for ABA of the monoclonal antibody (MAC 252, provided by Dr S.A. Quarrie, Cambridge Laboratory, John Innes Centre, Cambridge, UK) was verified in xylem sap by comparing a radioimmunoassay (RIA) of crude sap samples with RIA of sap fractions recovered from thin layer chromatography (Tardieu *et al.* 1996).

4

5

Calculation of components of the Ball–Berry index

We calculated A , h_s and C_s for two different conditions: those occurring in the cuvette during gas-exchange measurements (superscripted as 'gx') and those projected to occur before the emplacement of the cuvette (superscripted '0'). The gas-exchange system reported A^{gx} directly. We had to compute h_s , which is defined as the ratio of two partial pressures of water vapour, in air at the leaf surface and in the leaf interior, e_s/e_i . The value of e_i can be well approximated as that for pure water at the leaf temperature, T_L ; the small correction factor for leaf water potential, $\exp(RV/RT)$ (Nobel 1991) can be ignored. The value of e_s was obtained by equating two expressions for transpiration rate,

$$g_s(e_i - e_s) = g_b(e_s - e_a) \quad (2)$$

with e_a being the partial pressure in free air outside the leaf boundary layer. The conductance of this layer is denoted as g_b . One can readily obtain

$$h_s = \left(\frac{e_a + \frac{g_s}{g_b}}{e_i + \frac{g_s}{g_b}} \right) \quad (3)$$

The gas-exchange system reported e_a^{gx} and T_L^{gx} , and it was routinely used to estimate g_b^{gx} under cuvette conditions according to the manufacturer's directions (Li-Cor, Inc. 1990). The value of C_s was approximated closely as:

$$C_s = C_a - A \times 1.37/g_b \quad (4)$$

with C_a as the mixing ratio of CO₂ in free air.

If stomatal conductance changes slowly, the Ball–Berry index should be computed using initial conditions just before leaf emplacement into the cuvette. Although g_s is assumed unchanged, leaf temperature, external CO₂ and e_a and g_b all change. One must recalculate A^0 , h_s^0 and C_s^0 . The contrast of initial and gx conditions is not marked; thus, the calculations are omitted here; they are detailed in a document available on our Web page, <http://biology-web.nmsu.edu/vince>.

6

Data quality control

Some ranges of data were eliminated from analyses. Specifically, we excluded data points for which the CO₂ mixing

ratio within the cuvette of the gas-exchange system was below 250 or above 380 μmol mol⁻¹. At both extremes, the leaf environment has been changed markedly from free-air conditions. It is not certain how rapidly the stomatal conductance re-equilibrates under all conditions, so we do not feel confident in using these points. Their inclusion does not change any of our conclusions, while adding a small additional variance. In addition, some individual data points were excluded. First, transport physics demands that Eqn 4 be satisfied. We rejected data for which the value of C_s reported by the gas-exchange system differed by more than 10 μmol mol⁻¹ from the value calculated with Eqn 4. Such deviations can occur when a bolus of air of very different composition is suddenly introduced into the cuvette, or if mixing is poor, etc.

RESULTS

Structure of the data sets

In all cases, measurements of g_s , the stomatal conductance for water vapour were coupled with analysis of the ABA concentration in xylem sap of the same leaf. From primary measurements of gas exchange, we have the variables needed to estimate with good precision the components A , h_s and C_s of the Ball–Berry model, both as values occurring during gas-exchange measurements (superscripted as 'gx') and as values occurring in free air just before measurements began (superscripted as '0'). More precise and time-intensive measurements of pre-measurement conditions were not practical. The same calculation methods (described in 'Materials and Methods') can also be used to calculate vapour-pressure deficits from leaf to air, $D = e_i - e_a$, that are used in the model of Dewar (1995). In the earlier data set from 1994, four treatments were included: field; greenhouse; greenhouse with transparent chambers to reduce air circulation and raise humidity around the leaf; and injection of plants with ABA. The four treatments showed no significant differences in behaviour and are considered as a group with 193 valid members (all variables measured; CO₂ within acceptable limits during gas exchange). The second data set from 1999 contained 145 valid members, obtained on four separate days of experimentation. In contrast to the 1994 data set, all data were obtained under field conditions. Furthermore, the treatments cover a smaller range of stress (smaller range of [ABA]) but a wider range of irradiance and in more detail.

Initial tests: forms of local-response models

We have noted the form of the Ball–Berry model, following Eqn 1. Its possible limitations are analysed in the 'Discussion'. Two other forms were also tested. The first, proposed by Leuning (1995), differs principally in posing the humidity response in terms of the vapour-pressure deficit from leaf to air, D :

$$g_s = m \frac{A}{C_{sx}(1 + D/D^*)} \quad (5a)$$

The parameter D^* is adjusted for the best least-squares fit. The factor C_{sx} may be taken variously as C_s itself or as $C_s - \Gamma$. We found negligible differences among the two forms for C_{sx} in our data sets and used C_s only in results reported herein. This model has been analysed by Dewar (1995). Lhomme *et al.* (1998) have also shown that it effectively incorporates the responsiveness of g_s to the transpiration rate that was found by Mott & Parkhurst (1991) and Monteith (1995). The actual responsiveness is to epidermal transpiration rate (Bunce 1996), which commonly parallels whole-leaf rate. The empirical fit is observed to vary with leaf temperature (Mott & Parkhurst 1991). Matzner & Comstock (2001) provide an interpretation in terms of leaf hydraulic conductance. The net response to transpiration and temperature does not scale exactly as relative humidity (Matzner & Comstock 2001) as in the Ball–Berry model, but is often close.

An older empirical form (Jarvis 1976; Stewart 1988; Noilhan & Planton 1989) also uses the vapour-pressure deficit but in the numerator:

$$g_s = m \frac{A}{C_{sx}} (1 - D/D^*) \quad (5b)$$

Without incorporating a factor for stress response, we would not expect a strong fit to the data, although ABA-induced stomatal closure appears to induce the local response (lower g_s reduces A and h_s) and thus mimics an aerial response. The local environment and thus A , h_s or D and C_s changes upon inserting a leaf into the gas-exchange cuvette. It is possible (see ‘Materials and Methods’) to estimate the original environment from the gas-exchange environment. If stomata respond rapidly, one may expect that the use of gas-exchange environment (‘gx’) is more appropriate than the original environment (‘0’) and will yield a better statistical fit to the data. We tested the use of both environments in composing the indices in the Ball–Berry, Leuning and Jarvis models. The differences were minor, with the exception of the 1999 data analysis with the Leuning or Jarvis models (Eqns 5a & 5b); r^2 improves from 0.551 to 0.653 and from 0.637 to 0.691, respectively, upon using initial leaf conditions. For the other four combinations of years and models, use of initial conditions reduces r^2 by an amount ranging from –0.022 to –0.078.

Table 1 shows that the Ball–Berry model with gx conditions is appreciably superior to the Leuning and Jarvis models, for both data sets. The fits of both the Leuning and Jarvis models to the 1999 data were notably improved by using estimated initial environmental conditions. At best, the Jarvis model with such estimates was slightly superior to the Ball–Berry model using conditions during gas exchange. We have similarly found moderate statistical superiority of the Ball–Berry model over the other two models in diverse woody species in a mixed deciduous-coniferous forest (Gutschick *et al.* 2002) and in two riparian tree species in New Mexico, USA (Catalan-Valencia *et al.* in prep.) It was not possible to make a firm selection among the local-response models based on the comparisons here. However, the Ball–Berry model was always in the accept-

Table 1. Comparison of goodness-of-fit of three models of stomatal conductance to water vapour, g_s , as responding to the aerial environment

Year	Model	Slope (m) (mol m ⁻² s ⁻¹)	Intercept (b)	D^* (Pa)	r^2
1994	Ball–Berry	20.61	0.434	(–)	0.467
	Leuning	18.19	0.478	50	0.375
	Jarvis	18.01	0.49	80	0.377
1999	Ball–Berry	14.35	0.174	(–)	0.673
	Leuning	14.43	0.110	100	0.552
	Jarvis	14.42	0.138	110	0.637

Only data with low ABA concentrations in the xylem sap (< 100 nM) were used (127 data points for 1994, 136 for 1999). Note that the scaling parameter D^* does not occur in the Ball–Berry model. Values of the coefficient of determination, r^2 , in boldface are superior among the three alternative models and exceed 0.5; values in italic are acceptable (> 0.5) or, if less than but close to 0.5, are superior among the three models.

able group; thus, we used this model in the combined or ‘BB + ABA’ model, in the next section. In the case of the Leuning and Jarvis models, the goodness of fit was rather insensitive to the value of D^* . Commonly, a variation of 50%, relatively, changed r^2 by about 0.020. The slope, m , in the Ball–Berry model, differed significantly between the 1994 and 1999 data sets; see the ‘Discussion’ below. For all models, the fits for the 1994 data set were always markedly worse than for the 1999 data set. This is expected, because several aerial variables were estimated by less accurate methods in the 1994 data set. In particular, all variables are given as their averages over three observations rather than as the most reliable single observation, as used in the 1999 data set. This is typically the second observation, after changes in CO₂ in the cuvette have stabilized but before assimilation has decreased CO₂ as radically.

Tests of the multiplicative model, BB × ABA

Our combined model proposed that ABA modifies the Ball–Berry slope downward from its initial value, m_i :

$$g_s = g_{\text{fac}} m_i A h_s / C_s + b = e^{-\beta[\text{ABA}]} m_i A h_s / C_s + b \quad (6)$$

We assumed that the Ball–Berry model is good for describing the local response, as discussed above. The form of ABA response is appropriate to anisohydric species, of which sunflower is a representative (Tardieu & Simonneau 1998). The simplest test of the combined model is a linear regression of g_s against the new index, $g_{\text{fac}} A h_s / C_s$, also denoted as $g_{\text{fac}} I_{\text{BB}}$. We need to find the optimal value of β that maximizes r^2 for the linear regression.

Table 2 shows the results, using cuvette conditions for the local environment in composing the Ball–Berry index. The combined model was quite successful, explaining nearly 80% of the variance in g_s , in both the 1994 and 1999 data sets. The numerical values of the Ball–Berry parameters are well prescribed by non-linear least-squares fitting, as shown

Table 2. Improved fit of g_s with combined models of ABA action and Ball–Berry behaviour.

Year	Slope (m_0) (mol m ⁻²)	Intercept (b) (nM ⁻¹)	β	r^2
1994	28.08 (1.11)	0.157 (0.054)	0.0030 (0.0010)	0.770
1999	15.46 (0.77)	0.200 (0.073)	0.0030 (0.0010)	0.795

ABA action reduces (by the factor $\exp(-\beta [\text{ABA}])$) the slope, m_0 , in the Ball–Berry model, Eqn 6. The Ball–Berry portion is expressed in variables reflecting the environment during gas exchange, as in Table 1. All parameters are determined by non-linear least-squares fitting with equal weighting. Values in parentheses following the parameter values are adjusted standard errors; 95% confidence bounds are closely twice as large. All of the fits are highly significant statistically ($P \ll 0.000001$).

by the small standard error. The 95% confidence interval (Wald) in slope m is 8% (10%) above and below the mean for 1994 (1999) data. The error bounds for intercept b are larger on a relative scale but change g_s itself by only about 10% or less under average leaf conditions. The ABA response parameter, β , has a larger relative error, which would affect calculation of g_s more strongly under strong water stress. Inclusion of more high-stress data points in determining β would probably help, as might a search for a more robust functional form for the ABA response; see the Discussion. Figure 1b (open circles) shows that there is no significant systematic bias in the fit according to range of g_s in the 1994 or 1999 data; the few outliers do tend to be over-predictions at high g_s . There are some subtleties in comparing this performance with that of the Ball–Berry model alone or the ABA model alone. Most significantly, the Ball–Berry model should not be applied to the entirety of either data set. Those data points obtained with high [ABA] include a partial mimicking of the Ball–Berry response by ABA, which reduces g_s , in turn reducing both A and the Ball–Berry index. Thus, we offer the limited comparison from Table 1 earlier. There, the Ball–Berry model alone explains only about 47 and 67% of the variances in 1994 and 1999, respectively. The ABA model alone explains 58% of the variance in 1994 (when ABA dominated in the control of g_s), and 8% in 1999. It is notable that virtually the same value of the parameter β applies to both data sets. Both of these values require that we adjust β to be significantly higher than in the combined model; we shall return to this point later. The improvement in r^2 afforded by the combined model was on the order of 12 to 20%. This is significant for the practical application of predicting g_s . It is, admittedly, limited, for several reasons. Foremost, perhaps, there is limited room for improvement, given the inherent random errors in measuring g_s . The relative errors are largest at low conductance (low signal-to-noise in added water vapour, in the gas-exchange system) or very high conductance (minor errors in g_b require large, artifactual changes in g_s to preserve the measured total conductance).

Consistent with the combined model gaining explanatory power by incorporating both the local and ABA models, we find that typically all the components of the combined model are statistically significant. We evaluate this on data transformed to the form

$$\ln g_s = -\beta[\text{ABA}] + c_1 \ln(A) + c_2 \ln(h_s) - c_3 \ln(C_s) \quad (7)$$

(One may also adjust this to $\ln(g_s - \beta)$.) With this linearization, we may apply the simplest and most robust tests of significance of each factor ([ABA], $\ln(A)$, $\ln(h_s)$ and $\ln(C_s)$). We may also test that each factor in the Ball–Berry index is raised to the appropriate power: A and h_s as first powers and C_s as the -1 power. This would be indicated by the coefficients c_1 , $-c_2$ and c_3 being close to unity.

Table 3 presents results obtained: (1) with all the data points in each year; this generates some bias toward high g_s that ‘anchors’ the regression there; (2) consequently, re-sampling data randomly to have approximately equal numbers of points in four ranges of $\ln(g_s)$. Here, we chose the lower values, $\ln(g_s) < -1$ ($g_s < 0.37$ mol m⁻² s⁻¹). For these data points, the slope of $\ln(A)$ against $\ln(g_s)$ appears to be nearly constant (Fig. 2); this is useful in analysing the con-

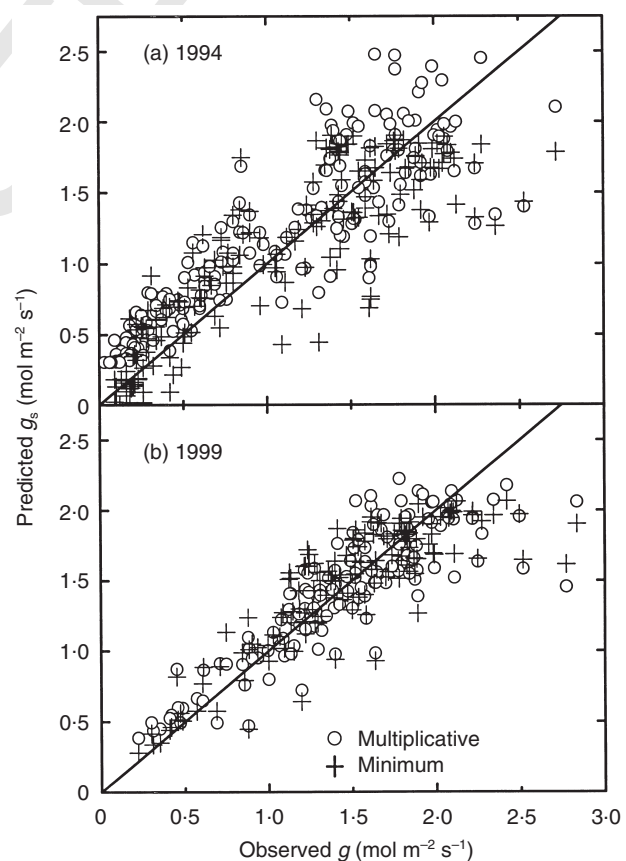


Figure 1. Stomatal conductance (g_s) predicted from two models of combined response to local environment and to root-sourced ABA signal: the multiplicative model (O) and the model of the minimum of local and ABA responses (+). Data for 1994 (a) and for 1999 (b) include all valid data with CO₂ mole fraction in the range (250, 380) $\mu\text{mol mol}^{-1}$ (see text).

Year	Data set	Coefficients of				r^2 value
		[ABA]	$\ln(A)$	$\ln(h_s)$	$\ln(C_s)$	
1994	All data	-0.00037 _(-3.03)	0.628 _(8.80)	2.643 _(15.92)	-0.404 _(-0.56)	0.848 **
	Re-sampled	-0.00108 _(-3.72)	0.886 _(7.78)	2.429 _(7.63)	-0.275 _(-3.41)	0.887 **
1999	All data	<i>-0.00091</i> _(-2.35)	0.582 _(8.53)	2.235 _(15.64)	0.102 _(0.46)	0.891 **
	Re-sampled	<i>-0.00003</i> _(-0.13)	0.669 _(5.34)	3.093 _(8.48)	-0.654 _(-0.96)	0.837 **

The nature of the data sets for the two different years is described in the text, as also is the manner in which data sets were re-sampled for even coverage in ranges of $\ln(g_s)$. As a subscript to each partial regression coefficient is its corresponding Student's t -value. Coefficients that are statistically significant at the $P = 0.05$ level are indicated in italics; those that are significant at the $P = 0.005$ level are in boldface.

Table 3. Multiple linear regression of $\ln(g_s)$ against [ABA] and the natural logarithms of A , h_s and C_s .

trol coefficient of g_s upon A for other analyses, presented later. This subsampling yielded 59 data points for the 1999 data set and 48 for the 1994 data set. We also discarded all data points in the 1994 data set with very large values of [ABA], which also 'anchor' the regressions in a region where: (1) the ABA effect is far beyond saturation; (2) $\ln(g_s)$ is less reliably measured; and (3) the effect of a minimal or 'floor' value of g_s should be included but is difficult to model.

Table 3 shows, first, that β in the ABA response is statistically significant ($|t| > 1.8$, yielding $P < 0.05$) for all cases except the full 1999 data set, which is dominated by low-[ABA] data points, giving an unreliable fit. Second, the factors A and h_s are also highly significant statistically. The coefficients of $\ln(A)$ are near 1, but those of $\ln(h_s)$ are always greater than 1, in the range 2–3. This discrepancy is revisited in the Discussion. The coefficient of $\ln(C_s)$ is not statistically significant; very small variance in C_s is sampled in either data set, because measurement conditions were near ambient CO_2 levels.

Consistent with the ABA effect being amplified by the local response, increasing levels of ABA appears to decrease the Ball–Berry slope progressively. We may partition the data into smaller ranges of [ABA], or, similarly, into ranges of $g_{\text{fac}} = \exp(-\beta[\text{ABA}])$ for g_{fac} in the range 0.5–1.0, the effective slope is 26.54 in the 1994 data. For progressive halvings of the g_{fac} range (0.25–0.5; 0.125–0.25; 0.0625–0.125), the slope decreases to 18.84, then 9.67 and finally 7.74. One might similarly use multiple linear regression on $\ln(g_s) = a \ln(I_{\text{BB}}) - \beta[\text{ABA}] + b$ to express this effect (see later), although the functional effect is less readily comprehended (and the data are \ln -transformed, changing the statistical fit). The 1999 data do not have adequate samples in high-[ABA] ranges for a comparable analysis.

It appears that the best fit to data in the combined model requires a value of ABA-responsiveness, $\beta_{\text{direct}} = 0.003 \text{ nm}^{-1}$, that is only about one-half as large as in the model using only the ABA response ($\beta_{\text{total}} = 0.006 \text{ nm}^{-1}$). We interpret this as the local response causing an amplification of the ABA effect. That is, the ABA-induced decreases in leaf assimilation A and in leaf-surface relative humidity h_s ,

induce the appropriate Ball–Berry-like response in g_s . A simplified model of A and of h_s supports this interpretation. Let us resolve a direct effect of ABA upon g_s , as a factor G (a 'direct-only' g_{fac}) multiplying the BB slope. The total effect of ABA, including the feedback multiplication via the local response, is proposed to be a power of the direct effect: $g_s \approx g_{s0} G^n$, as a change from the original conductance g_{s0} . Similarly, assimilation is proposed to change as a power of g_s itself: $A \approx A_0(g_s/g_{s0})^C$. We also take surface relative humidity as a power function, $h_s \approx h_{s0}(g_s/g_{s0})^Q$. Then, in the Ball–Berry model of local response, we may write

$$g_s \approx (m_0 G)(A_0 G^{n \cdot C})(h_{s0} G^{n \cdot Q}) = (m_0 A_0 h_{s0}) G^{(1 + n \cdot [C + Q])} = g_{s0} G^{(1 + n \cdot [C + Q])} \quad (8a)$$

or

$$G^n = G G^{n \cdot C} G^{n \cdot Q}$$

This yields simply that

$$n = 1 + n(C + Q) \quad n = 1/(1 - [C + Q]) \quad (8b)$$

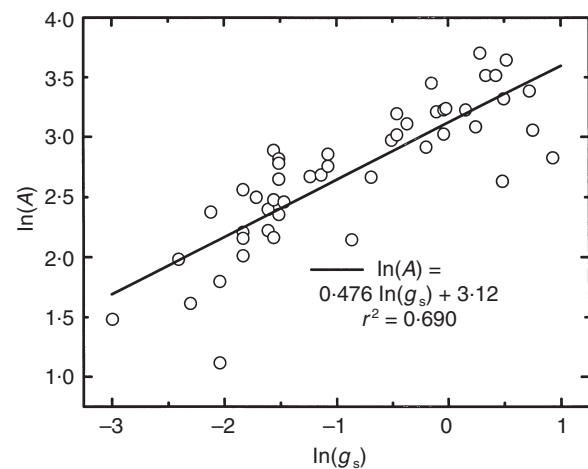


Figure 2. Nearly linear relation between logarithms of A [CO_2 assimilation rate ($\mu\text{mol m}^{-2} \text{ s}^{-1}$)] and g_s [stomatal conductance ($\text{mol m}^{-2} \text{ s}^{-1}$)], for 1999 data set, over entire range of both variables.

The amplification factor, n , is expected to vary with the range of g_s or, equivalently, [ABA]. The factor is larger if C and Q are larger and this occurs at small values of g_s . We may see this by approximating carboxylation kinetics with a model having two resistances, $1/g_s$ and an effective biochemical or mesophyll resistance, $1/g$. Taking the ambient partial pressure of CO_2 as C_a , one obtains $A = g_m g_s C_a / (g_s + g_m)$. The exponent C is simply the logarithmic derivative, $M\ln(A)/M\ln(g_s)$, which equals $1/(1 + g_s/g)$. It is large when g_s is small.

By using the whole data set, we average these effects and obtain mean values of C , Q and n . We must determine if these values are consistent with the apparent value $n \approx 3$. For this, we obtain the exponent C as the slope of $\ln(A)$ against $\ln(g_s)$, and analogously Q as the slope of $\ln(h_s)$ against $\ln(g_s)$. Equation 8b enables us to predict the amplification factor as n_{pred} . The apparent or observed amplification factor, n_{obs} , is the ratio of the β_{total} to β_{direct} . We obtain β_{total} via single regression of $\ln(g_s)$ against [ABA], and β_{direct} via multiple regression that includes $\ln(A)$, $\ln(h_s)$ and $\ln(C_s)$ in addition to [ABA]. For both data sets, each regression was done in two ways: (1) with all valid data points ($A > 0$, so that $\ln(A)$ is defined); and (2) re-sampled to put approximately equal emphasis on different regions of $\ln(g_s)$, as described earlier. The predicted and observed multipliers are comparable, as shown in Table 4. The values of C are consistent with a coupled model of assimilation (Farquhar, von Caemmerer & Berry 1980) and CO_2 transport, for a mean g_s of approximately 0.2 the maximal value of g_s (model details not shown; they are readily derived). The values of Q are specific to the boundary conditions for radiative and convective heat transfer and cannot be estimated with a single model.

Alternative model: g_s as the minimum of ABA and Ball–Berry responses

An alternative coupling may be proposed, as

$$g_s = \min[mAh_s/C_s + b_s g_{s,\max} e^{-\beta[\text{ABA}]} + g_{s,\min}] \quad (9)$$

The alternative model is in the spirit of a transition between two limiting factors or two controlling factors. It contrasts with the multiplicative model, which suggests a sequential biochemical action of ABA and some internal factor corresponding to the Ball–Berry ‘signal’. Transitional models are common, as in the widely applicable model of photosynthetic carboxylation (Farquhar *et al.* 1980 ff.). Commonly, they incorporate a smoothing of the transition with a convexity parameter (Collatz *et al.* 1990.). At present, we have little direct information on the biochemistry of stomatal control (see ‘Discussion’), and thus, no inherent preference for the multiplicative model over this model of the minimum, as we will call it. We tested the goodness of fit of this model to the same two data sets, with varied choices of m and of $g_{s,\max}$. We let $b = g_{s,\min}$ be the intercept of a regression incorporating the remaining factors. The fit was good, but not as good as that for the multiplicative model detailed above. For the 1994 data set, the optimal values $m = 28$, $g_{s,\max} = 1.6$ yielded $r^2 = 0.739$, compared with 0.770 for the multiplicative model. For the 1999 data set, the optimal values are $m = 16$, $g_{s,\max} = 2.0$, giving $r^2 = 0.791$. These regression coefficients are nearly as good as those for the multiplicative model. Thus, the choice between the two models is not clear on the basis of statistics. Neither is a functional basis readily apparent. Figure 1 indicates that the two models show similar patterns of goodness of fit according to range of g_s . A modest distinction is that the model of the minimum (symbol ‘+’) shows some systematic convexity at low g_s , in the fit to 1994 data.

DISCUSSION

Practical, empirical prediction of stomatal conductance

There has been significant discussion in the literature on the ability of various empirical models to predict stomatal conductance under broad ranges of environmental conditions and for diverse plant species. The most accurate and most appropriate form for the local response has been

		Amplification factor			
		C , in fit $A \propto (g_s)^C$	Q , in fit $h_s \propto (g_s)^Q$	Predicted, $n = 1/(1 - C - Q)$	Observed $\beta_{\text{total}}/\beta_{\text{direct}}$
1994	All data	0.412	0.163	1.7	2.6 = 0.00390/0.00150
	Re-sampled	0.476	0.132	2.6	2.8 = 0.00320/0.00115
1999	All data	0.566	0.141	3.3	7.7 = 0.00177/0.00023
	Re-sampled	0.684	0.122	5.2	– = 0.00125/ –

Table 4. Apparent amplification of ABA action on g_s by its action to reduce assimilation A and surface relative humidity h_s , thus reducing the Ball–Berry factor

Same data points are used as for Table 3. The power-law dependences of A and h_s are analysed as described in the text. Predicted amplification factor is from Eqns 8a and 8b in the text. Apparent coefficients of ABA response, β_{total} and β_{direct} are derived by fitting stomatal conductance g_s to ABA response only or as part of ABA + Ball–Berry response, respectively. Double dashes indicate that an analysis is not valid, because β_{direct} is not statistically significant for this data set.

debated (Aphalo & Jarvis 1993). The Ball–Berry form in particular has been characterized as a correlation rather than as a mechanistic equation. This is certainly true, while also true of all existing models. Some mechanistic aspects of stomatal control have been clarified, particularly that: (1) the stomata respond to internal CO₂ partial pressure and not to that at the surface (Mott 1988); (2) they respond to transpiration and more specifically to epidermal transpiration, as noted in the Introduction; a separate response is to leaf temperature (Matzner & Comstock 2001); (3) modification of PEP carboxylase enzyme by a kinase is involved (Du, Aghoram & Outlaw 1997); (4) there appear to be separate control loops responding to humidity and to assimilation rate (Santrucek & Sage 1996); (5) a number of elements in the molecular signalling cascade for ABA responses are known, whereas the ABA receptor itself is elusive; the same is true for direct sensing of osmotic stress independent of ABA (Luan 2002). However, no mechanistic synthesis is anywhere near at hand (Assmann 1999). Consequently, we need use empirical models. Accuracy of a chosen model in representing a specific data set is a strong criterion, although not a sole criterion. The Ball–Berry, Leuning and Jarvis models variously are superior for specific data sets.

Ability to estimate behaviour for partially characterized species and conditions is another significant criterion, for example, in climate modelling, in which large regions are poorly characterized physiologically or not at all (e.g. Sellers *et al.* 1996). We propose that the Ball–Berry model is useful, in that it requires only two parameters and these are commonly stable. The slope, m , is often near 10 among diverse C₃ species in various biomes (Ball *et al.* 1987; Leuning 1990; Collatz *et al.* 1991; Harley & Tenhunen 1991; de Pury 1995; Schultz & Lebon 1995). We note here that there is a significant difference in unstressed slope, m_0 , between our two data sets (1994, 1999) that is not readily explained other than by unmeasured or unanalysed differences in growth conditions. However, both slopes are so large that total (stomatal plus boundary-layer) conductance is dominated at low stress by the boundary layer and is accurately predicted with either value of slope.

It is worth noting that the Ball–Berry index is computed using the measured value of g_s itself (see Eqn 3 for h_s). Thus, the Ball–Berry equation may be regarded in one sense as an implicit equation for g_s , computationally, even if h_s approximates an independent mechanistic driving variable. In any event, the use of simple regression of g_s on I_{BB} may be regarded as including an artifactual correlation. One may remove this artefact by recasting the Ball–Berry equation as a quadratic in g_s and solving for g_s , then doing non-linear regression of g_s on the remaining environmental variables. We may write

$$g_s = \frac{mA[e_a/e_i + g_s/g_b]}{C_s[1 + g_s/g_b]} + b \quad (10)$$

One may then multiply both sides by $[1 + g_s/g_b]$ and gather terms into a quadratic equation, which has the formal solution

$$g_s = \frac{-B^* + \sqrt{(B^*)^2 - 4A^*C^*}}{2A^*} \quad (11)$$

with $A^* = 1/g_b^2$, $B^* = 1 - b/g_b - mA(C_s g_b)$, $C^* = b + mA h_a / C_s$, $h_a = e_a/e_i$

One may then regress g_s against the right-hand-side of Eqn 11. For the 136 data points of 1999 at low [ABA] (< 100 nM; negligible ABA control; best test of the Ball–Berry response), one obtains a fit with $r^2 = 0.618$. This may be compared with a fit to the unmodified Ball–Berry equation, which yields $r^2 = 0.676$.

The statistical artefact may thus be termed modest. The prediction of g_s from environmental conditions would still employ the full Ball–Berry equation, with modestly reduced confidence. It may be noted that alternative models of the local stomatal response are also implicit in a formal sense. For example, in predicting g_s , the vapour-pressure deficit in Eqns 5a and 5b is computed using leaf temperature, which is computed in turn using estimated g_s in the energy-balance equation. This only means that feedbacks are operating; the approximate representation of feedbacks differs among the Ball–Berry, Leuning and Jarvis models. The combined model, using the local and stress (ABA) responses multiplicatively, has high accuracy, with values of r^2 near 0.8, even for data sets that were not designed for accurate resolution of both local environment and ABA concentration. The gain in explanatory power, as r^2 , over a model with only the ABA response is significant. The gain over a model with only the Ball–Berry or similar local response is modest, but the local models are not useful predictors in stressed conditions; foremost, the values of A , h_s and C_s are strongly shifted by stress and cannot be computed from the local response alone. In explanatory power (as r^2), the multiplicative model is superior but not definitively superior to the alternative model of the minimum. Consideration of both models' relative success may help to guide mechanistic studies at the biochemical level, in seeking to resolve parallel versus serial action when local signals combine with signals from ABA (and IAA, pH and K⁺). Of course, our model is not intended to resolve rapid, hydraulic responses to short-term changes in water status (Zeiger *et al.* 1987; Assmann 1993).

The practical use of our combined model, in either the multiplicative or minimum form, merits some discussion. First, the algorithm is completely defined for computing g_s in any combination of local environment (irradiance, air temperature, etc.) and soil environment (soil water content and texture, thus, its water potential and hydraulic conductivity; rooting density and clumping factor (Tardieu *et al.* 1992). The xylem ABA concentration is computed in this process, adapting the methods of Tardieu & Davies (1993). The complete mathematical model, as a narrative description and as a Fortran program, is available from author Gutschick and on the Web site <http://biology-web.nmsu.edu/vince>. The full model includes numerous processes and their parameters, but is robust in the sense that parameters are readily measured or can be estimated for broad classes of plants and soils. Second, one

must determine the time scale on which the local environment is defined. Does the leaf respond to the irradiance and other variables on a short and essentially instantaneous time scale, or to a (weighted) average over a longer time scale? All stomatal models face such a challenge. For sunflower here, we find a rapid response, such that conditions during the 1.5 min of gas exchange are better predictors than initial conditions before the measurements began and perturbed the environment. In woody species, the time lags are commonly substantial. Ideally, a fully dynamic model is merited. Dynamic models of response to the local environmental alone have been made, for limited plant species and functional types (Gross, Kirschbaum & Pearcy 1991; Kirschbaum *et al.* 1998). They are most relevant for examining adaptations to contrasting environments, such as forest understories. For predicting total transpiration or assimilation of plant canopies, it is more practical to use effective (dynamic average) parameters in our existing model.

Other theoretical and conceptual implications

Our analyses imply an amplification of ABA action by the stomatal response to the local environment. Specifically, ABA induces partial closure, which decreases both assimilation and surface humidity. These decreases further reduce g_s , acting through unknown signals. From one point of view, this appears counterintuitive. A decrease in internal CO_2 partial pressure, C_i , typically induces stomatal opening (Mott 1988). This would counteract and not amplify ABA-induced stomatal closure. Amplification would require that stomatal controls responding to A (or C_i) and to ABA act in series rather than in parallel.

Santrucek & Sage (1996) have analysed stomatal control in terms of two feedback loops, one responding to C_i directly and another responding to A , which is clearly affected by C_i . In *Chenopodium album*, the gain of the first loop, g_s , was found to be near -0.15 at ambient CO_2 partial pressures. The gain of the A -responsive loop, G_A , was of larger absolute magnitude, near -0.25 . If one posits that the Ball–Berry model is an accurate description of g_s , the same mathematical calculations indicate a small response to C_i directly ($G_s \approx -0.05$) and a larger response to A ($G_A \approx -0.2$). Such behaviour is consistent with our proposed amplification, if the ABA control loop is in series with the A -responsive loop. The drop in C_i is less important than the drop in A , such that stomatal closure can be enhanced. It is also worth noting that other empirical models such as that of Leuning (1995) have similar patterns of feedback-loop gains. They differ from the Ball–Berry model primarily in the gain of a parallel feedback loop that responds to transpiration.

Jarvis, Mansfield & Davies (1999) offer another model of combined stomatal responses to assimilation and humidity. The humidity response is consistent with that proposed by Leuning (1995). The formulation of A is somewhat simplified, allowing an intriguing symmetry in A and E responses to occur. The model does not incorporate the water-stress response, but it does consider acclimation to growth at

elevated CO_2 . It might be profitably combined with an ABA model.

In an action that is equivalent to changing the feedback-loop strengths, we may loosen some constraints on our model, by representing g_s as $m A h_s^Q / C_s + b$, where m is again a function of $[ABA]$ but Q need not be unity. Our earlier analysis of $\ln(g_s)$ implied that Q equals or exceeds 2. This result implies that either the Ball–Berry model should be amended to allow h_s to appear to a power significantly different from 1, or that ABA may mediate a higher-order (quadratic) response to humidity (compare Bunce 1996). Our data support the former idea. We consider only those data with low $[ABA]$, less than 100 nM. Even for these, a multiple linear regression of $\ln(g_s)$ against $\ln(A)$, $\ln(h_s)$ and $\ln(C_s)$ yields a coefficient of $\ln(h_s) = 2.32 \pm 0.14$ for the 1999 data set and $2.38 (\pm 0.20)$ for the 1994 data set. In the original Eqn 6, before logarithmic transformation, a regression of g_s against the modified index $A h_s^2 / C_s$ does make a small improvement over use of the normal Ball–Berry index with $Q = 1$. For the low-ABA data of 1999, r^2 improves from 0.676 to 0.735. For the low-ABA data of 1994, the improvement is from 0.473 to 0.516. In summary, the evidence for enhanced sensitivity to humidity in our system is modest, especially given the inherently empirical nature of the Ball–Berry formulation. It is known, however, that sensitivity to humidity (as h_s or VPD) does vary with growth conditions, including CO_2 level (Bunce 1998; Heath 1998).

We should also examine if the exponent C for the response of g_s to assimilation, A , is appropriately unity. Indicative of the challenges in representing this coupling, the coefficient c_1 for $\ln(A)$ in the regression analysis of $\ln(g_s)$ changes in magnitude as the other driving variables (h_s , C_s , $[ABA]$) are introduced in a regression analysis and also as data with higher ranges of $[ABA]$ are included. Table 5 shows that c_1 is only near unity when ABA effects are essentially absent ($[ABA] < 10$ nM). As data with greater ABA content are included, the value of c_1 decreases. This indicates that the multiplicative model is partially confusing some control loops, but we have little guidance for modifying the formulation, in the absence of further biochemical studies. Table 5 shows that the value of c_1 also varies with the completeness of the set of driving variables. This sensitivity is a normal result of significant correlations among the driving variables. For example, a high assimilation rate is linked mechanistically to high g_s , and thus to strong humidification of the boundary layer (high h_s) and also to depression of C_s . A path analysis can include the direct linkages (in which the ‘direct’ coefficient $c_{1,d}$ might be near unity with *any* data) and the correlations. An adequate path analysis is unlikely to be constructed, given that: (1) mechanistic links and their directionality should all be known, whereas we lack information on some intermediate biochemistry; and (2) the mathematical relations among variables such as $\ln(A)$ and $\ln(C_s)$ are non-linear and often of the form of transcendental (non-algebraic) equations. The results of Table 5 may then be construed as further argument to pursue deeper biochem-

Table 5. Variation of the effective algebraic power of A in the Ball–Berry equation as other driving variables are introduced or data ranges are changed

Data range	Coefficients of				r^2	n
	$\ln(A)$	$\ln(h_s)$	$\ln(C_s)$	[ABA]		
[ABA] < 100	1.33 ± 0.09**				0.614	136
		2.96 ± 0.12**			0.820	136
			−3.51 ± 0.36**		0.431	136
	0.56 ± 0.07**	2.25 ± 0.13**			0.882	136
	0.56 ± 0.07**	2.27 ± 0.15**	0.39 ± 0.23		0.882	136
	0.60 ± 0.07**	2.22 ± 0.15**	0.12 ± 0.23	−0.00194 ± 0.00071	0.888	136
[ABA] < 30	0.75 ± 0.09**	1.94 ± 0.17**	−0.06 ± 0.32	−0.00730 ± 0.00230*	0.915	91
[ABA] < 10	0.92 ± 0.17**	2.06 ± 0.37**	1.46 ± 0.95	−0.00680 ± 0.01340	0.873	23
All ABA	0.58 ± 0.07**	2.03 ± 0.14**	0.10 ± 0.22	−0.00091 ± 0.00039	0.894	145

13

Stepwise multiple linear regression was performed on 1999 data, in which low [ABA] values predominate, allowing the Ball–Berry response to dominate. Note that [ABA] has been put as the last driving variable, differing from the order in Table 3.

ical studies of stomatal control. It is of interest that the coefficient c_2 for $\ln(h_s)$ is very stable, near 2, independent of the data range and the inclusion of other variables. This adds to the evidence that the response to h_s is stronger than in the simple Ball–Berry model. Equivalently, in models of g_s responding to VPD, the response is stronger than linear – for example, in the data of Leuning (1995).

Future research

Biochemical studies are progressing and might be aided by testing the hypothesis that ABA and aerial signals act sequentially. The ABA response appears to have additional dimensions that merit investigation. First, the pH of xylem sap appears to modulate ABA action in the leaf, probably by changing the degree of ionization of ABA and thus its ability to move freely in the apoplast (Hartung, Wilkinson & Davies 1998; Wilkinson *et al.* 1998; Wilkinson 1999; Netting 2000). Quantitative prediction of xylem-sap pH and its concurrent effect on the ABA response is not yet possible, although progress is being made (Auge *et al.* 2000). Second, K^+ nutrition may affect ABA action, given that K^+ is intimately involved in ABA action on guard cells (see, e.g. Assmann 1999; Netting 2000). Ecotypes that differ in K^+ dynamics may differ in ABA responses. Third, vapour-pressure deficits may modulate ABA action, or, as one may rephrase it, may act in part through their effect on ABA concentration or delivery rate. It is certainly conceivable that changes in epidermal transpiration rates, linked to VPD, may alter the delivery of ABA to the guard cells (Wilkinson & Davies 2002).

The action of ABA in some trees is similar to its action in herbaceous plants (Niinemets *et al.* 1999), in that g_s responds to xylem [ABA]. In other trees, the sensitivity of g_s varies with leaf water potential, in a manner that could be consistent with the isohydric model of Tardieu & Simonneau (1998), but the analysis is not complete (Fuchs & Livingston 1996; Correia *et al.* 1997). Redistribution of

ABA stored within leaves may control g_s . This independent route of action would require a major extension of the latter model.

ACKNOWLEDGMENTS

V.G. gratefully acknowledges the support of a travel grant as a supplement to the Jornada Long-Term Ecological Research grant (DEB-111971) from the National Science Foundation. He also acknowledges the National Institutes for Global Environmental Change, a program of the US Department of Energy, for cumulative support of research during which many of the ideas were developed. V.G. thanks Igr. Francois Tardieu for hosting his visit, for extensive discussions on ABA action, and for suggesting the model of the minimum; Phillipe Naudin for setting up computer services, and the other staff and students of the Laboratoire d'Ecophysiologie des Plantes sur Stress Environnementaux for discussions and assistance in field work. The authors thank Tanguy Lafarge for use of the 1994 data set, and two anonymous reviewers for many substantive comments.

REFERENCES

- Aphalo P.J. & Jarvis P.G. (1993) An analysis of Ball's empirical model of stomatal conductance. *Annals of Botany* **72**, 321–327.
- Assmann S.M. (1993) Signal transduction in guard cells. *Annual Review of Cell Biology* **9**, 345–375.
- Assmann S.M. (1999) The cellular basis of guard cell sensing of rising CO_2 . *Plant, Cell and Environment* **22**, 629–637.
- Auge R.M., Green C.D., Stodola A.J.W., Saxton A.M., Olinick J.B. & Evans R.M. (2000) Correlations of stomatal conductance with hydraulic and chemical factors in several deciduous tree species in a natural habitat. *New Phytologist* **145**, 483–500.
- Ball J.T., Woodrow I.E. & Berry J.A. (1987) A model predicting stomatal conductance and its contribution to the control of photosynthesis under different environmental conditions. In *Progress in Photosynthesis Research* Vol. 4 (ed. J. Biggins), pp. 5 221–5.224. M. Nijhoff, Dordrecht, The Netherlands.

- Barrieu P. & Simonneau T. (2000) The monoclonal antibody MAC252 does not react with the (-) enantiomer of ABA. *Journal of Experimental Botany* **51**, 305–307.
- Bunce J.A. (1996) Does transpiration control stomatal responses to water vapour pressure deficit? *Plant, Cell and Environment* **19**, 131–135.
- Bunce J.A. (1998) Effects of environment during growth on the sensitivity of leaf conductance to changes in humidity. *Global Change Biology* **4**, 269–274.
- Collatz G.J., Ball J.T., Grivet C. & Berry J.A. (1991) Physiological and environmental regulation of stomatal conductance, photosynthesis and transpiration: a model that includes a laminar boundary layer. *Agricultural and Forest Meteorology* **54**, 107–136.
- Collatz G.J., Berry J.A., Farquhar G.D. & Pierce J. (1990) The relationship between the Rubisco reaction mechanism and models of photosynthesis. *Plant, Cell and Environment* **13**, 219–225.
- Correia M.J., Rodrigues M.L., Ferreira M.I. & Pereira J.S. (1997) Diurnal change in the relationship between stomatal conductance and abscisic acid in the xylem sap of field-grown peach trees. *Journal of Experimental Botany* **48**, 1727–1736.
- Cowan I.R., Raven J.A., Hartung W. & Farquhar G.D. (1982) A possible role for abscisic acid in coupling stomatal conductance and photosynthetic carbon metabolism in leaves. *Australian Journal of Plant Physiology* **9**, 489–498.
- Davies W.J. & Zhang J. (1991) Roots signals and the regulation of growth and development of plants in drying soil. *Annual Reviews of Plant Physiology and Plant Molecular Biology* **42**, 55–76.
- Dewar R.C. (1995) Interpretation of an empirical model for stomatal conductance in terms of guard cell function. *Plant, Cell and Environment* **18**, 365–372.
- Du Z., Aghoram K. & Outlaw W.H. Jr (1997) *In vivo* phosphorylation of phosphoenolpyruvate carboxylase in guard cells of *Vicia faba* L. is enhanced by fusicoccin and suppressed by abscisic acid. *Archives of Biochemistry and Biophysics* **337**, 345–350.
- Dunleavy P.J. & Ladley P.D. (1995) Stomatal responses of *Vicia faba* to indole acetic acid and abscisic acid. *Journal of Experimental Botany* **46**, 95–100.
- Farquhar G.D., von Caemmerer S. & Berry J.A. (1980) A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta* **149**, 78–90.
- Fuchs E.E. & Livingston N.J. (1996) Hydraulic control of stomatal conductance in Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] and alder [*Alnus rubra* (Bong.)] seedlings. *Plant, Cell and Environment* **19**, 1091–1098.
- Gross L.J., Kirschbaum M.U.F. & Pearcy R.W. (1991) A dynamic model of photosynthesis in varying light taking account of stomatal conductance, C₃-cycle intermediates, photorespiration and Rubisco activation. *Plant, Cell and Environment* **14**, 881–893.
- Gutschick V.P., Maxwell C.J., Montes-Helu M., Najera F., Jackson E., Mortenson E., & Soto A. (2002) Physiological control of carbon, water fluxes in the Chequamegon National Forest its variability and consequences. *Global Change Biology* **XX**, 00–00.
- Hartung W., Wilkinson S. & Davies W.J. (1998) Factors that regulate abscisic acid concentrations at the primary site of action at the guard cell. *Journal of Experimental Botany* **49**, 361–367.
- Heath J. (1998) Stomata of trees growing in CO₂-enriched air show reduced sensitivity to vapour pressure deficit and drought. *Plant, Cell and Environment* **21**, 1077–1088.
- Jarvis P.G. (1976) The interpretation of leaf water potential and stomatal conductance found in canopies in the field. *Philosophical Transactions of the Royal Society (London) B* **273**, 593–610.
- Jarvis A.J. & Davies W.J. (1998) The coupled response of stomatal conductance to photosynthesis and transpiration. *Journal of Experimental Botany* **49**, 399–406.
- Jarvis A.J., Mansfield T.A. & Davies W.J. (1999) Stomatal behaviour, photosynthesis and transpiration under rising CO₂. *Plant, Cell and Environment* **22**, 639–648.
- Kirschbaum M.U.F., Kuppers M., Schneider H., Giersch C. & Noe S. (1998) Modelling photosynthesis is fluctuating light with inclusion of stomatal conductance, biochemical activation and pools of key photosynthetic intermediates. *Planta* **204**, 16–26.
- Leuning R. (1990) Modelling stomatal behaviour and photosynthesis of *Eucalyptus grandis*. *Australian Journal of Plant Physiology* **17**, 159–175.
- Leuning R. (1995) A critical appraisal of a combined stomatal-photosynthesis model for C₃ plants. *Plant, Cell and Environment* **18**, 339–355.
- Lhomme J.-P., Elguero E., Chehbouni A. & Boulet G. (1998) Stomatal control of transpiration: Examination of Monteith's formulation of canopy resistance. *Water Resources Research* **34**, 2301–2308.
- Li-Cor Inc. (1990) *The LI-6200 Primer*. LI-COR Inc, Lincoln, NE, USA.
- Luan S. (2002) Signalling drought in guard cells. *Plant, Cell and Environment* **25**, 229–237.
- Matzner S. & Comstock J. (2001) The temperature dependence of shoot hydraulic resistance: implications for stomatal behaviour and hydraulic limitation. *Plant, Cell and Environment* **24**, 1299–1307.
- Monteith J.L. (1995) A reinterpretation of stomatal responses to humidity. *Plant, Cell and Environment* **18**, 357–363.
- Mott K.A. (1988) Do stomata respond to CO₂ concentrations other than intercellular? *Plant Physiology* **86**, 200–203.
- Mott K.A. & Parkhurst D.F. (1991) Stomatal responses to humidity in air and helox. *Plant, Cell and Environment* **14**, 509–515.
- Netting A.G. (2000) pH, abscisic acid and the integration of metabolism in plants under stressed and non-stressed conditions: cellular responses to stress and their implication for plant water relations. *Journal of Experimental Botany* **51**, 147–158.
- Niinemets Ü., Söber A., Kull O., Hartung W. & Tenhunen J.D. (1999) Apparent controls on leaf conductance by soil water availability and via light-acclimation of foliage structural and physiological properties in a mixed deciduous, temperate forest. *International Journal of Plant Sciences* **160**, 707–721.
- Nobel P.S. (1991) *Physicochemical and Environmental Plant Physiology*. Academic Press, San Diego, CA, USA.
- Noilhan J. & Planton S. (1989) A simple parametrization of land surface processes for meteorological models. *Monthly Weather Reviews* **117**, 536–549.
- de Pury D.D.G. (1995) *Scaling Photosynthesis and Water Use from Leaves to Paddocks*. PhD Thesis, Australian National University, Canberra, Australia.
- Quarrie S.A., Whitford P.N., Appleford N.E.J., Wang T.L., Cook S.K., Henson I.E. & Loveys B.R. (1988) A monoclonal antibody to (S)-abscisic acid: its characterisation and use in a radioimmunoassay for measuring abscisic acid in crude extracts of cereal and lupin leaves. *Planta* **173**, 330–339.
- Santrucek J. & Sage R.F. (1996) Acclimation of stomatal conductance to a CO₂-enriched atmosphere and elevated temperature in *Chenopodium album*. *Australian Journal of Plant Physiology* **23**, 467–478.
- Schultz H. & Lebon E. (1995) Modelling vineyard transpiration and assimilation under water limiting conditions. In *3eme Rapport d'Avancement Des Travaux*. INRA, Montpellier, France.
- Sellers P.J., Randall D.A., Collatz G.J., Field C.B., Dazlich D.A., Zhang C., Collelo G.D. & Bounoua L. (1996) A revised land

- surface parametrization (SiB2) for atmospheric GCMs. Part I: model formulation. *Journal of Climate* **9**, 676–705.
- Simonneau T., Barrieu P. & Tardieu F. (1998) Accumulation rate of ABA in detached maize roots correlates with root water potential regardless of age and branching order. *Plant, Cell and Environment* **21**, 1113–1122.
- Sperry J.A., Adler F.R., Campbell G.S. & Comstock J.P. (1998) Limitation of plant water use by rhizosphere and xylem conductance: results from a model. *Plant, Cell and Environment* **21**, 347–359.
- Stewart J.B. (1988) Modelling surface conductance of pine forest. *Agricultural and Forest Meteorology* **43**, 19–37.
- Tardieu F. & Davies W.J. (1993) Integration of hydraulic and chemical signalling in the control of stomatal conductance and water status of droughted plants. *Plant, Cell and Environment* **16**, 341–349.
- Tardieu F. & Simonneau T. (1998) Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. *Journal of Experimental Botany* **49**, 419–432.
- Tardieu F., Bruckler L. & Lafolie F. (1992) Root clumping may affect the root water potential and the resistance to soil-root water transport. *Plant and Soil* **140**, 291–301.
- Tardieu F., Katerji N., Bethenod O., Zhang J. & Davies W.J. (1991) Maize stomatal conductance in the field: its relationship with soil and plant water potentials, mechanical constraints and ABA concentration in the xylem sap. *Plant, Cell and Environment* **14**, 121–112.
- Tardieu F., Lafarge T. & Simonneau T. (1996) Stomatal control by fed or endogenous xylem ABA in sunflower: interpretation of correlations between leaf water potential and stomatal conductance in anisohydric species. *Plant, Cell and Environment* **19**, 75–84.
- Tardieu F., Zhang J. & Gowing C.J.G. (1993) Stomatal control by both [ABA] in the xylem sap and leaf water status: a test of a model for droughted or ABA-fed field-grown maize. *Plant, Cell and Environment* **16**, 413–420.
- Tenhunen J.D., Hanano R., Abril M., Weiler E.W. & Hartung W. (1994) Above- and below-ground environmental influences on leaf conductance of *Ceanothus thyrsiflorus* growing in a chaparral environment: drought response and the role of abscisic acid. *Oecologia* **99**, 306–314.
- Tenhunen J.D., Sala Serra A., Harley P.C., Dougherty R.I. & Reynolds J.F. (1990) Factors influencing carbon fixation and water use by Mediterranean sclerophyll shrubs during summer drought. *Oecologia* **82**, 381–393.
- Wilkinson S. (1999) pH as a stress signal. *Plant Growth Regulators* **29**, 87–89.
- Wilkinson S. & Davies W.J. (2002) ABA-based chemical signalling: the co-ordination of responses to stress in plants. *Plant, Cell and Environment* **25**, 195–210.
- Wilkinson S., Corlett J.E., Oger L. & Davies W.J. (1998) Effects of xylem pH on transpiration from wild-type and *flacca* mutant tomato leaves: a vital role for abscisic acid in preventing excessive water loss even from well-watered plants. *Plant Physiology* **117**, 703–709.
- Zeiger E., Farquhar G.D. & Cowan I.R. (1987) *Stomatal Function*. Stanford University Press, Stanford, CA, USA.

Received 8 March 2002; received in revised form 7 June 2002; accepted for publication 11 June 2002

AUTHOR QUERY FORM

Journal: Plant, Cell and Environment

Article: 937

Dear Author,

During the preparation of your manuscript for publication, the questions listed below have arisen. Please attend to these matters and return this form with your proof.

Many thanks for your assistance.

Query References	Query	Remarks
1	Au: Is the text OK here in title and at definition in main text: abscisic acid (ABA)	
2	Au: is subscript r alone here intended or should it be Pr	
3	Au: Please supply company details/address of Hoagland	
4	Au: Is the text OK: Cambridge, UK	
5	Au: Is the text OK: radioimmunoassay (RIA)	
6	Au: Is the text OK: gx	
7	Au: citation repeated - OK	
8	Au: please check table headings - possibly some misalignments introduced during file transfers	
9	Au: Is the text OK: Gutschick et al. 2002	
10	Harley & Tenhunen 1991 has not been included in the list	
11	Au: please add volume and page range if available now	
12	Au: please add text to define use of double asterisks	
13	Au: please add text to define use of double asterisks	

MARKED PROOF

Please correct and return this set

Any errors in this proof which have been noticed by the printer's reader have been marked in green. If you see any more printer's errors, please mark them in red: there is no charge for correcting these mistakes. For your own alterations, please use black or blue or any colour other than green or red. Please use the proof correction marks shown below for all alterations and corrections.

<i>Instruction to printer</i>	<i>Textual mark</i>	<i>Marginal mark</i>
Leave unchanged	... under matter to remain	Stet
Insert in text the matter indicated in the margin	⤴	New matter followed by ⤴
Delete	⤵ through matter to be deleted	⤵
Delete and close up	⤵ through matter to be deleted	⤵
Substitute character or substitute part of one or more word(s)	/ through letter or ⤵ through word	New letter or new word
Change to italics	— under matter to be changed	ƒ
Change to capitals	≡ under matter to be changed	≡
Change to small capitals	= under matter to be changed	=
Change to bold type	~ under matter to be changed	~
Change to bold italic	≡ under matter to be changed	≡
Change to lower case	Encircle matter to be changed	⊖
Change italic to upright type	(As above)	⤴
Insert 'superior' character	/ through character or ⤴ where required	γ under character e.g. γ
Insert 'inferior' character	(As above)	⤵ over character e.g. ⤵
Insert full stop	(As above)	⦿
Insert comma	(As above)	,
Insert single quotation marks	(As above)	γ and/or γ
Insert double quotation marks	(As above)	γ and/or γ
Insert hyphen	(As above)	Ⓜ
Start new paragraph	⤴	⤴
No new paragraph	~	~
Transpose	⤴	⤴
Close up	linking c letters	∩
Insert space between letters	⤴ between letters affected	#
Insert space between words	⤴ between words affected	#
Reduce space between letters	↑ between letters affected	↑
Reduce space between words	↑ between words affected	↑