

Long-term growth of soybean at elevated [CO₂] does not cause acclimation of stomatal conductance under fully open-air conditions

ANDREW D. B. LEAKEY¹, CARL J. BERNACCHI², DONALD R. ORT^{1,3,4} & STEPHEN P. LONG^{1,4}

¹Institute for Genomic Biology and Department of Plant Biology, University of Illinois at Urbana-Champaign, 1207 West Gregory Drive, Urbana, IL 61801, USA, ²Center for Atmospheric Science, Illinois State Water Survey, 2204 Griffith Dr, Champaign, IL 61820, USA, ³Photosynthesis Research Unit, US Department of Agriculture/Agricultural Research Service, 1201 West Gregory Drive, Urbana, IL 61801, USA and ⁴Department of Crop Sciences, University of Illinois at Urbana-Champaign, 1201 West Gregory Drive, Urbana, IL 61801, USA

ABSTRACT

Accurately predicting plant function and global biogeochemical cycles later in this century will be complicated if stomatal conductance (g_s) acclimates to growth at elevated [CO₂], in the sense of a long-term alteration of the response of g_s to [CO₂], humidity (h) and/or photosynthetic rate (A). If so, photosynthetic and stomatal models will require parameterization at each growth [CO₂] of interest. Photosynthetic acclimation to long-term growth at elevated [CO₂] occurs frequently. Acclimation of g_s has rarely been examined, even though stomatal density commonly changes with growth [CO₂]. Soybean was grown under field conditions at ambient [CO₂] (378 $\mu\text{mol mol}^{-1}$) and elevated [CO₂] (552 $\mu\text{mol mol}^{-1}$) using free-air [CO₂] enrichment (FACE). This study tested for stomatal acclimation by parameterizing and validating the widely used Ball *et al.* model (1987, *Progress in Photosynthesis Research*, vol IV, 221–224) with measurements of leaf gas exchange. The dependence of g_s on A , h and [CO₂] at the leaf surface was unaltered by long-term growth at elevated [CO₂]. This suggests that the commonly observed decrease in g_s under elevated [CO₂] is due entirely to the direct instantaneous effect of [CO₂] on g_s , and that there is no longer-term acclimation of g_s independent of photosynthetic acclimation. The model accurately predicted g_s for soybean growing under ambient and elevated [CO₂] in the field. Model parameters under ambient and elevated [CO₂] were indistinguishable, demonstrating that stomatal function under ambient and elevated [CO₂] could be modelled without the need for parameterization at each growth [CO₂].

Key-words: *Glycine max*; climate change; FACE; model; photosynthesis.

INTRODUCTION

Across C₃ and C₄ species, long-term growth at concentrations of atmospheric CO₂ ([CO₂]) anticipated for later this

Correspondence: Andrew D. B. Leakey. Fax: +1 217 265 6800; e-mail: leakey@life.uiuc.edu

century leads consistently to lower stomatal conductance to water vapour (g_s ; reviewed in Long *et al.* 2004). This can in turn reduce plant water use, improve water use efficiency and enhance carbon (C) gain during drought stress (Field, Jackson & Mooney 1995; Kimball *et al.* 1999; Owensby *et al.* 1999; Leakey *et al.* 2004; Leakey *et al.* 2006).

The Ball, Woodrow & Berry (1987) model predicts leaf g_s on the basis of a linear, empirical relationship:

$$g_s = g_0 + m \frac{Ah}{[\text{CO}_2]} \quad (1)$$

where A is the net rate of photosynthetic CO₂ assimilation; h is the fractional atmospheric relative humidity; [CO₂] is the atmospheric [CO₂] at the leaf surface; g_0 is the y-axis intercept, and m is the slope of the line. The Ball *et al.* (1987) model, solved simultaneously with the Farquhar, von Caemmerer & Berry (1980) steady-state model of leaf photosynthesis, provides a parsimonious and effective means of predicting intact leaf photosynthesis and transpiration (Leuning 1990; Collatz *et al.* 1991; Harley & Tenhunen 1991). The combination of these two models and their derivatives are at the core of a number of contemporary models of terrestrial biosphere C and water cycling (e.g. Foley *et al.* 1996; Sellers *et al.* 1996; Bounoua *et al.* 1999). Critical to their use for predicting the responses of vegetation to rising [CO₂] is the issue of whether there is physiological acclimation during growth under elevated [CO₂], in the sense that g_0 or m of Eqn 1 are altered. This would occur if sensitivity of g_s to [CO₂], h and/or A changed with growth at elevated [CO₂]. In the case of photosynthetic acclimation, g_s would decrease alongside A at a given [CO₂] and h (Eqn 1). However, without independent stomatal acclimation to [CO₂], there would be no change in g_0 and m , and thus no change in the sensitivity of g_s to [CO₂], h and/or A . Acclimation of the key parameters of the Farquhar *et al.* (1980) photosynthesis model (maximum apparent carboxylation capacity, V_{cmax} ; and maximum apparent electron transport rate, J_{max}) under elevated [CO₂] is well documented (reviewed: Wullschlegel 1993; Drake, Gonzalez-Meler & Long 1997; Ainsworth & Long 2005).

However, relatively little is known of whether there is acclimation of the parameters of the Ball *et al.* (1987) g_s model under elevated $[\text{CO}_2]$, even though acclimation of stomatal frequency has been widely reported (reviewed in Woodward & Kelly 1995).

Parameters of the Ball *et al.* (1987) model were compared for six tree species at four field sites after exposure to ambient or elevated $[\text{CO}_2]$ in chambers and branch bags, in the most complete study to date (Medlyn *et al.* 2001). In seven out of eight species/site combinations, there was no change in g_0 or m with growth $[\text{CO}_2]$. Only in *Phillyrea angustifolia* at a water-stressed site in Italy was m different between ambient and elevated $[\text{CO}_2]$. This suggests that stomatal acclimation to growth $[\text{CO}_2]$ may be unusual in trees. Reports of crop responses are less consistent. There was no difference in g_0 and m of *Lolium perenne* (rye grass) grown at 350 and 600 $\mu\text{mol mol}^{-1} \text{CO}_2$ in a pseudo-replicated free-air $[\text{CO}_2]$ enrichment (FACE) study (Nijs *et al.* 1997). There was a marginally significant, lower m (–7%) in *Gossypium hirsutum* (cotton) grown at 650 versus 350 $\mu\text{mol mol}^{-1} \text{CO}_2$ in controlled environmental cabinets (Harley *et al.* 1992). In contrast, a large decrease in m at elevated $[\text{CO}_2]$ (49%) was reported for *Glycine max* (soybean), which responded more strongly than *Solanum tuberosum* (potato), *Phaseolus vulgaris* (bean) or *Sorghum bicolor* (sorghum) when grown at 350 and 700 $\mu\text{mol mol}^{-1} \text{CO}_2$ in open-top chambers (Bunce 2004). Possibly, herbaceous crops, which typically have high g_s and shorter lived leaves, are more likely to undergo stomatal acclimation at elevated $[\text{CO}_2]$ than trees. In addition, the extent and nature of stomatal acclimation may differ between chamber and FACE studies, as photosynthetic acclimation does (reviewed in Ainsworth & Long 2005). FACE experiments treat plants with elevated $[\text{CO}_2]$ without altering the plant–soil–atmosphere continuum, providing the most realistic simulation of future $[\text{CO}_2]$ without disturbing the potential feedbacks among stomatal function, canopy micrometeorology and root–shoot signalling.

This study tested the hypothesis that soybean growth at elevated $[\text{CO}_2]$ under field conditions does not result in acclimation of g_s (i.e. there is no change in the response of g_s to $[\text{CO}_2]$, h and A). This was tested by parameterizing and validating the model of g_s (Ball *et al.* 1987) in the youngest fully expanded leaves of soybean growing under ambient and FACE treatments of elevated $[\text{CO}_2]$ in central Illinois. Soybean may be particularly appropriate for this test because a previous study reports a very strong acclimation response when it is grown at elevated $[\text{CO}_2]$ in a controlled environment (Bunce 2004). Previous studies at this FACE facility [i.e. Soybean Free Air Concentration Enrichment (SoyFACE)] demonstrated that growth at elevated $[\text{CO}_2]$ resulted in photosynthetic acclimation [i.e. a statistically significant lower V_{cmax} and $V_{\text{cmax}}/J_{\text{max}}$ (Bernacchi *et al.* 2005)]. Soybean is inbred, and the agricultural management of the site provides relatively uniform growth conditions. This genetic and environmental uniformity increases the power to detect subtle $[\text{CO}_2]$ treatment effects. While providing a model field system for testing

general hypotheses, it is also a key regional ecosystem. The US Corn Belt accounted for 36% of global soybean production in 2003/2004 (USDA 2005). The soybean–corn agroecosystem is also arguably the largest single ecosystem type in the USA, covering 61.8 million hectares of the contiguous states in 2003 (USDA 2004). Therefore, this was a unique opportunity to investigate stomatal acclimation with a high degree of sensitivity, under fully open-air conditions with direct relevance to future agricultural performance and surface–atmosphere exchange of a major portion of the US land surface. There was no evidence for stomatal acclimation in terms of altered sensitivity of g_s to A , h and $[\text{CO}_2]$ in soybean grown at elevated $[\text{CO}_2]$. The Ball *et al.* (1987) model accurately predicted g_s for soybean growing in the field, regardless of the $[\text{CO}_2]$ at which plants were grown. This demonstrated that in this system, at least, stomatal function under ambient and elevated $[\text{CO}_2]$ can be modelled without the need for growth $[\text{CO}_2]$ -specific parameterization.

MATERIALS AND METHODS

Field site and cultivation

This experiment was conducted at the SoyFACE facility in Champaign, IL, USA (40°02'N, 88°14'W, 228 m above sea level; <http://www.soyface.uiuc.edu>). Situated on 32 ha of Illinois farmland, soybean [*Glycine max* (L.) Merr. cv. 93B15 (Pioneer Hi-Bred International, Des Moines, IA, USA)] and maize [*Zea mays* cv. 34B43 (Pioneer Hi-Bred International)] were planted over 16 ha each and rotated annually. The soil is typical of northern and central Illinois, being organically rich, deep and highly productive. The site is tile drained and has been in cultivation for over 100 years. For a detailed description of the site, see Leakey *et al.* (2004) and Rogers *et al.* (2004). The experiment was conducted as a randomized complete block design. A portion of the field was divided into four blocks. Within each block, one plot was maintained at current ambient $[\text{CO}_2]$, and another plot was fumigated to a target $[\text{CO}_2]$ of 550 $\mu\text{mol mol}^{-1}$, which is the $[\text{CO}_2]$ that the Intergovernmental Panel on Climate Change projects for the year 2050 (Prentice *et al.* 2001). Details of the FACE technology used at SoyFACE have been described previously (Miglietta *et al.* 2001; Leakey *et al.* 2004). On average across the season, $[\text{CO}_2]$ was 378 in the ambient and 552 $\mu\text{mol mol}^{-1}$ in the elevated $[\text{CO}_2]$ treatments. The $[\text{CO}_2]$ in the elevated $[\text{CO}_2]$ plots was within 10% of the target concentration for 84% of the time, on the basis of 1 min averages.

Soybean was planted on 27 May 2003 with row spacing of 0.38 m (15 in.). Cultural practices used were standard for the region, with no fertilizer added to the soybean crop and following annual rotation with corn. For a detailed description of agronomic practices, see Morgan *et al.* (2005). A hailstorm on 17 July caused a uniform ~ 50% defoliation of the crop, but rapid and vigorous regrowth produced a final yield close to the annual average for the region (Morgan *et al.* 2005).

Model parameterization

Laboratory-based gas exchange measurements to parameterize the Ball *et al.* (1987) model of g_s were made on nine dates between 15 August and 12 September. At pre-dawn on each date, an uppermost fully expanded leaf was selected at random from one field plot of each treatment. The petioles were cut, recut underwater and kept in water for the duration of the measurements. Leaves collected in this manner achieve light-saturated rates of photosynthesis (Ainsworth *et al.* 2004; Morgan *et al.* 2004) that equal or exceed rates observed in the field (Rogers *et al.* 2004). Leaf gas exchange was measured using two open-path gas exchange systems incorporating infrared CO₂ and water vapour analysers and a 2 cm² leaf chamber (LI-6400; Li-Cor, Lincoln, NE, USA). The two systems were calibrated against a standard known concentration of CO₂ in air (21.4% O₂/balance N₂; CO₂ 503 $\mu\text{mol mol}^{-1}$; SJ Smith Welding Supply, Decatur, IL, USA) and known h from a precision water vapour generator (LI-610, Li-Cor). Leaf A and g_s were calculated according to von Caemmerer & Farquhar (1981). All measurements were performed at a common leaf temperature of 25 ± 1 °C. Photosynthesis was initiated with an incident photosynthetic photon flux density (PPFD) of $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$, [CO₂] of $370 \mu\text{mol mol}^{-1}$ and atmospheric saturation vapour pressure deficit (D) of < 1 kPa. Once photosynthesis had attained steady-state rates, the effects of varying [CO₂], PPFD and D were tested in three consecutive phases (Fig. 1a), across ranges representative of growing conditions for *G. max* at SoyFACE (Rogers *et al.* 2004). Firstly, the [CO₂] of air entering the chamber was varied stepwise (370, 150, 250, 350, 450, 650, 850, 1200, $1500 \mu\text{mol mol}^{-1}$) as PPFD was held constant at $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$. Changes in leaf transpiration with [CO₂] caused variation in D , but this was minimized ($D < 1$ kPa) by manually adjusting the flow of air through a desiccant column to control the water vapour pressure of air entering the chamber. Secondly, PPFD incident on the leaf was varied stepwise (1500, 1000, 700, 400, 200, 100, 75, $50 \mu\text{mol m}^{-2} \text{s}^{-1}$) as [CO₂] of air entering the chamber was held constant at growth [CO₂] (370 or $550 \mu\text{mol mol}^{-1}$). Again, variation in D with transpiration was minimized by manual adjustments. Thirdly, vapour pressure was varied stepwise in six increments from ~ 0.5 – 1.0 kPa to 2.5 – 3.5 kPa while PPFD was held constant at $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$, and [CO₂] of air entering the chamber was maintained at growth [CO₂] (370 or $550 \mu\text{mol mol}^{-1}$). The shift from wetter to drier air was characterized in D because the deficit in vapour pressure from the intercellular leaf space to the atmosphere is a direct determinant of transpiration. In turn, changes in the rate of transpiration impact g_s (Mott & Parkhurst 1991). However, the Ball *et al.* (1987) model does not deal with this response mechanistically. Therefore, for the purposes of model parameterization, the progressive increase in D imposed on the leaf related to a progressive decrease in h . Throughout the measurements, gas exchange was allowed to reach steady state before the results were recorded and the next stepwise change initiated. The intercept (g_0) &

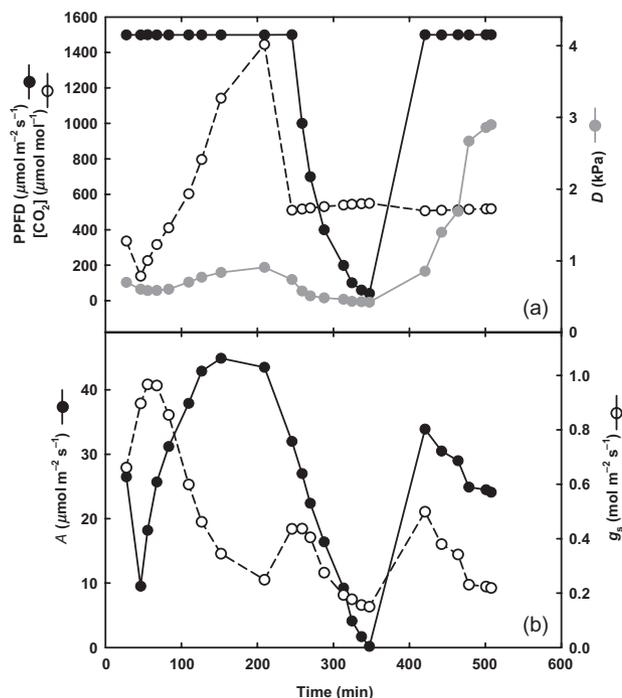


Figure 1. Representative course of (a) incident photosynthetic photon flux density (PPFD), atmospheric [CO₂] and atmospheric saturation vapour pressure deficit (D) in the leaf chamber during gas exchange measurements of (b) steady-state net photosynthetic CO₂ assimilation (A) and stomatal conductance (g_s) in the uppermost fully expanded leaves of field-grown *Glycine max* at SoyFACE in 2003. Leaves were harvested pre-dawn, recut underwater and measured in the laboratory. The response of gas exchange to variation in [CO₂], PPFD and D was tested consecutively while minimizing variation in the other variables. Steady-state gas exchange was attained after each stepwise change in conditions. Varying [CO₂] and PPFD altered leaf transpiration and caused variation in D , which was minimized by manually adjusting the water vapour pressure of air entering the chamber.

slope (m) of the Ball *et al.* (1987) model were determined by linear least squares regression. Parameterization was performed with gas exchange data from each individual leaf measured. Two leaves were measured from each of four ambient [CO₂] plots and four elevated [CO₂] plots. The effect of growth [CO₂] on the intercept and slope of the Ball *et al.* (1987) model was tested with the plot averages ($n = 4$), using a mixed model analysis of variance in the MIXED procedure of SAS (SAS Institute, Cary, NC, USA). Growth [CO₂] was treated as a fixed effect, while block was a random effect. Because of the low true replicate size and to avoid Type II errors, a probability level of $P = 0.1$ was set as the threshold for significance.

Model validation with *in situ* gas exchange and micrometeorological data

Air temperature, h and PPFD were measured, at a height of 3 m, at a central location at SoyFACE (details in Leakey

et al. 2004). Data were averaged and logged at 10 min intervals throughout the growing season.

The diurnal course of g_s and photosynthesis of the uppermost, fully expanded leaves of *G. max* in each plot at SoyFACE was measured *in situ* on four dates during the 2003 growing season: 23 June (day of year or DOY 176), 16 July (197), 6 August (218) and 26 August (238). The measurements were performed every ~ 2 h from sunrise to sunset with four open-path gas exchange systems (LI-6400, Li-Cor), which had been calibrated from a single standard source as previously described. At each time point during the day, each gas exchange system measured the pair of plots within a single block. Gas exchange systems were rotated among blocks between sampling periods – each of approximately 60 min. Measurements on all individuals were made at growth $[\text{CO}_2]$. Immediately before each sampling time point during the day, PPFD and air temperature above the canopy was measured. These conditions were then replicated in the leaf chamber, throughout that sampling period. Atmospheric water vapour pressure entering the chamber was not controlled and therefore tracked ambient conditions (as in Leakey *et al.* 2004). The four dates of measurements were selected to correspond to the four discrete developmental stages of: completion of fifth leaf expansion, beginning bloom, full bloom and beginning seed set (as defined in Ritchie *et al.* 1993) and to reflect a range of meteorological conditions. On each date, the diurnal course of g_s was predicted using the intercept and slope constants of the Ball *et al.* (1987) model that were generated from the laboratory measurements. The model input variables were measured A , $[\text{CO}_2]$ in the leaf chamber and h from the SoyFACE weather station. For comparison, the values of g_s predicted by the model, were regressed against measured values. Data collected from 09:00 to 13:00 PM on DOY 197 and at 9 AM on DOY 218, and 238 were excluded from the analysis. At those times, during *in situ* measurements of g_s , PPFD in the cuvette of the gas exchange system was lower than in the field. This reduced the observed A , which would have caused a systematic underestimation of g_s as calculated by the Ball *et al.* (1987) model.

RESULTS

In the controlled environment parameterization, systematic variation of $[\text{CO}_2]$, PPFD and D in the leaf chamber during gas exchange measurements (Fig. 1a) resulted in a wide but predictable range of values for g_s and A (Fig. 1b). Variation in g_s was linearly related to an index comprising A , h and $[\text{CO}_2]$, as described by the Ball *et al.* (1987) model of g_s (Fig. 2). The g_0 (Eqn 1) of this relationship was not significantly different from 0 under ambient $[\text{CO}_2]$ ($P = 0.86$) or elevated $[\text{CO}_2]$ ($P = 0.48$). The relationship is therefore reported as a straight line passing through the origin. There was no significant difference ($P = 0.57$) between m (Eqn 1) of the linear regression for plants grown under ambient $[\text{CO}_2]$ (10.6 ± 0.3) and elevated $[\text{CO}_2]$ (10.9 ± 0.5). Because standard errors were fairly small (*c.* 4% of m), the

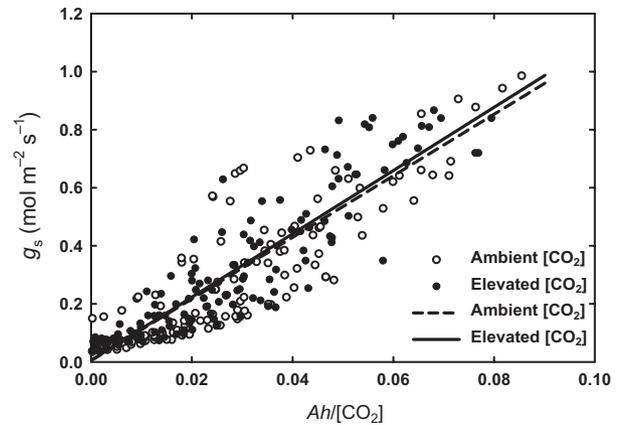


Figure 2. Relationship of measured stomatal conductance (g_s) with the product of net photosynthetic CO_2 assimilation (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and fractional relative humidity (h , unitless) divided by the atmospheric $[\text{CO}_2]$ ($\mu\text{mol mol}^{-1}$) for uppermost fully expanded leaves of *Glycine max* grown under ambient ($378 \mu\text{mol mol}^{-1}$) and elevated $[\text{CO}_2]$ ($552 \mu\text{mol mol}^{-1}$) at SoyFACE in 2003. Individual points represent gas exchange measurements of a single leaf. Only data for which $[\text{CO}_2] > 150 \mu\text{mol mol}^{-1}$ were used. Regression lines for ambient and elevated $[\text{CO}_2]$ -grown plants represent the treatment means ($n = 4$) of linear functions fitted to data from individual leaves. Ambient $[\text{CO}_2]$: $g_s = 10.6(Ah/[\text{CO}_2]) + 0.008$; elevated $[\text{CO}_2]$: $g_s = 10.9(Ah/[\text{CO}_2]) + 0.007$.

lack of significance is unlikely to be the result of a Type II error, in which high variability relative to sample size could obscure an underlying treatment effect. A power test indicated that, with this data set, 10 and 15% differences in g_s between treatments had 70 and 95% probabilities of being detected, respectively, even with the Type I error rate of $P = 0.10$. In other words, there was no evidence of stomatal acclimation in *G. max* to long-term growth at elevated $[\text{CO}_2]$, and the probability of supporting the null hypothesis was high.

To ensure that measurements of excised leaves in the laboratory were relevant to crop performance in the field, the model parameterization previously described was validated against *in situ* gas exchange measurements of g_s over the diurnal period, during the 2003 growing season. Measured and modelled g_s over the growing season agreed closely (Fig. 3) and were highly correlated ($r^2 = 0.92$, measured = 0.96 modelled + 0.01) with the slope and intercept statistically indistinguishable from 1 and 0, respectively (slope $P = 0.92$, intercept $P = 0.26$). In addition, the model successfully predicted the presence or absence of a CO_2 effect on g_s in the field (Fig. 4). For example, the course of g_s during the day in soybean grown at ambient and elevated $[\text{CO}_2]$ is presented in Fig. 4 for two of the 4 d on which field measurements were conducted. The model correctly predicted that there was a significant CO_2 effect on g_s on day of year 218, but not on day of year 238. This coincided with an average stimulation of A under elevated $[\text{CO}_2]$ that was low on day of year 218 (+12%) but high on day of year 238 (+44%).

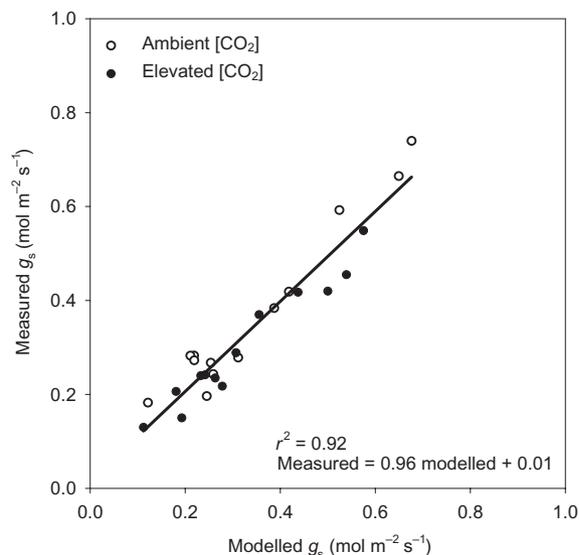


Figure 3. Relationship between stomatal conductance (g_s) measured over the diurnal period *in situ* (measured g_s) and g_s predicted by the model parameterized from laboratory gas exchange measurements (modelled g_s) for *Glycine max* grown under ambient ($378 \mu\text{mol mol}^{-1}$) and elevated $[\text{CO}_2]$ ($552 \mu\text{mol mol}^{-1}$) at SoyFACE in 2003.

DISCUSSION

Consistent with our prediction, there was no evidence of altered stomatal sensitivity to systematic variation of h , $[\text{CO}_2]$ and/or A in soybean grown under FACE treatments of ambient and elevated $[\text{CO}_2]$. This represents a comprehensive test for stomatal acclimation to elevated $[\text{CO}_2]$ in a major crop grown under fully open-air conditions, in the primary region of its production. This is despite the fact that there was a statistically significant acclimation of photosynthesis in the same crop, characterized by decreased $V_{c,\text{max}}$ and $V_{c,\text{max}}/J_{\text{max}}$ (Bernacchi *et al.* 2005). This acclimation is an optimization of photosynthesis to elevated $[\text{CO}_2]$ in that (1) the decrease in $V_{c,\text{max}}$ would result in lower rates of A if plants grown at ambient and elevated $[\text{CO}_2]$ were measured at a common $[\text{CO}_2]$ below the inflexion point of the A/c_i curve but (2) there was little or no difference in A when the common measurement $[\text{CO}_2]$ was above the inflexion point of the A/c_i curve. In this study, the Ball *et al.* (1987) model was parameterized with gas exchange measurements performed at $[\text{CO}_2]$ both above and below the inflexion point of the A/c_i curve. The sensitivity of g_s to h and A was consistent across this variation in $[\text{CO}_2]$, as well as between growth $[\text{CO}_2]$ treatments. These results suggest that the well-characterized decrease in g_s of crops under elevated $[\text{CO}_2]$ (Bryant, Taylor & Frehner 1998; Bunce 2004; Rogers *et al.* 2004) may be due entirely to the direct instantaneous effect of decreased conductance in response to elevated $[\text{CO}_2]$. However, this experiment does not test for changes in stomatal number or density, nor does it rule out the possibility of interdependent changes in stomatal and photosynthetic physiology that, combined, could have no net affect on the

sensitivity of g_s to A , h and $[\text{CO}_2]$. This expands upon the finding that $c_i/[\text{CO}_2]$ remains remarkably constant with growth $[\text{CO}_2]$ in FACE experiments when averaged across crop and tree studies (reviewed in Drake *et al.* 1997; Long *et al.* 2004; Ainsworth & Long 2005). Specifically, it adds evidence that the response of g_s to substantial, systematic variation in $[\text{CO}_2]$, h and A does not change between the current $[\text{CO}_2]$ and that projected for 2050. The result is consistent with the general absence of stomatal acclimation under elevated $[\text{CO}_2]$ found in a survey of tree responses (Medlyn *et al.* 2001). However, it does contradict a chamber study of soybean in which m was reported to be lower by a remarkable -49% under elevated $[\text{CO}_2]$ (Bunce 2004). This striking difference between a chamber and FACE study perhaps underlines the importance of validating chamber studies with tests under fully open-air conditions (reviewed in Long *et al.* 2005). Growth at elevated $[\text{CO}_2]$ also resulted in lower m (-7%) in cotton, but the effect in this case was only marginally significant ($P = 0.064$; Harley *et al.* 1992). There is evidence for and against acclimation in the two

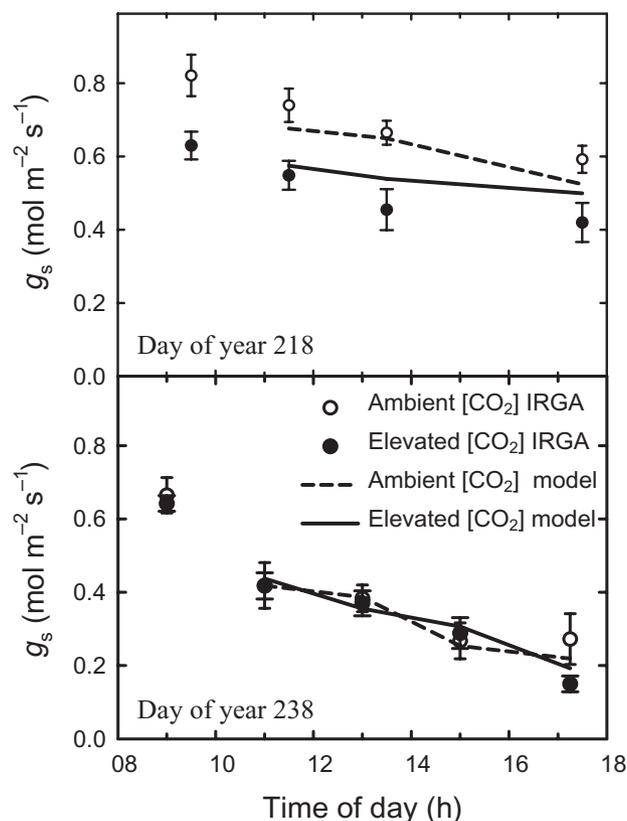


Figure 4. Diurnal course of stomatal conductance (g_s) measured *in situ* at SoyFACE (point values) and predicted by the model parameterized from laboratory gas exchange measurements (line values), on days of year 218 and 238 for *Glycine max* grown under ambient ($378 \mu\text{mol mol}^{-1}$) and elevated $[\text{CO}_2]$ ($552 \mu\text{mol mol}^{-1}$) at SoyFACE in 2003. Measured data are mean values for four replicate plots ± 1 SE. Modelled data are means of values modelled independently for each of the four plots per treatment. IRGA, infrared gas analyzer.

parameters, g_0 and m , of the Ball *et al.* (1987) model in response to varying growth conditions, mainly soil moisture (Harley & Tenhunen 1991; Valentini, Gamon & Field 1995; Sala & Tenhunen 1996; Colello *et al.* 1998; Medlyn *et al.* 2001; Xu & Baldocchi 2003). Some studies in chambers have reported stomatal acclimation as differences in g_s of plants from two growth $[\text{CO}_2]$ treatments, measured at a common $[\text{CO}_2]$ (e.g. Bunce 2001; Lodge *et al.* 2001). However, it follows from the Ball *et al.* (1987) model (Eqn 1) that if there is lower A as a result of photosynthetic acclimation, g_s will also be decreased. But this is not evidence of stomatal acclimation as defined in this study or by Šantrůček & Sage (1996). A more appropriate test is to compare the response of g_s to the A , $[\text{CO}_2]$ and h at different growth $[\text{CO}_2]$. At SoyFACE, there was good agreement between g_s measured under field conditions and that which was estimated with the Ball *et al.* (1987) model, regardless of whether the plants had been grown at current or elevated $[\text{CO}_2]$.

The magnitude of the CO_2 effect on g_s varied considerably between day of year 218 and 238 (Fig. 4). This corresponded with changes in the degree to which A was stimulated by elevated $[\text{CO}_2]$. It is difficult to determine whether another, unknown factor affected g_s and then photosynthesis in a treatment-specific manner, or vice versa. Drying soils stimulate abscisic acid signalling from the root to shoot which induces stomatal closure (Davies, Tardieu & Trejo 1994). Elevated $[\text{CO}_2]$ reduces soil water depletion between rainfall events by soybean at SoyFACE (Leakey, unpublished results). In the 2 weeks before day of year 218, there was 31 mm rain, compared with 8 mm in the 2 weeks before day of year 238. Because g_s is consistently lower in elevated $[\text{CO}_2]$ and leaf area is only marginally higher (Rogers *et al.* 2004; Dermody, Long & DeLucia 2006), it is likely that evapotranspiration is lower. As a result, it can be speculated that soil moisture would have been less depleted during this dry period under elevated $[\text{CO}_2]$, potentially minimizing a drought-induced decrease in g_s . This would be consistent with the smaller effect of growth at elevated $[\text{CO}_2]$ on g_s , alongside greater stimulation of photosynthesis by elevated $[\text{CO}_2]$, on day 238 versus day 218.

The results of this study complement the previous validation of the Ball *et al.* (1987) model in predictions of canopy C and water fluxes from crops and forests under ambient $[\text{CO}_2]$ (Baldocchi & Meyers 1998). Together they provide unique validation for the use of the model to estimate C and water fluxes in soybean under future CO_2 -rich atmospheres. This study was conducted under fully open-air conditions and suggests that, at least for soybean in the field, growth at elevated $[\text{CO}_2]$ does not require a reparameterization of the model. This makes the combined use of the Farquhar *et al.* (1980) and the Ball *et al.* (1987) models a more viable option for predicting plant, ecosystem and landscape responses to rising $[\text{CO}_2]$.

ACKNOWLEDGMENTS

We thank T. Mies and F. Dohleman for operating and maintaining the SoyFACE experimental facility. This research

was supported by the Illinois Council for Food and Agricultural Research (C-FAR), Archer Daniels Midland Co., the International Arid Lands Consortium and the US Department of Agriculture/Agricultural Research Service (USDA/ARS). ADBL was supported by a Fulbright Scholarship sponsored by Astra-Zeneca. The views expressed in this work are those of the authors and do not necessarily reflect those of the Illinois State Water Survey (ISWS).

REFERENCES

- Ainsworth E.A. & Long S.P. (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2 . *New Phytologist* **165**, 351–371.
- Ainsworth E.A., Rogers A., Nelson R. & Long S.P. (2004) Testing the 'source-sink' hypothesis of down-regulation of photosynthesis in elevated CO_2 in the field with single gene substitutions in *Glycine max*. *Agricultural and Forest Meteorology* **122**, 85–94.
- Baldocchi D. & Meyers T. (1998) On using eco-physiological, micrometeorological and biogeochemical theory to evaluate carbon dioxide, water vapor and trace gas fluxes over vegetation: a perspective. *Agricultural and Forest Meteorology* **90**, 1–25.
- 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* (ed. J. Biggens), pp. 221–224. Martinus-Nijhoff Publishers, Dordrecht, the Netherlands.
- Bernacchi C.J., Morgan P.B., Ort D.R. & Long S.P. (2005) The growth of soybean under free air $[\text{CO}_2]$ enrichment (FACE) stimulates photosynthesis while decreasing in vivo Rubisco capacity. *Planta* **220**, 434–446.
- Bounoua L., Collatz G.J., Sellers P.J., *et al.* (1999) Interactions between vegetation and climate: radiative and physiological effects of doubled atmospheric CO_2 . *Journal of Climate* **12**, 309–324.
- Bryant J., Taylor G. & Frehner M. (1998) Photosynthetic acclimation to elevated CO_2 is modified by source: sink balance in three component species of chalk grassland swards grown in a free air carbon dioxide enrichment (FACE) experiment. *Plant, Cell & Environment* **21**, 159–168.
- Bunce J.A. (2001) Direct and acclimatory responses of stomatal conductance to elevated carbon dioxide in four herbaceous crop species in the field. *Global Change Biology* **7**, 323–331.
- Bunce J.A. (2004) Carbon dioxide effects on stomatal responses to the environment and water use by crops under field conditions. *Oecologia* **140**, 1–10.
- von Caemmerer S. & Farquhar G.D. (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **153**, 376–387.
- Colello G.D., Grivet C., Sellers P.J. & Berry J.A. (1998) Modeling of energy, water, and CO_2 flux in a temperate grassland ecosystem with SiB2: May–October 1987. *Journal of the Atmospheric Sciences* **55**, 1141–1169.
- 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.
- Davies W.J., Tardieu F. & Trejo C.L. (1994) How do chemical signals work in plants that grow in drying soil. *Plant Physiology* **104**, 309–314.
- Dermody O., Long S.P. & DeLucia E.H. (2006) How does

- elevated CO₂ or ozone affect the leaf-area index of soybean when applied independently? *New Phytologist* **169**, 145–155.
- Drake B.G., Gonzalez-Meler M.A. & Long S.P. (1997) More efficient plants: a consequence of rising atmospheric CO₂? *Annual Review of Plant Physiology and Plant Molecular Biology* **48**, 609–639.
- 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.
- Field C.B., Jackson R.B. & Mooney H.A. (1995) Stomatal responses to increased CO₂ – implications from the plant to the global-scale. *Plant, Cell & Environment* **18**, 1214–1225.
- Foley J.A., Prentice I.C., Ramankutty N., Levis S., Pollard D., Sitch S. & Haxeltine A. (1996) An integrated biosphere model of land surface processes, terrestrial carbon balance, and vegetation dynamics. *Global Biogeochemical Cycles* **10**, 603–628.
- Harley P.C. & Tenhunen J.D. (1991) Modelling the photosynthetic response of C₃ leaves to environmental factors. In *Modelling Crop Photosynthesis – from Biochemistry to Canopy* (eds K.J. Boote & R.S. Loomis), pp. 1–16. CSSA, Madison, WI, USA.
- Harley P.C., Thomas R.B., Reynolds J.F. & Strain B.R. (1992) Modeling photosynthesis of cotton grown in elevated CO₂. *Plant, Cell & Environment* **15**, 271–282.
- Kimball B.A., LaMorte R.L., Pinter P.J., Wall G.W., Hunsaker D.J., Adamsen F.J., Leavitt S.W., Thompson T.L., Matthias A.D. & Brooks T.J. (1999) Free-air CO₂ enrichment and soil nitrogen effects on energy balance and evapotranspiration of wheat. *Water Resources Research* **35**, 1179–1190.
- Leakey A.D.B., Bernacchi C.J., Dohleman F.G., Ort D.R. & Long S.P. (2004) Will photosynthesis of maize (*Zea mays*) in the US Corn Belt increase in future CO₂ rich atmospheres? An analysis of diurnal courses of CO₂ uptake under free-air concentration enrichment (FACE). *Global Change Biology* **10**, 951–962.
- Leakey A.D.B., Uribealarea M., Ainsworth E.A., Naidu S.L., Rogers A., Ort D.R. & Long S.P. (2006) Photosynthesis, productivity and yield of maize are not affected by open-air elevation of CO₂ concentration in the absence of drought. *Plant Physiology* **140**, 779–790.
- Leuning R. (1990) Modeling stomatal behavior and photosynthesis of *Eucalyptus grandis*. *Australian Journal of Plant Physiology* **17**, 159–175.
- Lodge R.J., Dijkstra P., Drake B.G. & Morison J.I.L. (2001) Stomatal acclimation to increased CO₂ concentration in a Florida scrub oak species *Quercus myrtifolia* Willd. *Plant, Cell & Environment* **24**, 77–88.
- Long S.P., Ainsworth E.A., Leakey A.D.B. & Morgan P.B. (2005) Global food insecurity. Treatment of major food crops with elevated carbon dioxide or ozone under large-scale fully open-air conditions suggests recent models may have overestimated future yields. *Philos T Roy Soc B* **360**, 2011–2020.
- Long S.P., Ainsworth E.A., Rogers A. & Ort D.R. (2004) Rising atmospheric carbon dioxide: plants FACE the future. *Annual Review of Plant Biology* **55**, 591–628.
- Medlyn B.E., Barton C.V.M., Broadmeadow M.S.J., et al. (2001) Stomatal conductance of forest species after long-term exposure to elevated CO₂ concentration: a synthesis. *New Phytologist* **149**, 247–264.
- Miglietta F., Peressotti A., Vaccari F.P., Zaldei A., deAngelis P. & Scarascia-Mugnozza G. (2001) Free-air CO₂ enrichment (FACE) of a poplar plantation: the POPFACE fumigation system. *New Phytologist* **150**, 465–476.
- Morgan P.B., Bernacchi C.J., Ort D.R. & Long S.P. (2004) An *in vivo* analysis of the effect of season-long open-air elevation of ozone to anticipated 2050 levels on photosynthesis in soybean. *Plant Physiology* **135**, 2348–2357.
- Morgan P.B., Bollero G.A., Nelson R.L., Dohleman F.G. & Long S.P. (2005) Smaller than predicted increase in aboveground net primary production and yield of field-grown soybean under fully open-air [CO₂] elevation. *Global Change Biology* **11**, 1856–1865.
- Mott K.A. & Parkhurst D.F. (1991) Stomatal responses to humidity in air and helox. *Plant, Cell & Environment* **14**, 509–515.
- Nijs I., Ferris R., Blum H., Hendrey G. & Impens I. (1997) Stomatal regulation in a changing climate: a field study using free air temperature increase (FATI) and free air CO₂ enrichment (FACE). *Plant, Cell & Environment* **20**, 1041–1050.
- Owensby C.E., Ham J.M., Knapp A.K. & Auen L.M. (1999) Biomass production and species composition change in a tallgrass prairie ecosystem after long-term exposure to elevated atmospheric CO₂. *Global Change Biology* **5**, 497–506.
- Prentice I.C., Farquhar G.D., Fasham M.J.R., et al. (2001) The carbon cycle and atmospheric carbon dioxide. In *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change* (eds J.T. Houghton, Y. Ding, D.J. Griggs, M. Noguer, P.J. van der Linden & D. Xiaosu), pp. 183–238. Cambridge University Press, NY, USA.
- Ritchie S.W., Hanway J.J., Benson G.O., Herman J.C. & Lupkes S.J. (1993) *How a Corn Plant Develops* (Special report no. 48). Iowa State University of Science and Technology, Ames, IA, USA.
- Rogers A., Allen D.J., Davey P.A., et al. (2004) Leaf photosynthesis and carbohydrate dynamics of soybeans grown throughout their life-cycle under free-air carbon dioxide Enrichment. *Plant, Cell & Environment* **27**, 449–458.
- Sala A. & Tenhunen J.D. (1996) Simulations of canopy net photosynthesis and transpiration in *Quercus ilex* L. under the influence of seasonal drought. *Agricultural and Forest Meteorology* **78**, 203–222.
- Šantrůček 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.
- Sellers P.J., Randall D.A., Collatz G.J., Berry J.A., Field C.B., Dazlich D.A., Zhang C., Collelo G.D. & Bounoua L. (1996) A revised land surface parameterization (SiB2) for atmospheric GCMs.1. Model formulation. *Journal of Climate* **9**, 676–705.
- USDA (2004) *Crop Production 2003 Summary (Cr Pr 2-1 (04))*. Agricultural Statistics Board, National Agricultural Statistics Service, United States Department of Agriculture, Washington, D.C., USA.
- USDA (2005) *World Agricultural Production: World Grains by Commodity for December 2004*. Production Estimates and Crop Assessment Division, Foreign Agricultural Service, United States Department of Agriculture, Washington, D.C., USA.
- Valentini R., Gamon J.A. & Field C.B. (1995) Ecosystem gas-exchange in a California grassland – seasonal patterns and implications for scaling. *Ecology* **76**, 1940–1952.
- Woodward F.I. & Kelly C.K. (1995) The influence of CO₂ concentration on stomatal density. *New Phytologist* **131**, 311–327.
- Wullschlegel S.D. (1993) Biochemical limitations to carbon assimilation in C₃ plants – a retrospective analysis of the A/c_i curves from 109 species. *Journal of Experimental Botany* **44**, 907–920.
- Xu L.K. & Baldocchi D.D. (2003) Seasonal trends in photosynthetic parameters and stomatal conductance of blue oak (*Quercus douglasii*) under prolonged summer drought and high temperature. *Tree Physiology* **23**, 865–877.

Received 4 February 2006; received in revised form 24 April 2006; accepted for publication 27 April 2006