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Responses of stomatal conductance to light, humidity and temperature in winter wheat and barley grown at three concentrations of carbon dioxide in the field

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Abstract

Responses of leaf stomatal conductance to light, humidity and temperature were characterized for winter wheat and barely grown at ambient (about 350 μmol mol^{-1} in the daytime), ambient +175 and ambient +350 μmol mol^{-1} concentrations of carbon dioxide in open-topped chambers in field plots over a three year period. Stomatal responses to environment were determined by direct manipulation of single environmental factors, and those results were compared with responses derived from natural day to day variation in mid-day stomatal conductance. The purpose of these experiments was to determine the magnitude of reduction in stomatal conductance at elevated [CO_2], and to assess whether the relative response of conductance to elevated [CO_2] was constant across light, humidity and temperature conditions. The results indicated that light, humidity and temperature all significantly affected the relative decrease in stomatal conductance at elevated [CO_2]. The relative decrease in conductance with elevated [CO_2] was greater at low light, low water vapour pressure difference, and high temperature in both species. For measurements made at saturating light near mid-day, the ratio of mid-day stomatal conductances at doubled [CO_2] to that at ambient [CO_2] ranged from 0.42 to 0.86, with a mean of 0.66 in barley, and from 0.33 to 0.80, with a mean of 0.56 in wheat. Day-to-day variation in the relative effect of elevated [CO_2] on conductance was correlated with the relative stimulation of [CO_2] assimilation rate and with temperature. Some limitations of multiple linear regression, multiplicative, and 'Ball–Berry' models as summaries of the data are discussed. In barley, a better fit to the models occurred in individual years than for the combined data, and in wheat a better fit to the models occurred when data from near the end of the season were removed.

Keywords: barley, carbon dioxide, stomatal conductance, stomatal models, transpiration, wheat

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Introduction

A reduction in stomatal conductance is a common response of herbaceous plants to elevated concentrations of carbon dioxide. Decreased stomatal conductance in herbaceous crop plants as the atmospheric carbon dioxide concentration continues to rise has potential implications not only for crop water loss, but also for climate. This is because in many regions herbaceous crop communities cover a substantial fraction of the landscape, and changes in canopy conductance to water vapour would affect the partitioning of energy at the vegetation–atmosphere interface, which could in turn affect weather and climate (e.g. Henderson-Sellers *et al*. 1995; Sellers *et al*. 1996).

The atmospheric models which have been used to simulate the effects of increasing atmospheric [CO_2] on climate have primarily used fixed relative reductions in stomatal conductance, or reductions based on an empirical relationship between conductance, humidity, [CO_2] and photosynthesis (the 'Ball–Berry' model, Collatz *et al*. 1991), coupled with a biochemical model of photosynthesis (e.g. Sellers *et al*. 1996). One limitation to the latter approach has been that biochemical para-

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meters in the photosynthesis model may not be independent of humidity (e.g. Bunce 1993; Eamus *et al*. 1995; Goodfellow *et al*. 1997; Wilson & Bunce 1997). Because changes in humidity (or leaf to air water vapour pressure difference) are a major source of variation in stomatal conductance, there is a potential for substantial errors in predicting stomatal conductance from modelled values of photosynthesis.

In a study of four grass species grown in a growth cabinet at ambient [CO_2], Morison & Gifford (1983) found that the relative decrease in stomatal conductance caused by increased [CO_2] was the same over a range of humidity conditions. However, this was not the case for soybeans grown under field conditions at ambient and elevated [CO_2] (Wilson & Bunce 1997). Berryman *et al*. (1994) found that growth and measurement at elevated [CO_2] did not alter the relative responses of stomatal conductance to light or humidity in a tree species, but did alter the response to temperature. Limited data suggest that long-term exposure to elevated [CO_2] may alter stomatal responses to environment in some species (e.g. Stanghellini & Bunce 1993; Santrucek & Sage 1996; Wilson & Bunce 1997), although few studies of the acclimation of stomatal conductance to elevated [CO_2] have been conducted (cf. Morison 1998).

The purpose of this work was to determine, for winter wheat and barley plants grown in field plots at three concentrations of carbon dioxide, the magnitude of reduction in conductance at elevated [CO_2], and whether the relative decrease in stomatal conductance at elevated [CO_2] was the same for a range of light, temperature and humidity conditions. Response functions were obtained by directly manipulating individual environmental factors and by observations of how mid-day stomatal conductance varied with environmental conditions from day to day. We report here stomatal conductances of leaves measured only at the [CO_2] concentration at which the plants were grown. Therefore, the observed responses reflect the net effect of both growth and measurement [CO_2] conditions. Rather than confine measurements to a particular growth stage or year in order to reduce variation in the responses of conductance to environment, measurements were distributed over years and growth stages in order to be more generally representative of stomatal responses to elevated [CO_2] for these crops in this region.

Table 1 Parameter estimates for nonlinear regressions of stomatal conductance on PPFD in wheat and barley measured at the growth [CO_2] and a temperature of 20 °C. The equation used was: $g = a \times (1 - \exp(-b * \text{PPFD}))$. The values of conductance at each step in PPFD were divided by the conductance value at 2.0 mmol m^{-2} s^{-1} PPFD for the same leaf. This forces '*a*' to be approximately 1. Values in parentheses are standard errors

Species	[CO_2]	*a*	*b*	r^2
Wheat	350	0.97 (0.03)	0.0053 (0.0007)	0.757
Wheat	700	0.99 (0.05)	0.0027 (0.0005)	0.766
Barley	350	1.01 (0.02)	0.0039 (0.0003)	0.925
Barley	700	1.04 (0.02)	0.0023 (0.0002)	0.956

Materials and methods

Materials

Winter wheat, *Triticum aestivum* (L.) cv. Coker and winter barley, *Hordeum vulgare* (L.) cv. Wyson were grown in field plots at Beltsville, Maryland in 1994–95, 1995–96 and 1996–97. Seeds were sown in October at a rate of 20 and 13 g m^{-2} for wheat and barley, respectively, in rows 25 cm apart. Plots were fertilized in the spring at a rate of 26 and 19 g N m^{-2} for wheat and barley, respectively. The fertilizer contained 10% N, 10% K_2O and 10% P_2O_5. Plants were grown in rectangular open-topped chambers with clear acrylic walls. Chamber dimensions were $1 \times 1 \times 1.8$ m high for wheat and $1.1 \times 2.1 \times 2.7$ m high for barley. There were nine chambers per species, with three replicates of each of three [CO_2] treatments per species, except for the last year, when only six chambers and three replicates of the highest and lowest two [CO_2] treatments were used for wheat. [CO_2] treatments were ambient, ambient + 175 μmol mol^{-1}, and ambient + 350 μmol mol^{-1} of [CO_2]. The ambient air averaged about 350 μmol mol^{-1} in the daytime. Blowers pushed outside air into a perforated plastic pipe at the bottom centre of each chamber, with turnover times of about 20 s. Pure CO_2 was added to the inlet air upstream of the blower at constant flow rates for the elevated CO_2 treatments. There was no immediate feedback between CO_2 flow rates and [CO_2], but flow rates were adjusted every few days, as necessary. An air sampling system was used to monitor [CO_2] in chambers of all [CO_2] treatment by species combinations every hour. The monitoring system was in an air-conditioned shed adjacent to the plots, and also monitored photosynthetically active radiation, wind speed, and shaded, ventilated air temperature outside the chambers, and air temperatures inside representative chambers. Soil psychrometers were placed in the centre of each chamber, at a depth of 25 cm. Periodic readings of the psychrometers indicated that soil water potential was never below −0.2 MPa in any of the plots. It is typical for this region that precipitation exceeded potential evapotranspiration during the spring growth period of March to May, when the stomatal conductance measurements were made.

Stomatal conductance measurements

Three different types of leaf gas exchange measurements were made on plants from the interior rows of the chambers. First, at approximately weekly intervals, measurements of stomatal conductance and CO_2 exchange rate were made near mid-day on clear days from early spring until senescence. Mature, fully illuminated upper canopy leaves were measured at their nominal daytime growth [CO_2] of 350, 525, or 700 μmol mol^{-1}, using a CIRAS-1 (PP Systems, Haverhill, MA) portable, open system which measured stomatal conductance and CO_2 assimilation rate under nearly ambient conditions of light, temperature and humidity (Bunce 1998b). For these measurements, six leaves per species and [CO_2] treatment were sampled (two per open topped chamber) on the same day. Measurements commenced in the spring after stem elongation started. For about the last two thirds of each season, the mature upper canopy leaves which were measured were 'flag' (ultimate) leaves in wheat and penultimate leaves in barley. This cultivar of barley has very small ultimate leaves. Secondly, the same system was used to determine short-term responses to changes in humidity. Stomatal conductance and CO_2 assimilation rates of leaves were first measured as above, with air at the ambient water vapour content entering the leaf chamber, and then the leaf was kept in the cuvette and measurements continued as the relative humidity of

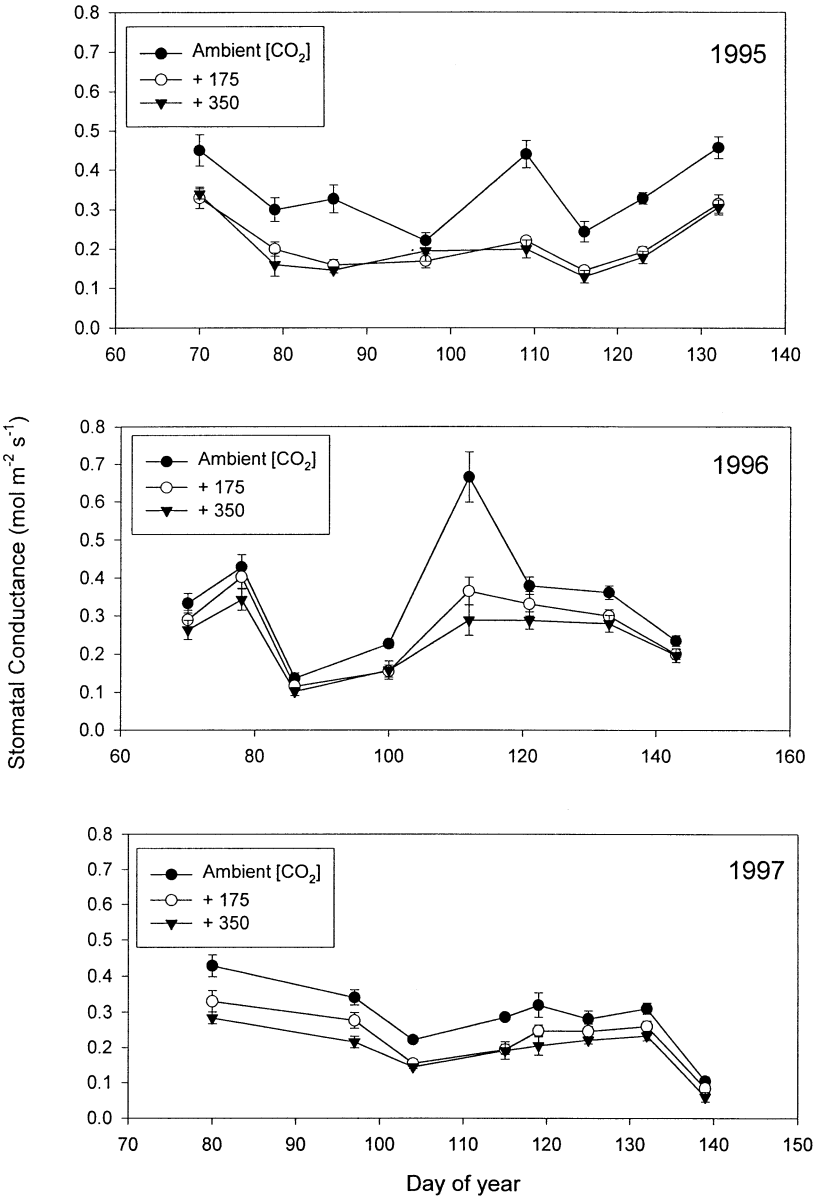

Fig. 1 Seasonal patterns of mid-day stomatal conductance in barley grown at ambient, ambient +175 and ambient +350 μmol mol^{-1} [CO_2] in open-topped chambers. Each point represents a mean value for six leaves from three replicate chambers. Error bars represent standard errors for $n = 3$ chambers per treatment. Air temperature during the measurements is given in Fig. 3.

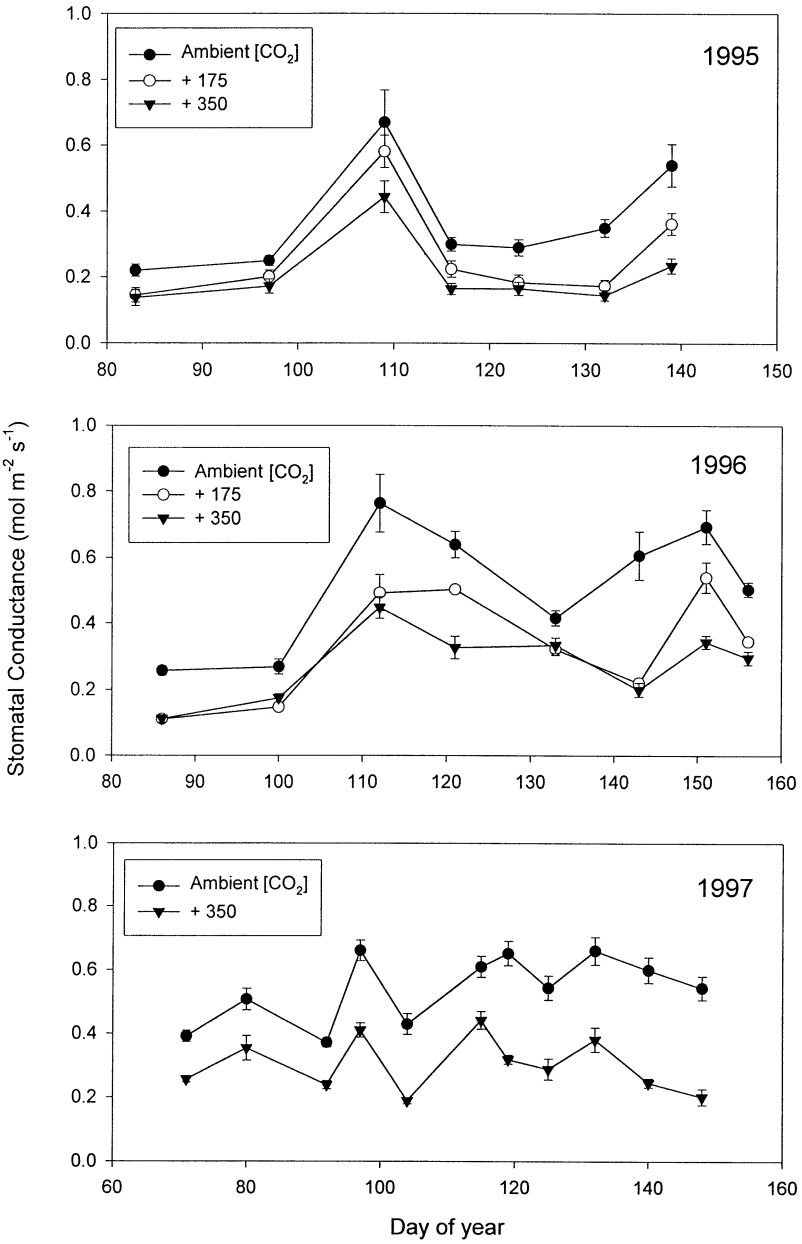

Fig. 2 Seasonal patterns of mid-day stomatal conductance in wheat grown at ambient, ambient +175 and ambient +350 μmol mol^{-1} [CO_2] in open-topped chambers. Each point represents a mean value for six leaves from three replicate chambers. Error bars represent standard errors for $n = 3$ chambers per treatment. Air temperature during the measurements is given in Fig. 3.

air entering the chamber was decreased to zero in three steps. The gas exchange system displayed the time course of stomatal conductance graphically, and steady-state values of gas exchange parameters were recorded at each step of humidity. It took about 10 min at each step to achieve steady-state values of conductance. Leaf temperatures were not controlled actively, but in the steady-state varied by less than 2 °C with humidity changes. These measurements were made on a few clear days each year, chosen for contrasting air temperatures. Three or four leaves from the lowest and highest [CO_2] treatments for a given species were measured on each measurement date. The third type of gas exchange measurement was the determination of the response of stomatal conductance to light at constant temperature and humidity, for plants grown at the highest and lowest [CO_2]. In these measurements a leaf chamber incorporating automatic temperature and light control was utilized with the CIRAS-1 system. This leaf chamber was available only in the last year of the study. Stomatal conductance and CO_2 exchange rate of leaves were first measured at the nominal growth [CO_2], at a photosynthetic photon flux density (PPFD) of 2.0 mmol m^{-2} s^{-1}, at a temperature a few degrees above the outside air temperature (in order to avoid problems of condensation within the cuvette). The PPFD was then reduced in steps to values of 1.5, 1.0,

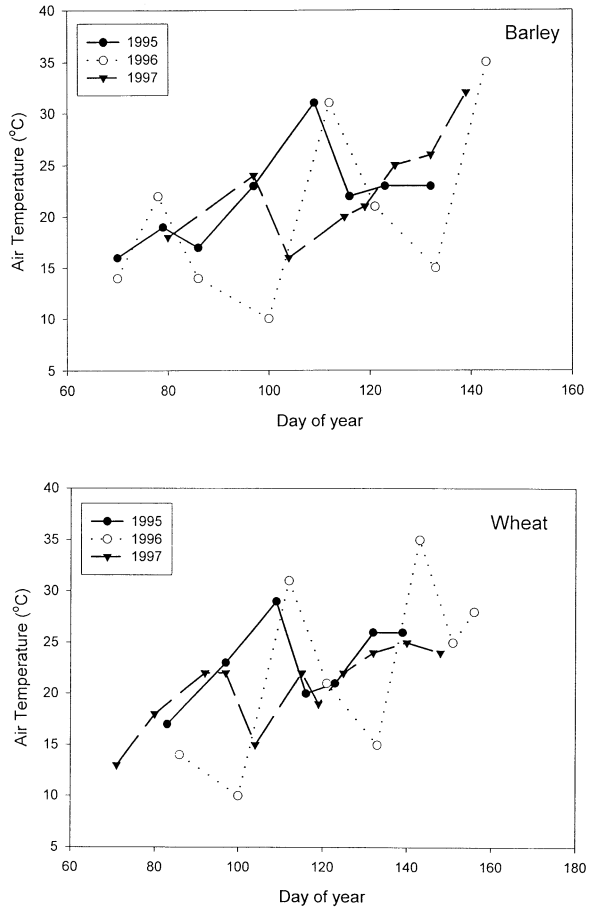

Fig. 3 Seasonal patterns of air temperature during the mid-day measurements of stomatal conductance (given in Figs 1 and 2) in barley and wheat grown at ambient, ambient + 175 and ambient + 350 μmol mol^{-1} $[CO_2]$.

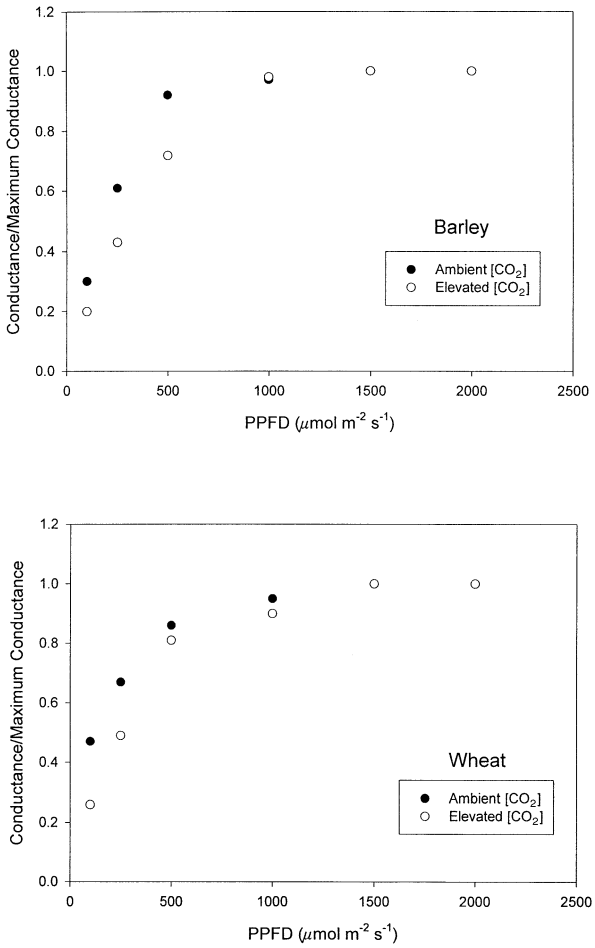

Fig. 4 Relative response of stomatal conductance to photosynthetic photon flux density (PPFD) for wheat (a) and barley (b) leaves grown and measured at ambient or elevated (ambient + 350 μmol mol^{-1}) concentrations of CO_2. All measurements were at 20 °C, and each point represents the mean for 5 leaves measured on 2 days. Statistical comparison of the curves is presented in the text.

0.50, 0.25, and 0.10 mmol m^{-2} s^{-1}, while $[CO_2]$ and leaf temperature were held constant. Steady-state values of stomatal conductance and photosynthesis were recorded at each step in PPFD. When stomatal conductance changed with PPFD, it reached a new steady value within 10 min. A minimum of a 10 minute equilibration time was therefore used at each step in PPFD. Leaves from both $[CO_2]$ treatments were measured on the same days. These measurements were made on clear days chosen for contrasting air temperatures.

The calibration of the infrared carbon dioxide and water vapour analysers of the leaf gas exchange measurement system was checked each measurement day, using standard gas mixtures for carbon dioxide, and a dew point generator (LI-610, Li-Cor, Inc., Lincoln, Nebraska) for water vapour. Calibration of the temperature, PPFD and mass flow sensors was checked monthly.

Data analysis

Stomatal conductance responses to changes in humidity at different temperatures were analysed by determining separately for each leaf a linear regression of stomatal conductance on the leaf to air water vapour pressure difference (D). Simple nonlinear regressions did not provide higher r^2 values. Measurements of the response of stomatal conductance to D on the different days provided a range of measurement temperatures. Each day was characterized by a 'nominal' temperature, which was the mean temperature for all measurements of a given species on that date. The effects of $[CO_2]$ treatment at the different nominal temperatures on the relative

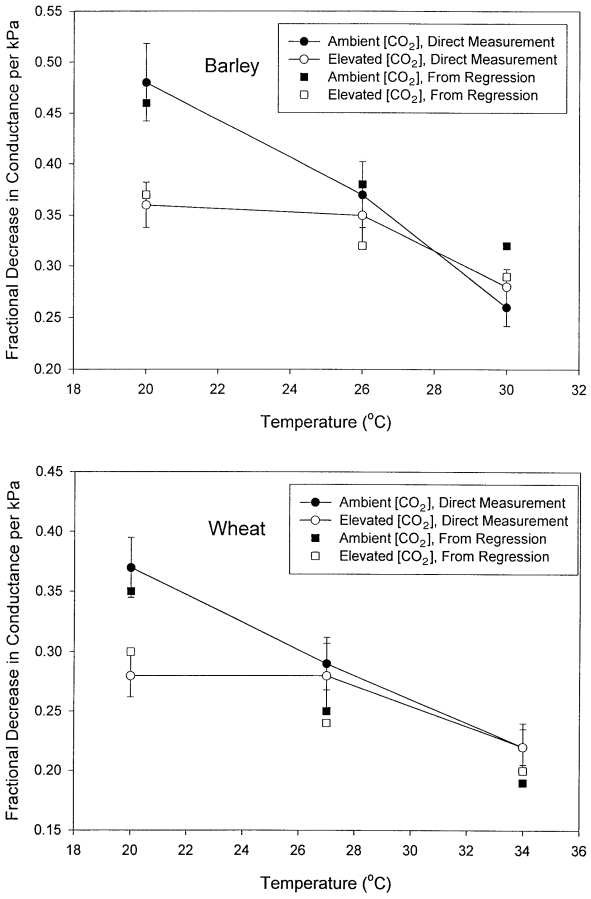

Fig. 5 Relative decreases in stomatal conductance with increasing water vapour pressure difference in wheat (a) and barley (b) leaves grown and measured at ambient or elevated (ambient + 350 μmol mol^{-1}) concentrations of CO_2. Relative decreases were obtained either by direct measurement of responses to increases in water vapour pressure difference or from multiple linear regressions relating mid-day values of conductance to temperature and water vapour pressure difference. For the direct measurements, relative decreases were determined separately for each leaf from the slope of a linear regression relating conductance to vapour pressure difference, divided by the value of conductance at the lowest value of vapour pressure difference. These are expressed as fractional decreases per kPa increase in water pressure difference. Vertical bars indicate standard errors, based on three replicate chambers per $[CO_2]$ treatment.

slope, i.e. the slope divided by the absolute value of conductance at the lowest value of D, were compared using analysis of variance on mean values for the three replicate chambers per $[CO_2]$ treatment.

Responses of stomatal conductance to PPFD were compared between $[CO_2]$ treatments and temperatures. Statistical comparisons were made after transformation of the response of conductance to PPFD into an approximately linear form followed by analysis of covariance to test for differences in the slopes of the linear regressions. Linear regressions of conductance/maximum conductance against the natural log of PPFD were used. The data were also summarized by nonlinear regressions, using a typical equation for a light–saturation curve (Jones 1983) of the form: $g = a \times (1 - \exp(-b \times \mathrm{PPFD}))$. The r^2 values of the linearized regressions were very similar to those of the nonlinear regressions shown in Table 1.

The day-to-day variation in the mean value of mid-day stomatal conductance for each $[CO_2]$ treatment was analysed using three approaches. In a multiple linear regression approach, stomatal conductance was the dependent variable and temperature (T), or the square of temperature and leaf to air water vapour pressure difference (D), were used as independent variables. The rate of CO_2 assimilation (A) was also tested as an independent variable. Responses to D at different temperatures determined from these multiple linear regressions were compared with the responses determined from the short-term manipulation of D on days with different temperatures. In addition to the multiple linear regressions, the stomatal responses to D and T were also summarized by developing a multiplicative model of stomatal conductance as a function of D and T (cf. Jones 1983), for each $[CO_2]$ treatment and species. For comparison with the two other approaches, the Ball–Berry model (Collatz *et al*. 1991) of stomatal conductance as a function of the product of assimilation rate, fractional humidity and the reciprocal of external $[CO_2]$ was also fitted to the data.

Results

The time course of mid-day stomatal conductance values (Fig. 1 and Fig. 2) illustrates the wide variation in mid-day conductances and the wide variation in the difference between $[CO_2]$ treatments. The air temperatures corresponding to these mid-day stomatal conductance measurements are given in Fig. 3. For each year, analysis of variance indicated significant effects of $[CO_2]$ treatment, day of year, and a significant interaction between $[CO_2]$ treatment and day of year. Very high values of stomatal conductance occurred on infrequent days characterized by high temperatures and low water vapour pressure differences, and averaged 1040 and 650 mmol H_2O m^{-2} s^{-1} at ambient $[CO_2]$ in wheat and barley, respectively, over the three years. Average mid-day values were 490 and 330 mmol m^{-2} s^{-1} in wheat and barley, respectively.

Stomatal conductance saturated at PPFDs of about half of full sunlight or less. The relative responses at 20 °C are shown in Fig. 4. The relative responses for a given $[CO_2]$ treatment and species at temperatures of 12, 16, and 25 °C

Table 2 Multiple regression models of stomatal conductance for wheat and barley at three growth [CO_2]. Data are for day-to-day variation in mid-day values measured at > 1.4 mmol m^{-2} s^{-1} PPFD. The multiple linear regressions used mean stomatal conductance in mol m^{-2} s^{-1} as the dependent variable and the leaf to air water vapour pressure difference (D) in kPa and either temperature (T) (barley) in °C or T^2 (wheat) as independent variables, depending on which gave higher R^2 values. CO_2 assimilation rate (A) in μmol m^{-2} s^{-1} was also significant as an independent variable (at $P = 0.05$) for wheat, but not for barley. Values in parentheses are standard errors. Measurements were made on 23 days for barley, 26 days for wheat at 350 and 700 μmol mol^{-1} CO_2, and 15 days for wheat at 525 μmol mol^{-1}

Species	[CO_2]	a	b (D)	c (T)	d (A)	R^2
Barley	350	0.206 (0.056)	−0.36 (0.06)	0.029 (0.005)		0.694
Barley	525	0.208 (0.047)	−0.25 (0.05)	0.017 (0.004)		0.598
Barley	700	0.213 (0.047)	−0.20 (0.05)	0.013 (0.004)		0.485
Wheat	350	0.428 (0.048)	−0.27 (0.05)	0.00092 (0.00013)		0.674
Wheat	525	0.343 (0.083)	−0.25 (0.08)	0.00072 (0.00019)		0.548
Wheat	700	0.276 (0.044)	−0.15 (0.05)	0.00042 (0.00012)		0.345
Wheat	350	0.083 (0.10)	−0.21 (0.05)	0.00079 (0.00011)	0.014 (0.004)	0.803
Wheat	525	0.030 (0.06)	−0.24 (0.04)	0.00061 (0.00009)	0.015 (0.002)	0.903
Wheat	700	0.019 (0.04)	−0.10 (0.03)	0.00032 (0.00007)	0.008 (0.001)	0.805

were very similar to those illustrated in Fig. 4 (not shown). Analysis of covariance of the linear form of the relative response indicated a significant [CO_2] effect on the slope for both species (at $P = 0.05$), indicating that at low PPFDs, high [CO_2] reduced the relative value of stomatal conductance (Fig. 4). A significant [CO_2] effect in both species was also indicated by the means and standard errors of the parameters of the nonlinear regressions (Table 1).

Increasing D reduced stomatal conductance in all leaves. There was no significant effect of measurement date within a [CO_2] treatment and nominal temperature on the slope of the response of conductance to D in either species (not shown). In both species, the highest temperatures decreased the relative sensitivity to D (Fig. 5). The relative sensitivity was significantly less (at $P = 0.05$) at high [CO_2] at the lowest temperatures in both species (Fig. 5), but did not differ significantly at other temperatures. The values of relative sensitivity of conductance to D at different temperatures calculated from the multiple regressions of day-to-day variation in mid-day stomatal conductances were in agreement with the results of the manipulations of D, in the sense of indicating decreasing relative sensitivity with increasing temperature and a larger effect of [CO_2] treatment at low temperature (Fig. 5).

The mid-day measurements of stomatal conductance covered a wide range of environmental conditions and stomatal conductances. Leaf temperatures ranged from 8 to 32 °C for barley and 10–35 °C for wheat. Values of D ranged from 0.6 to 2.6 kPa in barley and 0.9–3.6 kPa in wheat. Mid-day stomatal conductances at high PPFD varied by a factor of at least 5 in barley and 4 in wheat, for each [CO_2] treatment. CO_2 assimilation rates varied by a factor of 4 or more in barley, and 2 or more in wheat. In both species, and for all [CO_2] treatments, multiple linear regression indicated a significant negative response of conductance to D, and a positive response to temperature (T) (Table 2). In barley, T provided a better fit than T^2, but in wheat T^2 fitted better than T (only the better fitting regressions are shown). In wheat, but not in barley, CO_2 assimilation rate (A) had a significant positive effect on conductance even after D and T effects were included (Table 2). The regression coefficients for T and D for the intermediate [CO_2] were closer to the lower than to the higher [CO_2] in wheat, but in barley, the medium [CO_2] treatment had coefficients closer to those at the higher than the lower [CO_2] treatment (Table 2). The relative increase in stomatal conductance with increasing T at a constant value of D was less at high [CO_2] in both species (Fig. 6).

As the multiple linear regressions indicated that increasing temperature increased stomatal conductance over the temperatures measured, an exponential response of conductance to temperature was incorporated into the multiplicative model of conductance (Table 3). The temperature function used was: $\exp(-b(T_1-T)$, where T_1 is the value of temperature at which the function returns a value of 1. T_1 was set to 40 °C, so that the function produced values from 0 to 1 over the temperature range of 0–40 °C. The R^2 values for the multiplicative model were nearly the same as for the multiple linear regressions in barley and also in wheat for the multiple linear regressions not incorporating CO_2 assimilation rate (Table 2).

For barley grown at the highest [CO_2] treatment, the Ball–Berry model (Table 4) gave a substantially poorer fit to the data on day to day variation in conductance than did either the multiple linear regression or the multiplicative models (Table 2). For wheat, the Ball–Berry

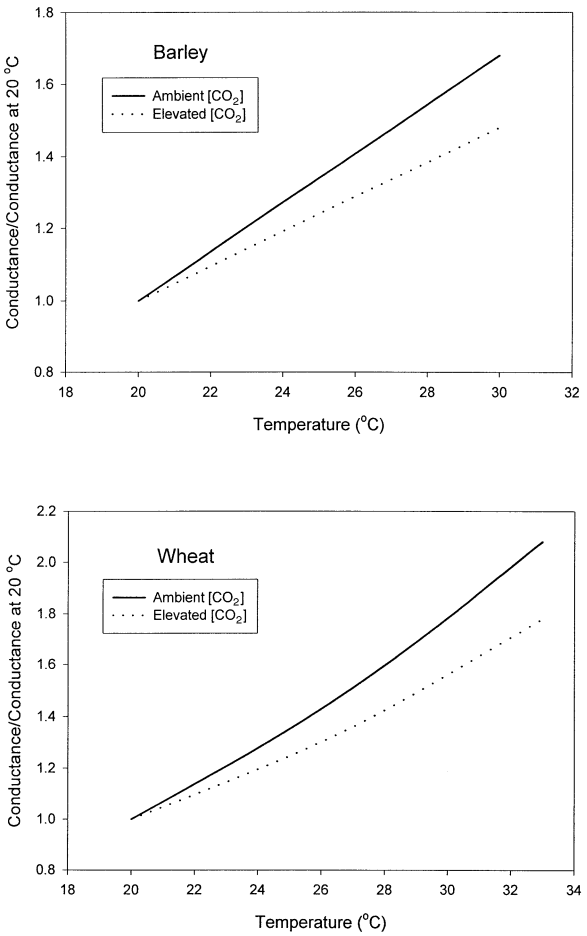

Fig. 6 Relative increase in stomatal conductance with temperature in wheat (a) and barley (b) leaves grown and measured at ambient or elevated (ambient + 350 μmol mol^{-1}) concentrations of CO_2. Absolute values of conductance were calculated from multiple linear regressions (Table 2) relating mid-day stomatal conductance to temperature (barley) or the square of temperature (wheat) and water vapour pressure difference. Conductances were calculated assuming a constant water vapour pressure difference of 1 kPa.

model (Table 4) gave higher R^2 values than the multiplicative model or the multiple linear regression model which did not incorporate A, but lower R^2 values than the multiple linear regression model which included A (Table 2). Although the combined data seem to define a single linear relationship between conductance and the Ball–Berry index for each species (Fig. 7), analysis of covariance indicated that, at the overall mean value of the Ball–Berry index, the ambient [CO_2] treatment had significantly higher values of stomatal conductance than either elevated treatment, for both species (Table 5).

Because the R^2 values of these three models based on day to day variation in mid-day stomatal conductance were smaller than the R^2 values obtained by manipulating individual environmental factors, causes of low R^2 values in the models were examined. In wheat, excluding data from the last three weeks before harvest substantially increased the R^2 values of the multiple linear regressions and eliminated the significance of the CO_2 assimilation rate term (Table 6). In barley, elimination of data from specific parts of the season did not increase R^2 values, but year-to-year variation in the absolute values of stomatal conductances reduced the R^2 values compared with values obtained separately for each year. However, the patterns of how the regression coefficients differed between [CO_2] treatments were consistent from year to year and were the same as presented for the combined data. Separating data by year or by stage of growth did not significantly affect the regression between stomatal conductance and the Ball–Berry index, for either species.

The ratio of mid-day stomatal conductance at elevated [CO_2] compared to that at ambient [CO_2], varied by a factor of at least 2 in both species across measurement days (Table 7). The ratio of conductances increased as the ratio of CO_2 assimilation rate at elevated to that at ambient [CO_2] increased, and decreased with increasing

Table 3 Multiplicative models of stomatal conductance in wheat and barley at three growth [CO_2]. Data are for day-to-day variation in mid-day values measured at > 1.4 mmol m^{-2} s^{-1} PPFD. The mean value of stomatal conductance (g) in mol m^{-2} s^{-1} was the dependent variable and the leaf to air water vapour pressure difference (D) in kPa and temperature (T) in °C were used as independent variables. The model was: $g = g_{max} \times \exp(bD) \times \exp(c(T_1 - T)$, with b and c as negative numbers. T_1 is the temperature at which the temperature function equals 1, and was set to 40 °C. This was analysed as a multiple linear regression: $\ln(g) = a + bD + c(T_1–T)$, because this resulted in smaller standard errors of parameter estimates than did nonlinear regressions. Numbers in parentheses are standard errors. Measurements were made on 23 days for barley, 26 days for wheat at 350 and 700 μmol mol^{-1} CO_2, and 15 days for wheat at 525 μmol mol^{-1}

Species	[CO_2]	a	b	c	R^2
Barley	350	1.99 (0.60)	−1.15 (0.22)	−0.085 (0.018)	0.588
Barley	525	1.52 (0.61)	−1.15 (0.22)	−0.078 (0.018)	0.594
Barley	700	1.26 (0.70)	−1.14 (0.25)	−0.070 (0.020)	0.513
Wheat	350	1.39 (0.40)	−0.49 (0.12)	−0.079 (0.013)	0.602
Wheat	525	1.57 (0.85)	−0.68 (0.25)	−0.099 (0.026)	0.551
Wheat	700	0.53 (0.56)	−0.50 (0.17)	−0.065 (0.019)	0.344

temperature in both species (Table 7). In these data, the ratios of CO_2 assimilation rate at 525 compared to 350 μmol mol^{-1} CO_2 ranged from 0.9 to 1.4 (mean 1.2) in barley, and from 0.9 to 1.5 (mean 1.2) in wheat. Ratios of CO_2 assimilation rate at 700 compared to 350 μmol mol^{-1} CO_2 ranged from 0.9 to 1.7 (mean 1.4) in barley, and from 0.8 to 1.9 (mean 1.3) in wheat. Although the correlation coefficients were not large in barley, both regression terms were significant at $P = 0.05$. It should be noted that water vapour pressure difference was correlated with the temperature (R^2 values between 0.6 and 0.7 in the data for both species), and similar regressions were obtained using vapour pressure difference in place of the temperature term. The ratio of CO_2 assimilation rates at elevated, compared with ambient [CO_2], was not significantly correlated with temperature in these data (not shown).

Discussion

The maximum values of mid-day stomatal conductance observed here for both species exceeded by factors of 1.5–2.5 the value of 400 mmol m^{-2} s^{-1} suggested as representative of herbaceous crops by Kelliher *et al*. (1995). However, the maximum values occurred on infrequent days in which high temperatures coincided with low water vapour pressure differences, and the mean mid-day stomatal conductances were close to the suggested value. This is to be expected, since this is essentially the nature of the data summarized by Kelliher *et al*. (1995). Therefore, the values given by Kelliher *et al*. (1995) should not be interpreted as physiologically optimum values of stomatal conductance.

Table 4 Ball–Berry models of stomatal conductance in wheat and barley at three growth [CO_2]. Data are for day-to-day variation in mid-day values measured at > 1.4 mmol m^{-2} s^{-1} PPFD. The mean value of stomatal conductance (g) in mol m^{-2} s^{-1} was the dependent variable and fractional humidity (H), and CO_2 assimilation rate (A) in μmol m^{-2} s^{-1} were used as independent variables. The model was $g = d + (e\ A\ *\ H/C_a)$, where C_a is the growth and measurement [CO_2]. Numbers in parentheses are standard errors. Measurements were made on 23 days for barley, 26 days for wheat at 350 and 700 μmol mol^{-1} CO_2, and 15 days for wheat at 525 μmol mol^{-1}

Species	[CO_2]	d	e	R^2
Barley	350	0.088 (0.051)	10.0 (2.0)	0.551
Barley	525	0.080 (0.034)	8.3 (1.7)	0.554
Barley	700	0.104 (0.040)	6.6 (2.2)	0.298
Wheat	350	0.203 (0.063)	9.6 (2.0)	0.499
Wheat	525	−0.024 (0.038)	18.4 (2.0)	0.869
Wheat	700	0.084 (0.029)	9.4 (1.3)	0.672

The hypothesis that the relative reduction in stomatal conductance caused by growth and measurement at elevated [CO_2] is independent of light, humidity and temperature was rejected separately for each variable, for each species. The relative reduction in conductance at elevated [CO_2] was greater at low light, low water vapour pressure difference (at low temperature), and at high temperature in both species. The large range in the ratios of mid-day stomatal conductances at elevated, relative to ambient, [CO_2] also indicates clearly that use of a constant relative decrease in conductance to simulate the effect of increasing atmospheric [CO_2] on conductance is unrealistic for these species.

Patterns of nonuniform relative decreases in stomatal conductance at elevated [CO_2] similar to those reported here, have also been reported in a few other cases. A larger relative [CO_2] effect at low light has been reported for beech (Heath & Kerstiens 1997). A larger relative effect of [CO_2] on conductance at low water vapour pressure difference has been reported for several other species (Hollinger 1987; Bunce 1993; Goodfellow *et al*. 1997; Wilson & Bunce 1997; Heath 1998). In soybean, as in wheat and barley, this was more evident at low temperatures (Wilson & Bunce 1997). In contrast, the short-term sensitivity of conductance to [CO_2] was often reduced at low values of water vapour pressure difference in wheat and barley as well as other species (Bunce 1998a). This indicates that growth [CO_2] predominantly controls the pattern of a relatively larger [CO_2] effect on stomatal conductance at low water vapour pressure difference. In contrast to the results presented here for wheat and barley, Wilson & Bunce (1997) found a larger relative [CO_2] effect on conductance at low temperature for soybean.

In terms of effects of increasing atmospheric [CO_2] on evapotranspiration, it is interesting that two conditions tending to maximize evapotranspiration, high light and high water vapour pressure difference, reduced the relative reduction in stomatal conductance by elevated [CO_2], but a third condition, high temperature, tending to maximize evapotranspiration (because it increases conductance) increased the relative effect of elevated [CO_2]. In the day-to-day variation in mid-day conductance, the temperature effect predominated over the effect of water vapour pressure difference, as high temperatures increased the reduction in conductance caused by elevated [CO_2]. If the [CO_2] in the atmosphere continues to rise, we would expect barley to show significant reductions in stomatal conductance sooner than wheat, based on the larger effect of the intermediate [CO_2] treatment on conductance in barley than in wheat relative to the effect of doubled [CO_2].

In addition to the effects of light, temperature and humidity on the relative reduction in stomatal conduc-

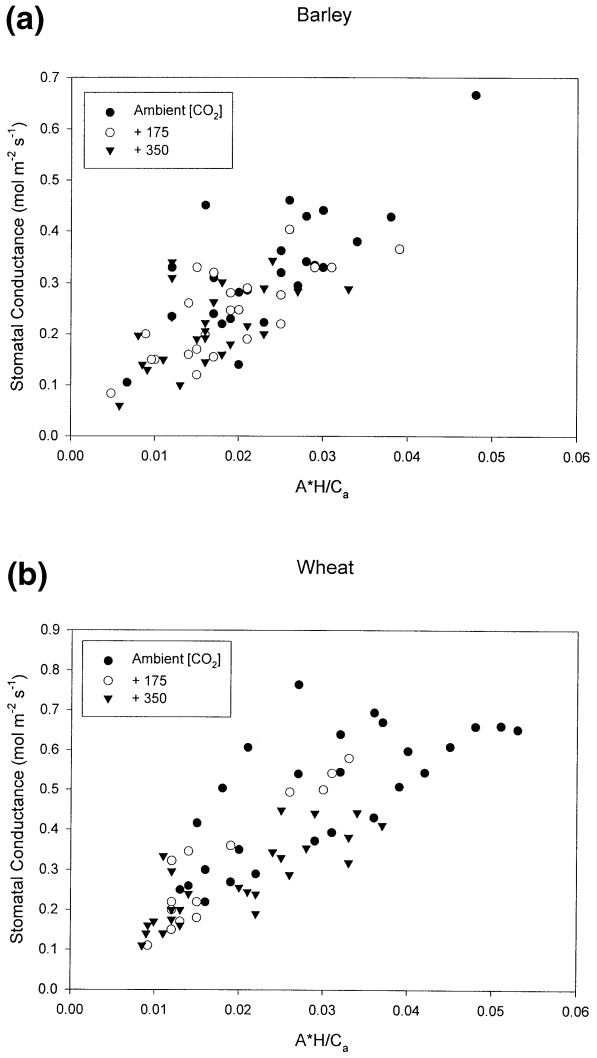

Fig. 7 Stomatal conductance as a function of a Ball–Berry index, in barley (a) and wheat (b) at three growth $[CO_2]$s. The index was $A \times H/C_a$, where A is CO_2 assimilation rate in μmol $m^{-2} s^{-1}$, H is fractional humidity, and C_a is the ambient $[CO_2]$ in μmol mol^{-1}. Data are for day-to-day variation in mid-day values measured at >1.4 mmol $m^{-2} s^{-1}$ PPFD. Each point represents a mean of six leaves from three replicate chambers.

tance by elevated $[CO_2]$, variation in the ratio of stomatal conductances was also correlated with the relative stimulation of $[CO_2]$ assimilation. The larger the relative stimulation of photosynthesis, the larger was the ratio of conductances, indicating relatively less reduction in conductance. The short-term stimulation of photosynthesis by elevated $[CO_2]$ in these plants was not affected strongly by temperature (Bunce 1998b), and variation in the ratio of assimilation rates primarily reflected changes in the degree of photosynthetic acclimation. Photosynthetic acclimation to elevated $[CO_2]$ increased

Table 5 Mean values of stomatal conductance at the overall mean value of the Ball–Berry index, as determined by analysis of covariance, for wheat and barley leaves grown at three $[CO_2]$s. Data are for day-to-day variation in mid-day values measured at >1.4 mmol $m^{-2} s^{-1}$ PPFD. Linear regression equations for each species and $[CO_2]$ treatment are given in Table 4. Values within species followed by different letters were significantly different at $P = 0.05$

$[CO_2]$ treatment (μmol mol^{-1})	Stomatal conductance (mol $m^{-2} s^{-1}$)	
	Barley	Wheat
350	0.287*a*	0.426*a*
525	0.245*b*	0.404*a*
700	0.234*b*	0.303*b*

Table 6 Multiple regression models of stomatal conductance in wheat grown at three $[CO_2]$s over 3 years. Data are for day-to-day variation in mid-day values measured at >1.4 mmol $m^{-2} s^{-1}$ PPFD, but exclude data for measurement days within 3 weeks of crop maturity. The multiple linear regressions used mean stomatal conductance in mol $m^{-2} s^{-1}$ as the dependent variable and the leaf to air water vapour pressure difference (D) in kPa and T^2 in °C as independent variables. Values in parentheses are standard errors. Regressions were calculated for measurements made on 14 days for wheat at 350 and 700 μmol mol^{-1} CO_2, and 7 days for wheat at 525 μmol mol^{-1}

$[CO_2]$	*a*	*b* (*D*)	*c* (*T*)	R^2
350	0.454 (0.057)	−0.39 (0.07)	0.012 (0.002)	0.852
525	0.234 (0.039)	−0.25 (0.04)	0.009 (0.001)	0.986
700	0.272 (0.034)	−0.26 (0.04)	0.008 (0.001)	0.884

through the season in both species (Sicher & Bunce 1997). In both species, plants grown at elevated $[CO_2]$ had lower soluble protein and ribulose bisphosphate carboxylase per unit of area (Sicher & Bunce 1997, 1998). Our observations therefore support the hypothesis of Sellers *et al*. (1996) that acclimation of photosynthesis to elevated $[CO_2]$ increases the relative reduction in stomatal conductance, although the mechanism remains unclear.

All three models of the response of stomatal conductance to temperature and humidity, the multiple regression, multiplicative and the 'Ball–Berry' models could be fitted to the data on mid-day stomatal conductances combined over three years, although the R^2 values were often not high. Some of the variability in the response of conductance to environment within a CO_2 treatment could be attributed to variation from year to year (barley) or within years (wheat). Such effects are to be expected, since the environment during leaf

Table 7 Day-to-day variation in the ratios of mean mid-day stomatal conductance (g) of plants grown and measured at 700 or 525 μmol mol^{-1} CO_2 to those grown and measured at 350 μmol mol^{-1}, and multiple linear regressions relating the conductance ratios to the ratio of CO_2 assimilation rate (A) in the elevated compared to the ambient [CO_2] and to the square of temperature

				Regression coefficients			
Species	Ratio	Mean	Range	I	$a(A)$	$b(T^2)$	R^2
Barley	$g525/g350$	0.74	0.46–0.94	0.32	0.41	−0.00015	0.221
Barley	$g700/g350$	0.66	0.42–0.86	0.39	0.30	−0.00032	0.357
Wheat	$g525/g350$	0.66	0.36–0.87	−0.02	0.67	−0.00018	0.611
Wheat	$g700/g350$	0.56	0.33–0.80	0.18	0.36	−0.00019	0.640

development can affect the response of stomatal conductance to environment (e.g. Bunce 1998c). The broader the dataset, the less certain one is likely to be of predictions of stomatal conductance by simple models such as these. However, a more restricted dataset with better fit to stomatal response models would raise concerns about the general applicability of the results. Although it is unlikely that soil water deficits reduced stomatal conductance in this dataset, it is possible that leaf water potentials were low enough on days with high transpiration rates to limit conductance. However, the general agreement between the responses of stomatal conductance to humidity determined by direct short-term manipulation of humidity with those determined from the regressions of mid-day stomatal conductance values on environment suggests that mid-day conductances were controlled by temperature and humidity rather than leaf water potential.

None of the modelling approaches to summarizing the data was clearly superior in all cases. Some limitations to these approaches should be noted. The multiplicative model as formulated here does not mimic the interaction with temperature in the effect of [CO_2] on sensitivity to water vapour pressure difference observed here in wheat and barley and also in soybean (Wilson & Bunce 1997). In applying the Ball–Berry and the multiple regression models, measured values of CO_2 assimilation rate were available. Having to predict CO_2 assimilation rates in these species for use in stomatal response models would introduce additional uncertainties for at least three reasons. First, the degree of photosynthetic acclimation to elevated [CO_2] varied through the season (Sicher & Bunce 1997). Secondly, responses of CO_2 assimilation rates to changes in water vapour pressure difference were not simply predictable from changes in calculated internal [CO_2], as previously shown in other species (Bunce 1993; Eamus *et al*. 1995; Wilson & Bunce 1997). Thirdly, the temperature dependence of the response of CO_2 assimilation rate to internal [CO_2] deviated substantially from that predicted by a standard biochemical model of C3 photosynthesis (Bunce 1998b). In light of these limitations, perhaps the most robust summary of stomatal responses capturing the interaction between temperature and water vapour pressure deficit was the multiple regression approach not utilizing CO_2 assimilation rates.

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