

# Direct and acclimatory responses of stomatal conductance to elevated carbon dioxide in four herbaceous crop species in the field

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## Abstract

In order to separate the net effect of growth at elevated  $[\text{CO}_2]$  on stomatal conductance ( $g_s$ ) into direct and acclimatory responses, mid-day values of  $g_s$  were measured for plants grown in field plots in open-topped chambers at the current ambient  $[\text{CO}_2]$ , which averaged  $350 \mu\text{mol mol}^{-1}$  in the daytime, and at ambient +  $350 \mu\text{mol mol}^{-1}$   $[\text{CO}_2]$  for winter wheat, winter barley, potato and sorghum. The acclimatory response was determined by comparing  $g_s$  measured at  $700 \mu\text{mol mol}^{-1}$   $[\text{CO}_2]$  for plants grown at the two  $[\text{CO}_2]$ . The direct effect of increasing  $[\text{CO}_2]$  from 350 to  $700 \mu\text{mol mol}^{-1}$  was determined for plants grown at the lower concentration. Photosynthetic rates were measured concurrently with  $g_s$ . For all species, growth at the higher  $[\text{CO}_2]$  significantly reduced  $g_s$  measured at  $700 \mu\text{mol mol}^{-1}$   $[\text{CO}_2]$ . The reduction in  $g_s$  caused by growth at the higher  $[\text{CO}_2]$  was larger for all species on days with low leaf to air water vapour pressure difference for a given temperature, which coincided with highest conductances and also the smallest direct effects of increased  $[\text{CO}_2]$  on conductance. For barley, there was no other evidence for stomatal acclimation, despite consistent down-regulation of photosynthetic rate in plants grown at the higher  $[\text{CO}_2]$ . In wheat and potato, in addition to the vapour pressure difference interaction, the magnitude of stomatal acclimation varied directly in proportion to the magnitude of down-regulation of photosynthetic rate through the season. In sorghum,  $g_s$  consistently exhibited acclimation, but there was no down-regulation of photosynthetic rate. In none of the species except barley was the direct effect the larger component of the net reduction in  $g_s$  when averaged over measurement dates. The net effect of growth at elevated  $[\text{CO}_2]$  on mid-day  $g_s$  resulted from unique combinations of direct and acclimatory responses in the various species.

*Keywords:* acclimation, barley, photosynthesis, potato, sorghum, transpiration, wheat

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## Introduction

A reduction in stomatal conductance ( $g_s$ ) is one of the more common responses to growth at elevated concentrations of carbon dioxide among herbaceous plants (cf. Sage 1994; Morison 1998; Assman 1999). Because  $g_s$  affects the energy balance at the interface between vegetation and the atmosphere, accurate predictions of the climatic effects of the rising concentration of carbon dioxide require a better understanding of the mechanisms underlying this reduction in  $g_s$  (e.g. Sellers *et al.*

1996). The net effect of growth at elevated  $[\text{CO}_2]$  on  $g_s$  has at least two components, a direct effect and an acclimation response (Morison 1998), and during drought often a third component related to the feedback effect of conservation of soil water (e.g. Hamerlynck *et al.* 1997; Huxman *et al.* 1998). While direct effects of  $[\text{CO}_2]$  on  $g_s$  have been studied widely under laboratory conditions, and net effects are commonly reported for field studies, direct and acclimatory responses have seldom been separated under field conditions. Acclimation can be detected as a growth  $[\text{CO}_2]$  effect on values of conductance measured under identical environmental con-

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ditions. This more general use of the term 'acclimation' (Morison 1998) is applied here, rather than the definition of Santrucek & Sage (1996) that attempts to separate stomatal acclimation from photosynthetic responses.

In modelling the effects of reduced  $g_s$  on climate, Sellers *et al.* (1996) hypothesized that the net decrease in conductance with rising atmospheric  $[\text{CO}_2]$  would be larger if down-regulation of photosynthesis occurred in addition to the direct effect of  $[\text{CO}_2]$  on  $g_s$ . This hypothesis was based on the frequently observed correlation between photosynthetic capacity and  $g_s$  (Wong *et al.* 1979), which results in the relative constancy of the ratio of internal to external  $[\text{CO}_2]$  (Collatz *et al.* 1991; Sage 1994). In other words, acclimation of photosynthesis would result in acclimation of  $g_s$ . The objective of the present paper was to test this hypothesis with data for several species of herbaceous crops grown under field conditions at the current ambient  $[\text{CO}_2]$  and at elevated  $[\text{CO}_2]$ . In previous work with three of the species examined here, the interaction of environmental factors on the net reduction in  $g_s$  in winter wheat and barley (Bunce 2000), the extent and biochemical nature of the acclimation of photosynthesis to elevated  $[\text{CO}_2]$  in wheat, barley (Sicher & Bunce 1997, 1998) and potato (Sicher & Bunce 1999), and in these same three species also the interaction between water vapour pressure deficit and the direct effect of  $[\text{CO}_2]$  on  $g_s$  of plants grown in ambient air have been described (Bunce 1998; Sicher & Bunce 1999). The present paper reports tests in winter wheat, winter barley, potato and sorghum of whether acclimation of  $g_s$  to growth at elevated  $[\text{CO}_2]$  occurred under field conditions, whether it was correlated with acclimation of photosynthesis, and whether direct or acclimatory responses contributed more to the net reduction in  $g_s$  in plants grown at elevated  $[\text{CO}_2]$ .

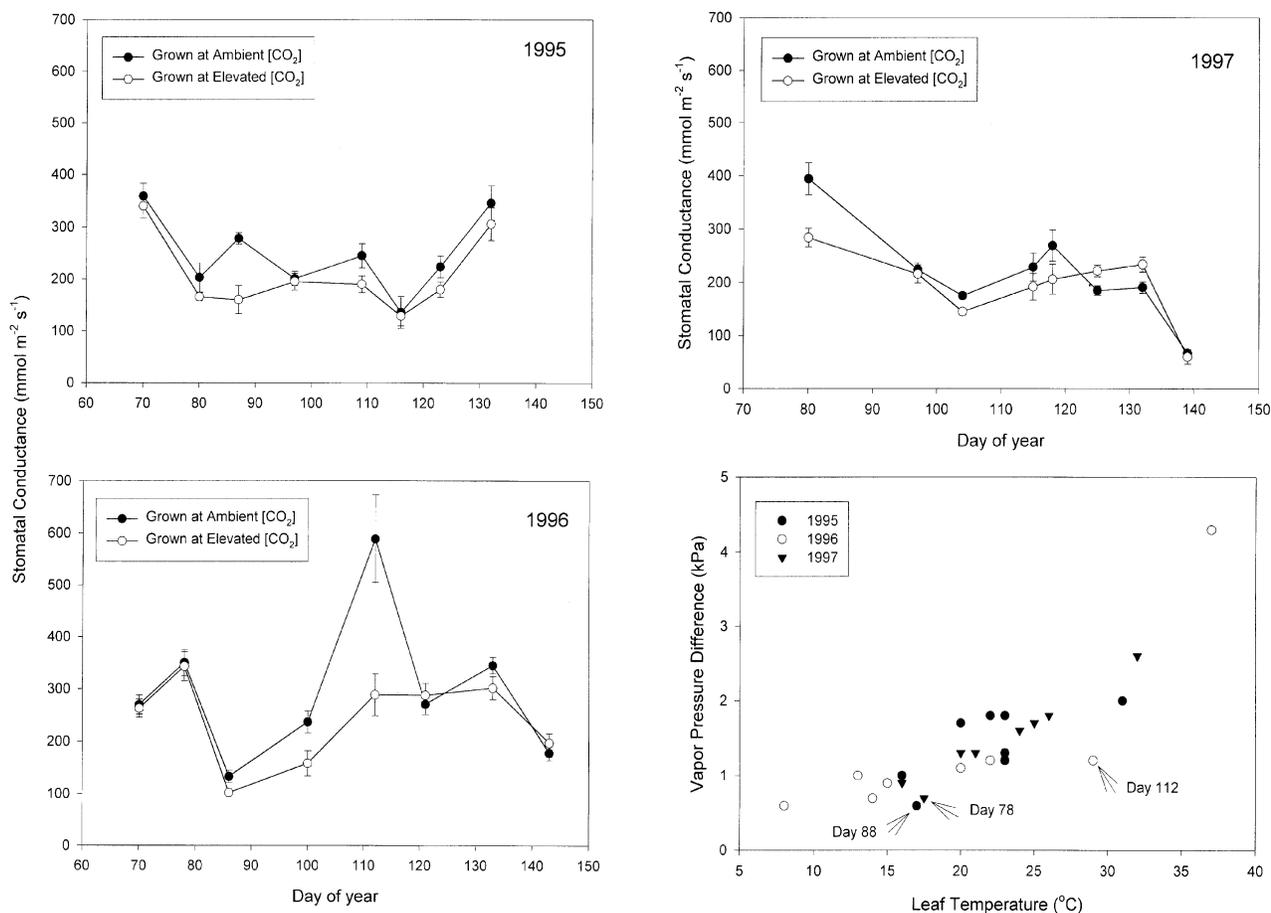
Because it was of interest to determine whether the  $g_s$  of plants grown and measured at elevated  $[\text{CO}_2]$  was affected by the long-term exposure to elevated  $[\text{CO}_2]$ , the  $g_s$  of plants grown and measured at elevated  $[\text{CO}_2]$  was compared with the  $g_s$  of plants grown at the current ambient  $[\text{CO}_2]$ , then briefly exposed to elevated  $[\text{CO}_2]$ .

## Materials and methods

All plants were grown in field plots at the South Farm of the Beltsville Agricultural Research Center, Beltsville, MD, during the period from 1994 to 1998. Species used were winter wheat (*Triticum aestivum* L. cv. Coker), winter barley (*Hordeum vulgare* L. cv. Wyson), potato (*Solanum tuberosum* L. cv. Atlantic), and sorghum (*Sorghum bicolor* L. Moench cv. AT × 399 × RT × 430, a commercial red hybrid). Winter wheat and barley were planted in October and leaf gas exchange was measured

in the spring. Potato and sorghum were planted in spring and gas exchange was measured in the summer. Seed densities were those recommended for the region for each species, and fertilizer treatments were based on soil tests and local recommendations for each species. Details of plant management for wheat and barley can be found in Bunce (2000), and for potato in Sicher & Bunce (1999). Sorghum was grown in 50 cm rows at a plant density of 15 plants  $\text{m}^{-2}$ , and fertilized after emergence with 25  $\text{g N m}^{-2}$ . The  $[\text{CO}_2]$  treatments were applied to plants in open topped rectangular clear acrylic chambers. Air was pushed into the bottom of each chamber through perforated plastic pipe and for half of the chambers pure  $\text{CO}_2$  was introduced into the inlet blower at a rate sufficient to raise the  $[\text{CO}_2]$  by  $350 \pm 50 \mu\text{mol mol}^{-1}$  above the current ambient  $[\text{CO}_2]$  concentration, which averaged  $350 \mu\text{mol mol}^{-1}$   $[\text{CO}_2]$  in the daytime.  $\text{CO}_2$  flow rates were adjusted every few days, as necessary. An air sampling system monitored  $[\text{CO}_2]$  in chambers of all  $[\text{CO}_2]$  treatment by species combinations every hour. Chambers had mixing fans positioned above the crop canopies that provided an air speed of about  $1.5 \text{ m s}^{-1}$  at canopy height. Chambers for barley, potato and sorghum covered  $2.4 \text{ m}^2$  of ground and were 2.7 m tall, and chambers for wheat covered  $1.2 \text{ m}^2$  of ground and were 1.8 m tall. The turnover time of the air in both sizes of chambers was about 20 s, and air temperatures averaged about  $1^\circ\text{C}$  above that of outside air. Soil psychrometers were placed in the centre of each chamber, at a depth of 25 cm. For wheat, barley and sorghum there were three replicate chambers per  $[\text{CO}_2]$  treatment, with two replicate chambers for potato. Three years of leaf gas exchange data were obtained for wheat, barley and potato, and two years for sorghum. All plots received normal precipitation.

Stomatal conductances and net photosynthetic rates were determined near mid-day on clear days every 1–2 weeks through the growing seasons for each crop, except in sorghum, where measurements were terminated shortly after anthesis because fungi growing on pollen sticking to leaves interfered with gas exchange measurements. Gas exchange was measured on fully illuminated mature upper canopy leaves of interior plants. Steady-state gas exchange was measured using a portable photosynthesis system (CIRAS-1, PP Systems, Haverhill, MA) with a broad leaf cuvette and automatic  $\text{CO}_2$  control system. Details of measurement protocols and system calibration can be found in Bunce (2000). The steps taken to assure that adequate time was allowed for short-term responses to increased  $[\text{CO}_2]$  to be complete are given in Bunce (1998). Plants from each  $[\text{CO}_2]$  treatment were measured at the growth  $[\text{CO}_2]$ , and plants from the chambers with the current ambient  $[\text{CO}_2]$  were also measured at  $700 \mu\text{mol mol}^{-1}$   $[\text{CO}_2]$ .



**Fig. 1** Seasonal patterns of mid-day stomatal conductance measured at  $700 \mu\text{mol mol}^{-1}$  [ $\text{CO}_2$ ] for barley plants grown at the current ambient [ $\text{CO}_2$ ] or at elevated [ $\text{CO}_2$ ] (ambient +  $350 \mu\text{mol mol}^{-1}$ ). Vertical bars represent SE of the mean, for  $n=3$  chambers per treatment.

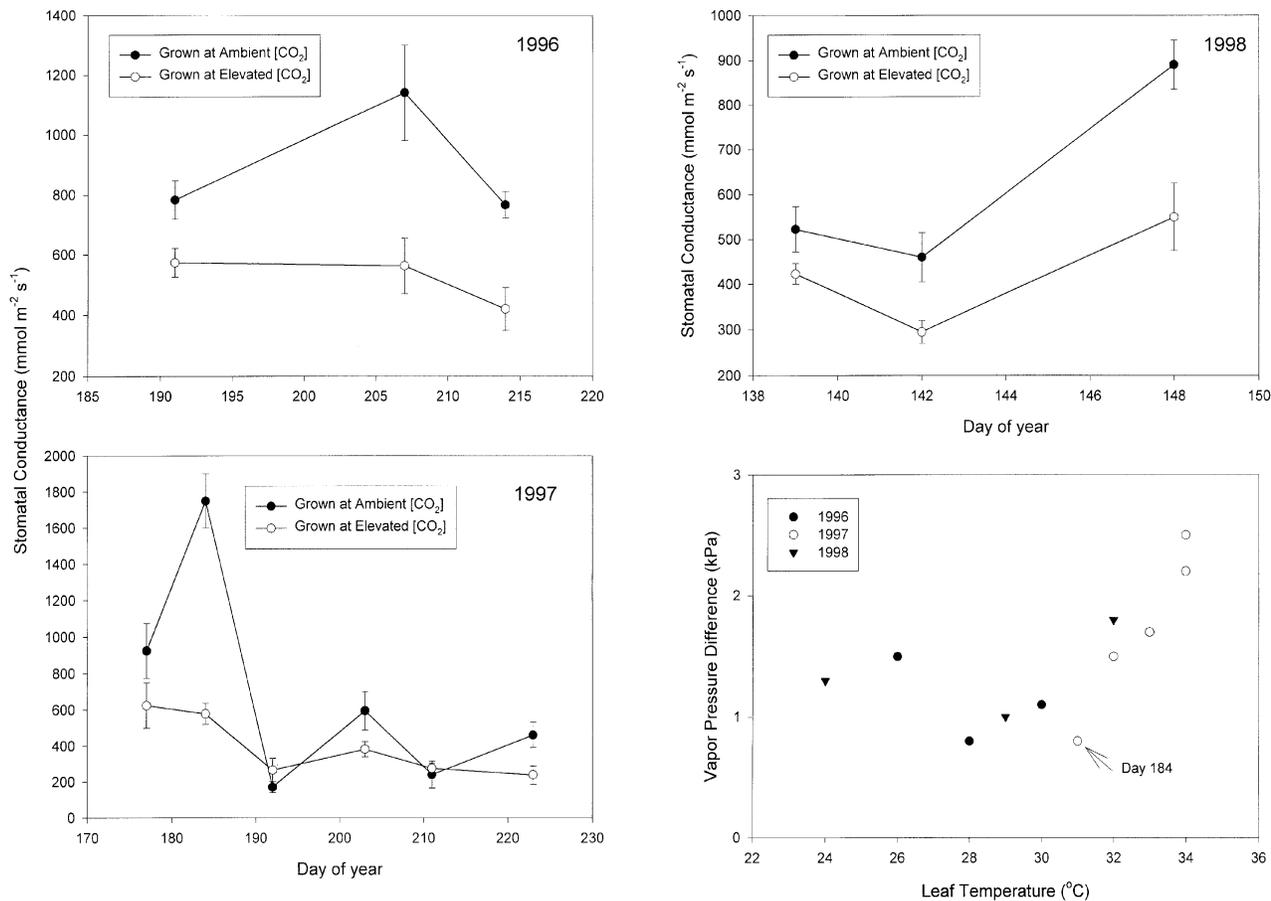
Measurements were made at nearly ambient conditions of air temperature and water vapour pressure difference, and at photosynthetic photon flux densities above  $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Leaf temperatures were no more than  $3^\circ\text{C}$  above ambient air temperatures. Mean leaf temperatures did not vary by more than  $1^\circ\text{C}$  between [ $\text{CO}_2$ ] treatments on a given measurement date. All leaf gas exchange data presented here are for dates when soil water deficits were unlikely to limit leaf gas exchange rates, based on soil water potential measurements and leaf gas exchange data. This criterion required elimination of only one measurement date for one species. Potato measured on one date in the midsummer of 1997 had lower  $g_s$ , photosynthetic rates and soil water potentials than for all other dates, and data for that date are not considered here. On each measurement date, six leaves per species were measured from a given [ $\text{CO}_2$ ] treatment, but analysis of variance tests were conducted on mean values of true replicates ( $n=2$  or 3 chambers per species per [ $\text{CO}_2$ ] treatment). A two-way analysis of variance was used to test for effects of growth [ $\text{CO}_2$ ],

measurement date, and the interaction between growth [ $\text{CO}_2$ ] and measurement date on  $g_s$ . The hypothesis of homogeneity of variance of  $g_s$  was not rejected at  $P=0.05$  for any of the species.

The acclimatory response of  $g_s$  was quantified as the ratio of  $g_s$  for plants grown at the elevated [ $\text{CO}_2$ ] to those of plants grown at the current ambient [ $\text{CO}_2$ ], when both were measured at  $700 \mu\text{mol mol}^{-1}$  [ $\text{CO}_2$ ]. The direct or short-term effect of increasing [ $\text{CO}_2$ ] was quantified as the ratio of  $g_s$  measured at  $700 \mu\text{mol mol}^{-1}$  [ $\text{CO}_2$ ] to that measured at  $350 \mu\text{mol mol}^{-1}$  [ $\text{CO}_2$ ] for plants grown at the lower concentration. The net effect of growth at elevated [ $\text{CO}_2$ ] on  $g_s$ , i.e. the ratio of  $g_s$  in the growth environments for plants grown at elevated [ $\text{CO}_2$ ] to those of plants grown at the current ambient [ $\text{CO}_2$ ], was thus the product of the acclimatory and the direct effects:

$$\frac{g_s(e,E)}{g_s(a,A)} = \frac{g_s(e,E)}{g_s(a,E)} \times \frac{g_s(a,E)}{g_s(a,A)}$$

Net effect = Acclimatory effect  $\times$  Direct effect,



**Fig. 2** Seasonal patterns of mid-day stomatal conductance measured at  $700 \mu\text{mol mol}^{-1}$  [ $\text{CO}_2$ ] for potato plants grown at the current ambient [ $\text{CO}_2$ ] or at elevated [ $\text{CO}_2$ ] (ambient +  $350 \mu\text{mol mol}^{-1}$ ). Vertical bars represent SE of the mean, for  $n=2$  chambers per treatment.

**Table 1** Mean values of mid-day stomatal conductance measured at  $700 \mu\text{mol mol}^{-1}$  [ $\text{CO}_2$ ] for plants of four species grown at the current ambient [ $\text{CO}_2$ ] or at elevated [ $\text{CO}_2$ ] (ambient +  $350 \mu\text{mol mol}^{-1}$ ). Standard errors and probability levels from two-way analysis of variance are given. There were 26 measurement dates for wheat, 24 for barley, 12 for potato and 11 for sorghum. There were three replicate chambers per [ $\text{CO}_2$ ] treatment in wheat, barley and potato, and two for sorghum.

Species	Conductance ( $\text{mmol m}^{-2} \text{s}^{-1}$ )		Probability level of effect:		
	Ambient	Elevated	Growth [ $\text{CO}_2$ ]	Date	Growth [ $\text{CO}_2$ ] $\times$ Date
Wheat	$420 \pm 29$	$270 \pm 20$	***	***	***
Barley	$252 \pm 22$	$214 \pm 15$	**	***	**
Potato	$714 \pm 93$	$447 \pm 51$	***	***	*
Sorghum	$461 \pm 78$	$296 \pm 34$	***	***	**

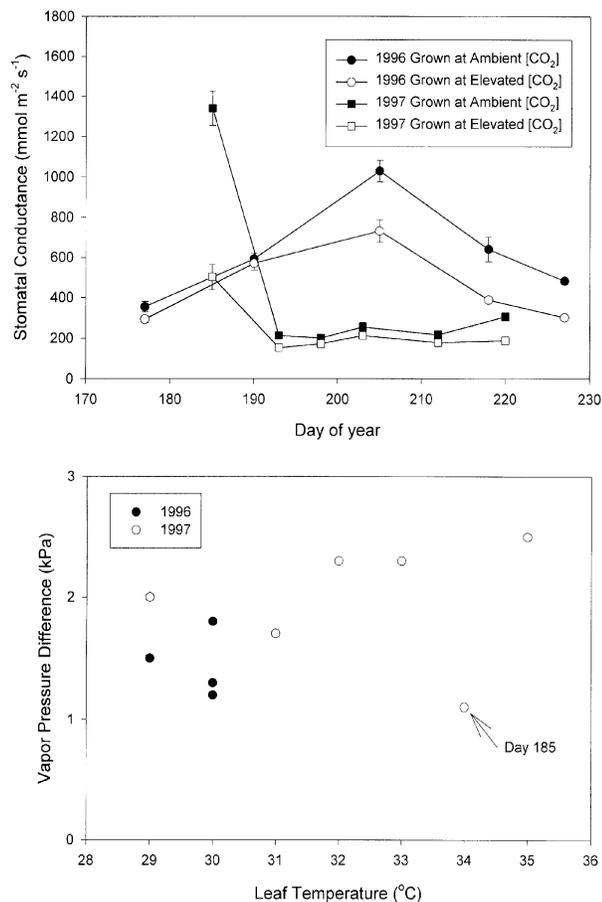
\*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$

where the lower case letters refer to the growth conditions of (a) the current ambient [ $\text{CO}_2$ ] or (e) elevated [ $\text{CO}_2$ ], and the upper case letters refer to the measurement conditions of (A) the current ambient [ $\text{CO}_2$ ] or (E) elevated [ $\text{CO}_2$ ].

The ratio of photosynthetic rates of plants grown at the elevated [ $\text{CO}_2$ ] to that of plants grown at the current ambient [ $\text{CO}_2$ ], when both were measured at  $700 \mu\text{mol mol}^{-1}$  [ $\text{CO}_2$ ] was used to quantify the acclimation of photosynthetic rate. All of these ratios had approximately normal distributions over measurement dates for each species. We tested whether the mean ratios of each species differed significantly from a value of one, using single-group  $t$ -tests.

## Results

Leaf gas exchange data were analysed for a total of 26 d in wheat, 24 d in barley, 12 d in potato and 11 d in sorghum. The effect of growth [ $\text{CO}_2$ ] was significant for all species at  $P \leq 0.01$ , with lower  $g_s$  in plants grown at the elevated [ $\text{CO}_2$ ] (Table 1). However, the effect of



**Fig. 3** Seasonal patterns of mid-day stomatal conductance measured at  $700 \mu\text{mol mol}^{-1}$  [ $\text{CO}_2$ ] for sorghum plants grown at the current ambient [ $\text{CO}_2$ ] or at elevated [ $\text{CO}_2$ ] (ambient +  $350 \mu\text{mol mol}^{-1}$ ). Vertical bars represent SE of the mean, for  $n=3$  chambers per treatment.

growth [ $\text{CO}_2$ ] varied significantly with measurement date for all species ( $P \leq 0.05$ ). The nature of this interaction was explored further.

In all species, the growth [ $\text{CO}_2$ ] by measurement date interaction effect occurred partly because the growth [ $\text{CO}_2$ ] effect was largest on days with unusually low leaf to air vapour pressure differences ( $D$ ) for a given temperature (Figs 1,2,3,4). For barley and wheat, the seasonal patterns of conductance of plants grown at elevated [ $\text{CO}_2$ ] in Fig 1 and 4 has been previously published in a different context (Bunce 2000). The low  $D$  days also had unusually high values of  $g_s$  for plants grown at the current ambient [ $\text{CO}_2$ ]. This source of the significant treatment-by-date interaction is further indicated by the fact that excluding the few days with the unusual values of  $D$  for a given temperature from the analysis (1 d each in sorghum and potato, 3 d in barley, shown in Figs 1,2,3) made the interaction term nonsignificant in these species. In wheat there was an additional

**Table 2** Seasonal patterns of direct and acclimatory responses of stomatal conductance to elevated [ $\text{CO}_2$ ] in wheat grown at the current ambient [ $\text{CO}_2$ ] or at elevated [ $\text{CO}_2$ ] (ambient +  $350 \mu\text{mol mol}^{-1}$ ), and the acclimation of photosynthetic rate. The direct effect is expressed as the conductance of plants grown at the current ambient [ $\text{CO}_2$ ] and measured at  $700 \mu\text{mol mol}^{-1}$  [ $\text{CO}_2$ ] relative to their conductance measured at  $350 \mu\text{mol mol}^{-1}$ . The acclimatory response of stomatal conductance and photosynthetic rate is expressed as the values for elevated-grown plants relative to those of ambient-grown plants when both measured at  $700 \mu\text{mol mol}^{-1}$ . The net effect of growth at elevated [ $\text{CO}_2$ ] on conductance is the product of the direct and acclimatory effects. Stage I refers to dates prior to flowering, II to early grain-filling, and III to late grain-filling. Mean ratios are given for a total of 26 measurement dates.

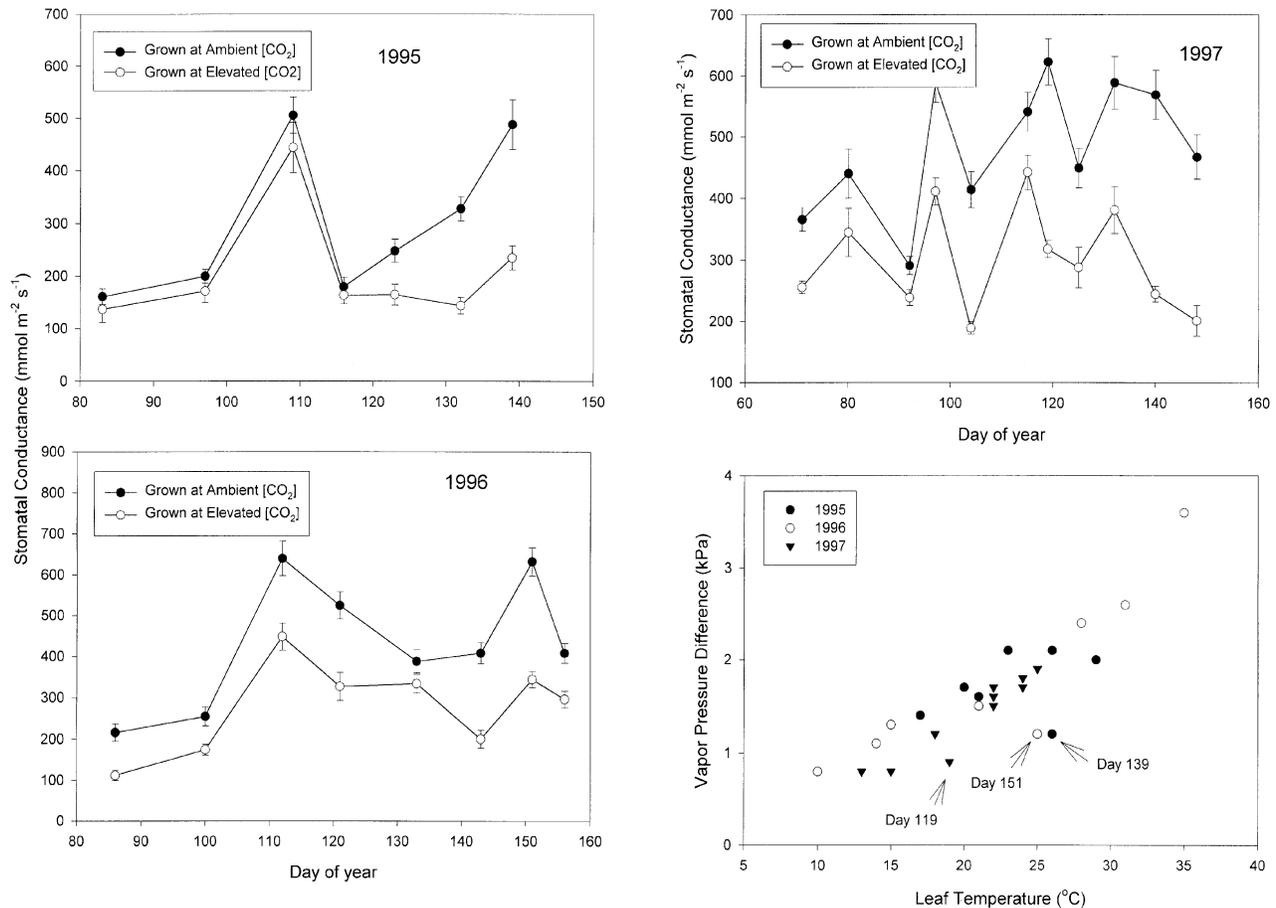
Effect	Stage			
	I	II	III	Mean
Direct	0.85*	0.83*	0.90*	0.85*
Acclimation of conductance	0.74*	0.69*	0.51*	0.66*
Net conductance	0.62*	0.57*	0.44*	0.56*
Acclimation of photosynthesis	0.80*	0.83*	0.61*	0.76*

\* indicates a ratio significantly different from 1.0, at  $P=0.05$

cause of the interaction term, which will be discussed later. Whether or not the significant interaction term occurred because the absolute difference in  $g_s$  between growth [ $\text{CO}_2$ ] treatments was larger on days with high absolute values of  $g_s$ , was tested by using the natural log of  $g_s$  in place of conductance in the two-way analysis of variance. However, the interaction term remained significant for each species.

A seasonal pattern in the acclimation of  $g_s$  occurred in wheat, which was associated with a seasonal pattern in photosynthetic acclimation (Table 2). Reductions in both  $g_s$  and photosynthetic rate of plants grown at elevated [ $\text{CO}_2$ ] were larger during late grain-filling than earlier. The direct response of  $g_s$  to a step increase in [ $\text{CO}_2$ ] changed little with season, and was consistently smaller (i.e. with a ratio closer to 1) than the acclimation response (Table 2).

Similarly, in potato, the magnitude of the acclimation of  $g_s$  was highly correlated with the magnitude of reduction in photosynthetic rate caused by growth at elevated [ $\text{CO}_2$ ], as well as with  $D$  (Table 3). In potato, the direct effect of increasing [ $\text{CO}_2$ ] on  $g_s$  was to increase  $g_s$  slightly on days with low vapour pressure difference and to decrease it on days with high  $D$  (Sicher and Bunce 2000). The mean direct effect was very small when averaged over days, and the acclimation response was



**Fig. 4** Seasonal patterns of mid-day stomatal conductance measured at  $700 \mu\text{mol mol}^{-1}$   $[\text{CO}_2]$  for wheat plants grown at the current ambient  $[\text{CO}_2]$  or at elevated  $[\text{CO}_2]$  (ambient +  $350 \mu\text{mol mol}^{-1}$ ). Vertical bars represent SE of the mean, for  $n=3$  chambers per treatment.

**Table 3** Multiple linear regression relating the stomatal conductance of potato plants grown at elevated  $[\text{CO}_2]$  relative to the conductance of plants grown at the current ambient  $[\text{CO}_2]$ , both measured at  $700 \mu\text{mol mol}^{-1}$   $[\text{CO}_2]$  to the same ratio of photosynthetic rates and to the leaf to air water vapour pressure deficit ( $D$ ) in kPa. The value of  $R^2$  was 0.887, for  $n=12$

Variable	Coefficient	SE	Probability of no linear relationship
Intercept	-0.881	0.23	0.004
Photosynthesis ratio	1.53	0.30	0.007
$D$	0.23	0.07	0.009

almost solely responsible for the net reduction in  $g_s$  (Table 3).

In barley, removing the three days with unusually low  $D$  for a given temperature not only made the interaction between growth  $[\text{CO}_2]$  and measurement date nonsigni-

ficant, but also made the growth  $[\text{CO}_2]$  effect nonsignificant, with mean  $g_s$  values differing by only 7% between plants grown at the two  $[\text{CO}_2]$  and measured at  $700 \mu\text{mol mol}^{-1}$   $[\text{CO}_2]$  (Table 4). Down-regulation of photosynthetic rate in barley occurred consistently throughout the season. For sorghum, stomatal acclimation occurred consistently, but there was no acclimation of photosynthetic rate. The acclimation effect was larger than the direct effect in sorghum, when averaged over measurement dates (Table 4).

The magnitude of the direct effect on  $g_s$  of increasing the measurement  $[\text{CO}_2]$  from  $350$  to  $700 \mu\text{mol mol}^{-1}$  for plants grown at the current ambient  $[\text{CO}_2]$  increased with  $D$  in all species. This is illustrated for sorghum in Fig. 5. Similar curves and regression equations for wheat, barley and potato have been previously published (Bunce 1998; Sicher and Bunce 2000).

While both stomatal acclimation and the direct response to elevated  $[\text{CO}_2]$  varied with  $D$ , the days with largest acclimation effects on  $g_s$  were also days with the

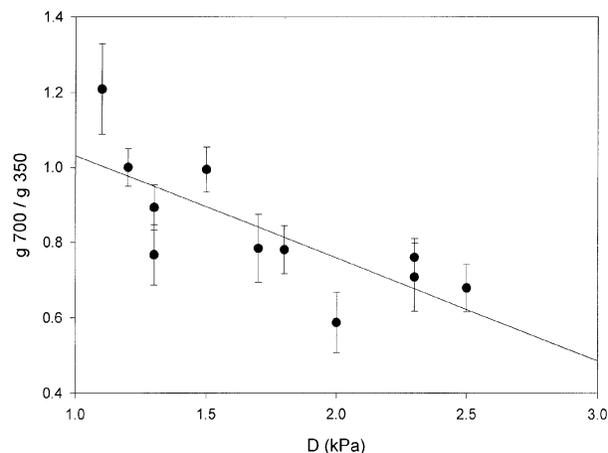
**Table 4** Direct and acclimatory responses of stomatal conductance to elevated [CO<sub>2</sub>] in barley, potato and sorghum grown at the current ambient [CO<sub>2</sub>] or at elevated [CO<sub>2</sub>] (ambient +350 μmol mol<sup>-1</sup>), and the acclimation of photosynthetic rate. The direct effect is expressed as the conductance of plants grown at the current ambient [CO<sub>2</sub>] and measured at 700 μmol mol<sup>-1</sup> [CO<sub>2</sub>] relative to their conductance measured at 350 μmol mol<sup>-1</sup>. The acclimatory response of stomatal conductance and photosynthetic rate is expressed as the values for elevated-grown plants relative to those of ambient-grown plants when both were measured at 700 μmol mol<sup>-1</sup>. The net effect on conductance is the product of the direct and acclimatory effects. Ratios are mean values for a total of 24 measurement dates for barley, 12 for potato and 11 for sorghum. Also shown in [] are mean ratios for barley excluding three measurement dates with extremely high conductances of plants grown at the current ambient [CO<sub>2</sub>].

Effect	Species		
	barley	potato	sorghum
Direct effect on conductance	0.77* [0.75*]	0.99	0.83*
Acclimation of conductance	0.89* [0.93]	0.67*	0.72*
Net conductance effect	0.67* [0.68*]	0.65*	0.60*
Acclimation of photosynthesis	0.83* [0.83*]	0.80*	0.98

\* indicates a ratio significantly different from 1.0, at  $P=0.05$

**Table 5** Correlations between direct and acclimatory responses of mid-day stomatal conductance to elevated [CO<sub>2</sub>] among days for four crop species, and correlations between the net decrease in mid-day conductance and the leaf to air water vapour pressure difference ( $D$ ) on different measurement days. The direct effect is expressed as the conductance of plants grown at the current ambient [CO<sub>2</sub>] and measured at 700 μmol mol<sup>-1</sup> [CO<sub>2</sub>] relative to their conductance measured at 350 μmol mol<sup>-1</sup>. The acclimatory response of stomatal conductance is expressed as values for plants grown at elevated [CO<sub>2</sub>] (ambient +350 μmol mol<sup>-1</sup>) relative to those of ambient-grown plants when both were measured at 700 μmol mol<sup>-1</sup> [CO<sub>2</sub>]. The net effect is expressed as the conductance of plants grown and measured at 700 μmol mol<sup>-1</sup> [CO<sub>2</sub>] relative to the conductance of plants grown and measured at 350 μmol mol<sup>-1</sup>, and is the product of the direct and acclimatory effects

Species	Direct vs. Acclimatory Effect		Net Effect vs. $D$	
	$r$	Probability of no correlation	$r$	Probability of no correlation
Wheat	-0.45	0.021	-0.26	0.20
Barley	-0.53	0.008	+0.05	0.83
Potato	-0.76	0.011	-0.39	0.24
Sorghum	-0.69	0.012	+0.36	0.17



**Fig. 5** The relationship between the direct effect of increasing the measurement [CO<sub>2</sub>] from 350 to 700 μmol mol<sup>-1</sup> [CO<sub>2</sub>] for sorghum plants grown at the current ambient [CO<sub>2</sub>] and the leaf to air water vapour pressure difference ( $D$ ) during the measurement. The direct effect is represented as the ratio of conductance at 700 to that at 350 μmol mol<sup>-1</sup> [CO<sub>2</sub>]. Each point represents the mean for 6 leaves on a given measurement date, and vertical bars represent SE for  $n=3$  chambers. The correlation coefficient was  $-0.757$ , which is significant at  $P=0.05$ .

smallest direct response. Thus, the acclimation effect and the direct effect were significantly negatively correlated in all of the species, although the correlation coefficients were not large (Table 5). The offsetting effects of smaller direct effects and larger acclimation effects on days with low  $D$  resulted in low and nonsignificant correlations between the net decrease in  $g_s$  at elevated [CO<sub>2</sub>] and  $D$  (Table 5).

Constant ratios of substomatal to external [CO<sub>2</sub>] ( $C_i/C_a$ ) were consistent with the existence of a relationship between the acclimation of  $g_s$  and the acclimation of photosynthesis in wheat and potato. For wheat, the  $C_i/C_a$  ratio averaged  $0.67 \pm 0.01$  for both growth [CO<sub>2</sub>] conditions in leaves measured at 700 μmol mol<sup>-1</sup> [CO<sub>2</sub>] and also in leaves grown and measured at the current ambient [CO<sub>2</sub>]. In potato, the  $C_i/C_a$  ratio averaged  $0.72 \pm 0.01$  in all growth and measurement [CO<sub>2</sub>] conditions. In contrast, for sorghum, plants grown at elevated [CO<sub>2</sub>] had a lower  $C_i/C_a$  ratio (mean = 0.42) when measured at 700 μmol mol<sup>-1</sup> [CO<sub>2</sub>], than in plants grown at the lower [CO<sub>2</sub>] (mean = 0.50). This is consistent with lower  $g_s$  but not photosynthesis in this species when grown at elevated [CO<sub>2</sub>]. In barley, lack of acclimation of  $g_s$  despite acclimation of photosynthesis was reflected in a higher mean  $C_i/C_a$  ratio of 0.67 for plants grown at elevated [CO<sub>2</sub>] compared with plants grown at the current ambient [CO<sub>2</sub>] ( $C_i/C_a$  ratio = 0.61), when both were measured at 700 μmol mol<sup>-1</sup> [CO<sub>2</sub>].

## Discussion

The results indicate that three types of stomatal acclimation to growth at elevated  $[\text{CO}_2]$  occurred in the various species. One type was present in all species, larger differences between plants grown at the current ambient and elevated  $[\text{CO}_2]$  in  $g_s$  measured at  $700 \mu\text{mol mol}^{-1} [\text{CO}_2]$  on days with low  $D$ . This was related to the small response of  $g_s$  of plants grown at the current ambient  $[\text{CO}_2]$  to the short-term increase in  $[\text{CO}_2]$  on those days. Low  $D$  reducing the stomatal response to  $[\text{CO}_2]$  has been reported previously for other species (Hall & Kaufman 1975; Bunce 1998). This type of acclimation is probably not very important to seasonal transpiration rates, because it occurred on infrequent days with low values of  $D$  for a given temperature, which are also days in which differences in  $g_s$  would have the least impact on transpiration rates.

The second type of acclimation was correlated with acclimation of photosynthesis, and occurred in wheat and potato. In wheat, acclimation of photosynthesis was evidenced by lower rates of mid-day photosynthesis measured at  $700 \mu\text{mol mol}^{-1} [\text{CO}_2]$  in plants grown at that  $[\text{CO}_2]$ , and was correlated with low Rubisco content and activity (Sicher & Bunce 1997). In potato, acclimation of photosynthesis was correlated with low activity of Rubisco, with no change in Rubisco content (Sicher & Bunce 1999). Photosynthetic acclimation was always present in wheat, but increased in magnitude through the season. In potato, photosynthetic acclimation showed no clear seasonal pattern. In these species the  $C_i/C_a$  ratio was the same for both growth  $[\text{CO}_2]$  conditions. Sage (1994) reviewed the literature and found that constant  $C_i/C_a$  ratios were common, but not universal for plants showing photosynthetic acclimation to different growth  $[\text{CO}_2]$  conditions. While Assman (1999) considered constant  $C_i/C_a$  ratios in such cases indicated lack of acclimation of  $g_s$ , until the mechanistic link between down-regulation of photosynthesis and reduced  $g_s$  is understood, it seems best to identify this response as parallel acclimation of  $g_s$  and photosynthesis to growth at elevated  $[\text{CO}_2]$ .

A third type of acclimation of  $g_s$  was evident in sorghum, in which  $g_s$  was consistently lower in plants grown at elevated  $[\text{CO}_2]$  on measurement days covering a wide range of  $D$ , and occurred despite no acclimation of photosynthesis. A partly irreversible decrease in  $g_s$  in plants grown at elevated  $[\text{CO}_2]$  has also been observed in *Acacia smallii* (Polley *et al.* 1997) and in *Ginkgo biloba* (Beerling *et al.* 1998). It was not practical in the present study to determine complete responses of  $g_s$  to sub-stomatal  $[\text{CO}_2]$  for plants grown in both  $[\text{CO}_2]$  treatments, because other environmental conditions were not constant over the time period required to determine a

single complete  $[\text{CO}_2]$  response function, let alone to compare growth  $[\text{CO}_2]$  treatments (Morison 1998). Hence, gains of the various feedback loops involving stomata, photosynthesis and  $[\text{CO}_2]$  (Santrucek & Sage 1996) could not be evaluated to identify the nature of the acclimation in  $g_s$  in the cases of sorghum and barley, in which the  $C_i/C_a$  ratio changed with growth  $[\text{CO}_2]$ .

It has been suggested that  $g_s$  may be more sensitive to  $[\text{CO}_2]$  in C4 than in C3 plants (e.g. Akita & Moss 1972; cf. Morison & Gifford 1983). However, in terms of the direct effect of  $[\text{CO}_2]$  on  $g_s$ , the acclimation of  $g_s$  to growth at elevated  $[\text{CO}_2]$ , and the net effect of growth and measurement at elevated  $[\text{CO}_2]$  on  $g_s$ , the C4 species examined here, sorghum, had responses intermediate between those of the three C3 species examined. The present data are thus consistent with the conclusion of Morison & Gifford (1983) of similar relative responses of  $g_s$  to  $[\text{CO}_2]$  in C3 and C4 species. The lack of photosynthetic acclimation to elevated  $[\text{CO}_2]$  in sorghum is consistent with most reports for C4 species (e.g. Ziska & Bunce 1997; but see Watling *et al.* 2000).

The results presented here do not consistently support the hypothesis that the amount of stomatal acclimation to elevated  $[\text{CO}_2]$  is correlated with the amount of acclimation of photosynthesis. Although the hypothesis was supported by the data for wheat and potato, in barley acclimation of photosynthesis occurred with little evidence of acclimation of  $g_s$ , and in sorghum acclimation of  $g_s$  occurred with no acclimation in photosynthesis. It is important that the ability to predict the acclimation of  $g_s$  to long-term exposure to increased  $[\text{CO}_2]$  is developed, as in three of the species examined here the acclimatory effect of elevated  $[\text{CO}_2]$  on  $g_s$  contributed more than the direct effect to reduced mid-day  $g_s$ .

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