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# Photosynthesis and conductance of spring-wheat leaves: field response to continuous free-air atmospheric CO<sub>2</sub> enrichment

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## ABSTRACT

Spring wheat was grown from emergence to grain maturity in two partial pressures of CO<sub>2</sub> (*p*CO<sub>2</sub>): ambient air of nominally 37 Pa and air enriched with CO<sub>2</sub> to 55 Pa using a free-air CO<sub>2</sub> enrichment (FACE) apparatus. This experiment was the first of its kind to be conducted within a cereal field without the modifications or disturbance of microclimate and rooting environment that accompanied previous studies. It provided a unique opportunity to examine the hypothesis that continuous exposure of wheat to elevated *p*CO<sub>2</sub> will lead to acclimatory loss of photosynthetic capacity. The diurnal courses of photosynthesis and conductance for upper canopy leaves were followed throughout the development of the crop and compared to model-predicted rates of photosynthesis. The seasonal average of midday photosynthesis rates was 28% greater in plants exposed to elevated *p*CO<sub>2</sub> than in controls and the seasonal average of the daily integrals of photosynthesis was 21% greater in elevated *p*CO<sub>2</sub> than in ambient air. The mean conductance at midday was reduced by 36%. The observed enhancement of photosynthesis in elevated *p*CO<sub>2</sub> agreed closely with that predicted from a mechanistic biochemical model that assumed no acclimation of photosynthetic capacity. Measured values fell below predicted only in the flag leaves in the mid afternoon before the onset of grain-filling and over the whole diurnal course at the end of grain-filling. The loss of enhancement at this final stage was attributed to the earlier senescence of flag leaves in elevated *p*CO<sub>2</sub>. In contrast to some controlled-environment and field-enclosure studies, this field-scale study of wheat using free-air CO<sub>2</sub> enrichment found little evidence of acclimatory loss of photosynthetic capacity with growth in elevated *p*CO<sub>2</sub> and a significant and substantial increase in leaf photosynthesis throughout the life of the crop.

*Key-words:* acclimation; atmospheric change; climate change; elevated carbon dioxide; FACE; phenology; photosynthesis; Rubisco; stomatal conductance.

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## INTRODUCTION

The mean partial pressure of CO<sub>2</sub> at sea level is expected to rise from 35.5 Pa to around 55 Pa by about 2060 (modified IS92a, Schimmel *et al.* 1996). In the short term, an increase in *p*CO<sub>2</sub> stimulates net photosynthetic rate in wheat and other C<sub>3</sub> plants because the present atmospheric *p*CO<sub>2</sub> is insufficient to saturate Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) and because CO<sub>2</sub> inhibits the competing process of photorespiration (Bowes 1991; Stitt 1991). Therefore net photosynthesis is increased by elevated *p*CO<sub>2</sub>, regardless of whether Rubisco activity or regeneration of ribulose-1,5-bisphosphate (RubP) are limiting, and regardless of whether light is saturating or limiting (Drake *et al.* 1997). Increased carbon uptake resulting from this initial stimulation of photosynthesis by elevated *p*CO<sub>2</sub> will alter the balance of supply and capacity to use carbohydrates, with the result that non-structural carbohydrate concentrations invariably increase per unit leaf area (reviewed in Drake *et al.* 1997). Such accumulations of carbohydrate may cause a short-term decrease in photosynthetic rate via sequestration of cytosolic inorganic phosphate and a long-term decrease in photosynthetic capacity by repression of specific photosynthetic genes, notably those for Rubisco (Sharkey 1994; Stitt 1991; Webber, Nie & Long 1994). Wheat is currently the world's most important food crop (Rosenzweig & Parry 1994). Do such losses of photosynthetic capacity occur in wheat crops growing in elevated *p*CO<sub>2</sub>?

Studies using controlled environments and field enclosures, in particular open-top chambers, have produced conflicting results. Habash *et al.* (1995), using artificially lit controlled environments, observed up-regulation of photosynthetic capacity in a winter wheat, including increased Rubisco activity with growth in elevated *p*CO<sub>2</sub>. By contrast, Barnes, Ollerenshaw & Whitfield (1995), studying two spring and three winter wheats in artificially lit controlled environments, found an average 15% loss of photosynthetic capacity with growth at elevated *p*CO<sub>2</sub>, with the acclimation most marked in the winter wheats. Delgado *et al.* (1994), using naturally lit, large climate-tracking chambers, found no loss of leaf photosynthetic capacity or

Rubisco with a season-long exposure of winter wheat to a  $p\text{CO}_2$  of 70 Pa, regardless of nitrogen supply. McKee & Woodward (1994) and McKee, Farage & Long (1995), studying a dwarf spring wheat in artificially lit controlled environments, and Tuba, Szente & Koch (1994) and Sicher & Bunce (1997), both studying winter wheats in the field in open-top chambers, all with a good nitrogen supply, found marked acclimatory loss of photosynthetic capacity.

With one exception, measurements of photosynthesis in wheat grown in elevated  $p\text{CO}_2$  have been limited to plants grown in protected environments ranging from artificially lit cabinets to open-top chambers. Even within open-top chambers, the crop environment is modified by reduced exposure to wind, altered coupling of canopy and atmosphere, increased temperature and humidity, and decreased precipitation and photosynthetic photon-flux density, such that the long-term effects of enclosure can be similar in magnitude to effects of elevated  $p\text{CO}_2$  (Allen *et al.* 1992). Miglietta, Giuntoli & Bindi (1996) grew a super-dwarf winter wheat in 'mini-FACE' systems, each an isolated raised plot of 1 m<sup>2</sup>. Although the microclimate of such small plots would differ from that of large plots within a field, this system would have escaped the other limitations of enclosures. In their study there was no significant loss of photosynthetic capacity for well fertilized plants, which showed an exceptional 50% increase in leaf photosynthetic rates under elevated  $p\text{CO}_2$ . However, the study's statistical sensitivity was limited by the use of only two replicate plots per treatment, only one developmental stage was reported and only light-saturated photosynthesis was considered. It is difficult to extrapolate this result to the wider context because other field studies have shown that acclimation depends on the growth stage of wheat (Nie *et al.* 1995a; Sicher & Bunce 1997). Further, many previous studies have considered only light-saturated or mid-day photosynthesis. The enhancement of photosynthesis in elevated  $p\text{CO}_2$  apparent at noon may be absent in the late afternoon as end-product inhibition develops. A significant proportion of total carbon assimilation will occur under light-limited conditions, where different factors control the response of photosynthesis to elevated  $p\text{CO}_2$  (Drake, González-Meler & Long 1997). The question therefore remains of whether the findings from these modified environments or small plots will be reproduced at the field scale, and how the findings will vary with time of day and stage of development. Finally,  $p\text{CO}_2$  is expected to reach 55 Pa by 2060, and is not expected to double before 2100 (Schimel *et al.* 1996). Many previous studies have considered the effects of an approximate doubling of  $p\text{CO}_2$ . It is possible that evaluations at excessively high  $\text{CO}_2$  concentrations increase the likelihood of acclimation and exaggerate its significance in considering climate change effects on crops within the next century.

Free-air  $\text{CO}_2$ -enrichment (FACE) allows the study of elevated  $p\text{CO}_2$  effects on crops grown under field conditions without any direct modification of microclimate (Hendrey, Lewin & Nagy 1993). Large areas of undisturbed canopy are available where it is possible to avoid

edge and wall effects and other disturbances typical of the small canopies enclosed within controlled-environment and open-top chambers. Similarly, the technique avoids the border effects of the 1 m<sup>2</sup> mini-FACE systems. Each plot in the FACE wheat project covered  $\approx 350$  m<sup>2</sup> of crop and contained  $\approx 46\,000$  plants. This allowed sampling of different leaves throughout several days without significant effect on the canopy. It was also possible to study simultaneously within the crop a range of other variables, from canopy microclimate and soil moisture to leaf proteins and gene expression, which aided interpretation of any observed changes in leaf photosynthesis (reviewed in Kimball *et al.* 1995 and Pinter *et al.* 1996). Therefore, the FACE wheat project (Kimball *et al.* 1995) provided an unrivalled opportunity to examine the response of wheat to elevated  $p\text{CO}_2$  in an open-field situation, with good nutrition and without limitation on rooting volume. In the present study we evaluated whether the enhancement that would be expected for leaf photosynthesis by elevated  $p\text{CO}_2$ , in the absence of acclimation or inhibition, occurs in an open field. This was determined over the course of entire days and throughout the life of the crop. The results were compared with the photosynthetic rate predicted for the leaves in the absence of acclimation or inhibition.

## MATERIALS AND METHODS

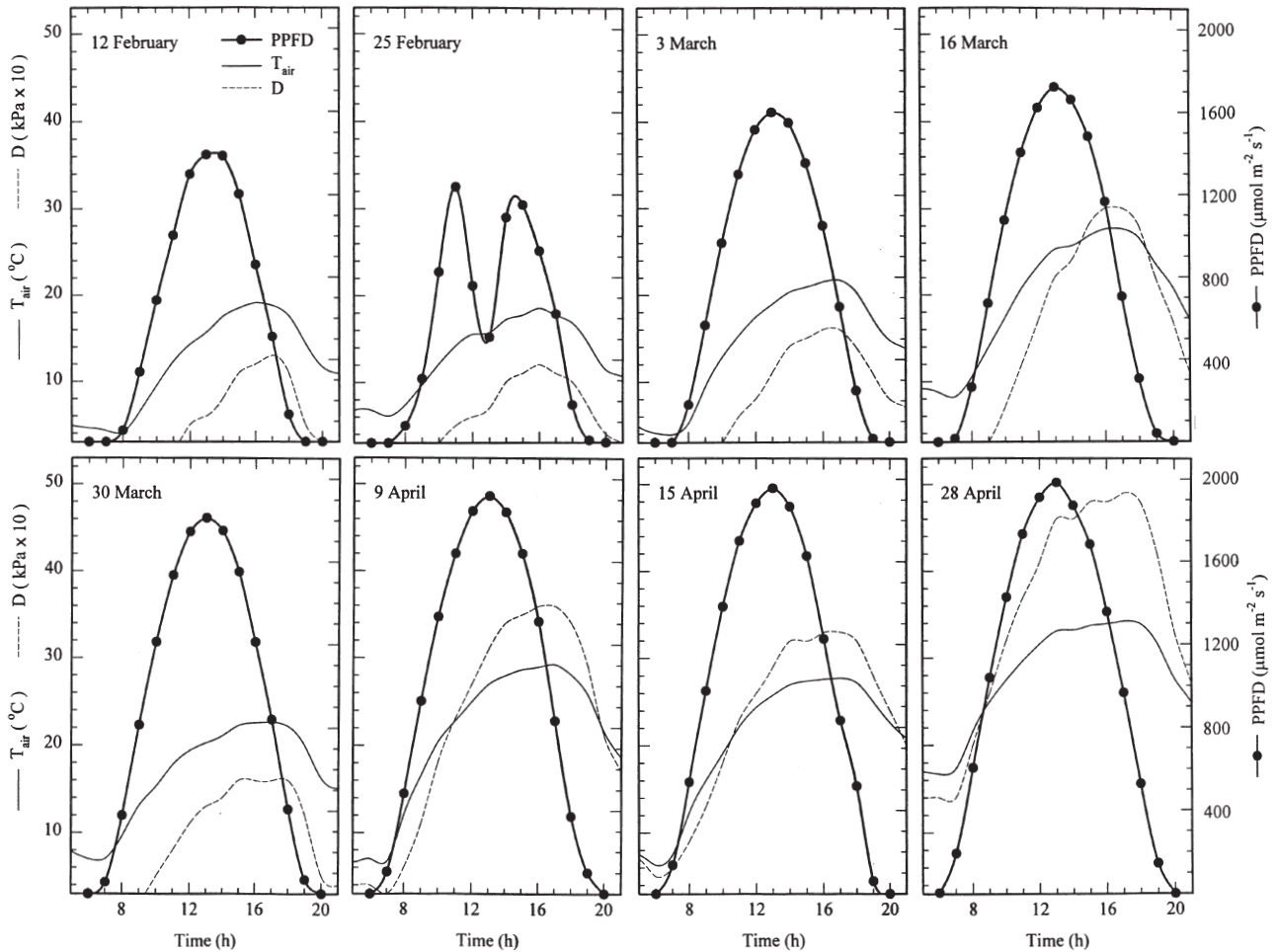
### The FACE system and cultivation

Spring wheat (*Triticum aestivum* L. cv. 'Yecora Rojo') was grown in a 10 ha field on the experimental farm of the Maricopa Agricultural Center, University of Arizona, Maricopa, AZ (33° 01' N, 112° 00' W). The crop emerged on 1 January 1993 (DAE 1) and the elevated  $p\text{CO}_2$  treatment was applied from this point until completion of grain maturation in May. Full details of the site, cultivation, irrigation, fertilization and the free-air  $\text{CO}_2$  enrichment (FACE) apparatus, site weather recording and soil moisture measurement have been given previously (Kimball *et al.* 1995; Nie *et al.* 1995a). The experimental design consisted of four replicate blocks each containing a 21 m diameter plot with elevated  $\text{CO}_2$  ( $p\text{CO}_2$  55 Pa) and a 21 m diameter control plot ( $p\text{CO}_2$  37 Pa).

The development stage and leaf area of the crop and the soil water conditions on the 8 d chosen for this study are summarized in Table 1. These represent a range of developmental stages from tillering through completion of grain filling. Leaf area index (L) rose from 2 in early February to about 5 by the end of the month and remained at 5–6 until late April (Pinter *et al.* 1996). Figure 1 shows the time course of photosynthetic photon flux density (PPFD), air temperature ( $T_{\text{air}}$ ) and vapour pressure deficit ( $D$ ) for Maricopa on the dates photosynthesis was measured. All days had predominantly clear skies, apart from 25 February. There was a seasonal rise in total PPFD and a progressive rise in daily maximum air temperatures from 20 °C at the start of the study to 35 °C at the end. Minimum temperatures remained in the range 4–8 °C until the final

Date	DAE	Developmental stage	Leaf area index		Soil water	
			FACE	Control	FACE	Control
12 February	42	Tillering	2.4	2.2	0.25	0.25
25 February	55	Stem elongation	5.2	4.6	0.23	0.23
3 March	62	Stem elongation	5.3	5.1	0.23	0.23
16 March	75	Inflorescence emergence	5.8	5.2	0.23	0.23
30 March	89	Anthesis	5.5	5.2	0.26	0.26
9 April	99	Milk ripe	5.7	6.0	0.22	0.22
15 April	105	Soft dough	5.6	6.0	0.25	0.25
28 April	118	Hard dough	4.3	5.1	0.25	0.25

**Table 1.** A summary of crop growth stage and the days after emergence (DAE); leaf area index and volumetric soil water content for 8 d of the 1993 spring wheat growing season at Maricopa, Arizona. FACE = elevated CO<sub>2</sub> partial pressure of ≈ 55 Pa; Control = current ambient CO<sub>2</sub> partial pressure of ≈ 37 Pa



**Figure 1.** Diurnal courses of air temperature ( $T_{air}$ ), air saturation deficit ( $D$ ), and photosynthetically active photon flux density (PPFD) at Maricopa, Arizona for 8 d of the 1993 growing season. These data, and the data in the other figures, were fitted with a best-fit non-linear regression (Marquardt–Levenberg algorithm). The actual data as well as the fitted curve are shown to better illustrate the daily progression.

measurement. Daily maximum air saturation deficits ranged from 1.2 kPa in February to nearly 5 kPa in late April. Soil water was maintained within 70% of field capacity. The crop received 277 kg nitrogen ha<sup>-1</sup> and 44 kg phosphorus ha<sup>-1</sup>, with other nutrients adjusted to avoid potential deficiencies (Kimball *et al.* 1995; Pinter *et al.* 1996).

**Leaf photosynthesis and conductance**

On the 8 d indicated in Table 1 and Fig. 1, leaf gas exchange was measured by two teams using portable closed (transient) gas-exchange systems with 250 cm<sup>3</sup> transparent chambers (LI-6200, LI-COR, Inc., Lincoln,

NE, USA). These measurements were repeated at intervals of  $\approx 90$  min from dawn until about 2 h after sunset. Each day before starting measurements, the infra-red gas analysers of the gas-exchange systems were calibrated against a gravimetrically prepared calibration mixture of  $\text{CO}_2$  in air ( $\pm 1\%$ , 'Primary standard', Matheson Gas Products, Inc., Cucamonga, CA, USA). The chamber humidity sensor was calibrated against known humidities provided by a dew-point controlled water vapour generator (LI-610, LI-COR, Inc.). Measurements were started at a leaf-chamber ambient  $p\text{CO}_2$  ( $p_a$ ) of  $55 \pm 3$  Pa in the elevated  $p\text{CO}_2$  plots and  $37 \pm 2$  Pa in the control plots. Measurements were confined to the uppermost fully expanded leaf of major tillers, as judged by ligule emergence and formation of a leaf collar. From 16 March, measurements were of flag leaves. The central portion of most leaves was approximately horizontal, and the leaf cuvette was clamped on this portion of the leaf and maintained in the horizontal position. The horizontal portion of the leaf was chosen to minimize within-leaf variation in photosynthetic capacity and the effect of leaf angle on incident photon flux. The rate of change in  $p\text{CO}_2$  was allowed to stabilize for  $\approx 25$  s before data-logging began, after which three 10 s observations were recorded. The total time for measurement of each leaf was about 75–100 s. In each 90 min interval, five leaves were measured in each of the four replicate elevated  $p\text{CO}_2$  and control plots. We calculated leaf net  $\text{CO}_2$  assimilation per unit area ( $A$ ), stomatal conductance to water vapour ( $g_s$ ) and  $\text{CO}_2$  partial pressure of the intercellular (substomatal) air space ( $p_i$ ) using the equations of von Caemmerer & Farquhar (1981).

The measurements from five leaves in each plot, in each 90 min interval, were pooled to obtain a single-plot measure and treated as a single replicate, to avoid pseudoreplication. These single-plot measures were used for all statistical analyses. The effects of plot  $p\text{CO}_2$  and days after emergence (DAE) on the midday rate of photosynthesis and on the dawn-to-dusk integral of photosynthesis (total  $\text{CO}_2$  uptake per unit leaf area,  $A'$ ) were examined by two-way analysis of variance (Sokal & Rohlf 1981). Individual means on a given date were compared for significance by the least significant difference (LSD) calculated from Student's  $t$  distribution (Snedecor & Cochran 1980).

### Predicting enhancement of photosynthesis by elevated $\text{CO}_2$

A mechanistic biochemical model of leaf photosynthesis linked to a phenomenological model for stomatal behaviour (Humphries & Long 1995) was used to analyse the observed effects of elevated  $p\text{CO}_2$  on photosynthesis. This model assumes a non-rectangular, hyperbolic response of electron transport to PPFD (Long & Drake 1992; Evans & Farquhar 1991) and uses the steady-state model of leaf photosynthesis of Farquhar *et al.* (1980) adapted for more recent data concerning temperature responses of the Rubisco-catalysed reactions (Lloyd *et al.* 1995). This biochemical model is linked to the phenomenological model

for  $g_s$  of Ball, Woodrow & Berry (1987), which assumes that  $g_s$  is a function of net assimilation, leaf surface  $\text{CO}_2$  concentration and relative humidity. The models are solved iteratively for  $p_i$  (Humphries & Long 1995). Variables for the leaf biochemistry and stomatal models were as given previously (Long & Drake 1992; Harley & Tenhunen 1991, respectively), except that the RubP- and  $\text{CO}_2$ -saturated catalytic activity of Rubisco ( $V_{c,\text{max}}$ ) was set to  $84 \mu\text{mol m}^{-2} \text{s}^{-1}$ , the maximum *in vivo* capacity whole-chain electron transport ( $J_{\text{max}}$ ) was set to  $190 \mu\text{mol m}^{-2} \text{s}^{-1}$ , and the Michaelis constants for Rubisco were those used by Lloyd *et al.* (1995). Values of  $V_{c,\text{max}}$  and  $J_{\text{max}}$  were estimated from  $A$  versus  $p_i$  data for flag leaves at inflorescence emergence from plants in the control plots (Osborne *et al.*, in press). This avoided the possibility that estimates of these parameters could be affected by transient photoinhibition, end-product inhibition, or water stress.

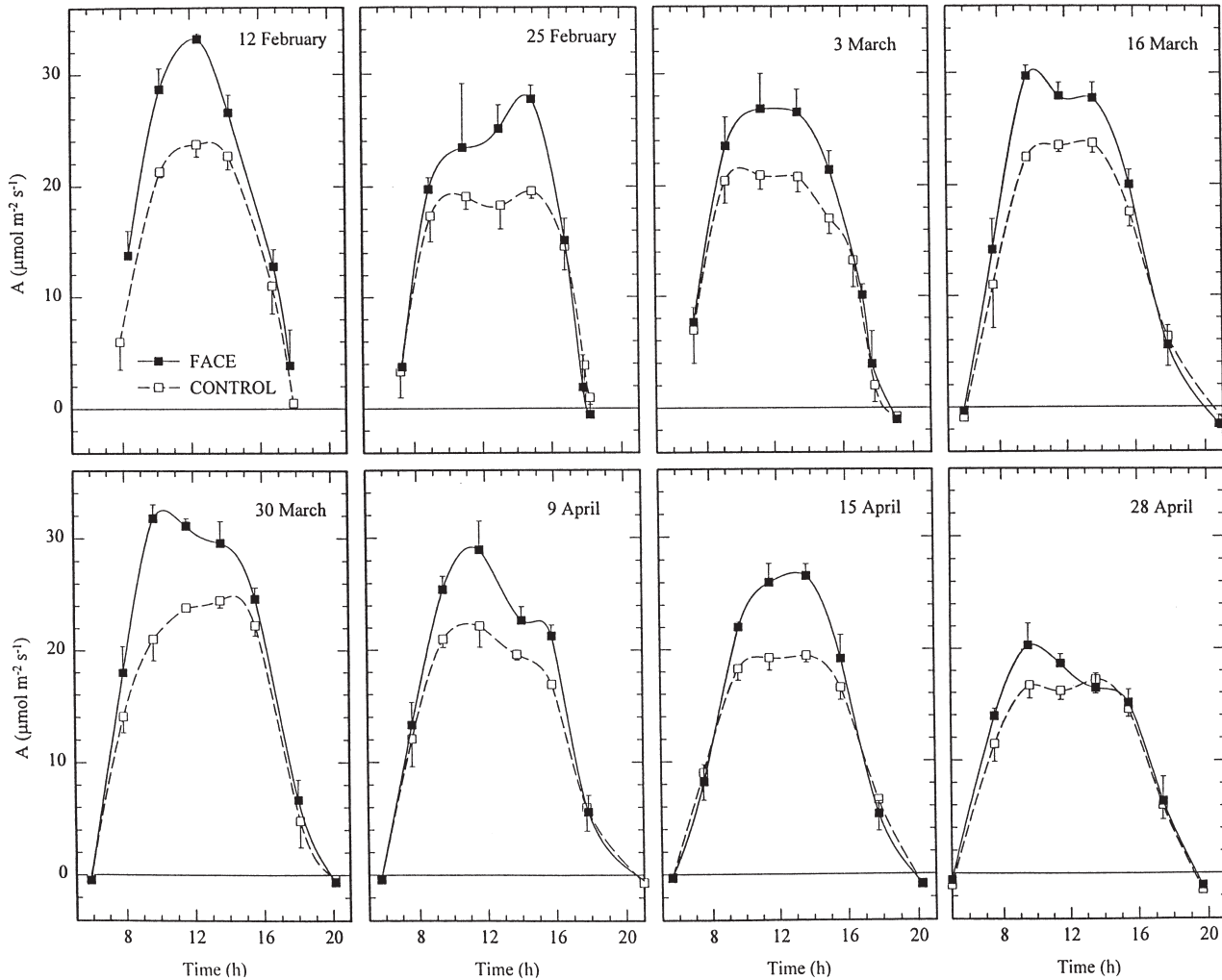
This model was used to estimate mean midday rates of  $A$  from the mean values of PPFD,  $T_{\text{air}}$ , relative humidity and  $p_a$  measured simultaneously by the gas-exchange systems. In addition, the model was used to predict the diurnal course of  $A$  for 16 March and 9 April 1993.

## RESULTS

Leaf net  $\text{CO}_2$  assimilation ( $A$ ) of plants grown in elevated  $p\text{CO}_2$  always appeared higher than that in plants grown in control plots (Fig. 2). However, this difference was least on the final day of measurement (28 April), when grain filling in the elevated  $p\text{CO}_2$  treatment was complete (Fig. 2). Except for 25 February, sky conditions were clear for all measurement days and diurnal courses of  $A$  (Fig. 2) reflect the general bell-shaped courses of PPFD (Fig. 1). Midday values of  $A$  ( $A_m$ ) within the control plots ranged from 17 to  $24 \mu\text{mol m}^{-2} \text{s}^{-1}$  and, with the exception of 28 April, ranged from 25 to  $33 \mu\text{mol m}^{-2} \text{s}^{-1}$  for the elevated  $p\text{CO}_2$  plots (Tables 2 & 3). The mean daily PPFD,  $T_{\text{air}}$  and  $D$  rose progressively through the season (Fig. 1). Despite this, the peaks in leaf photosynthetic rates within treatments showed little change with date, except for the final measurement (Fig. 2).

Averaged over all days,  $A_m$  was enhanced 28% and  $A'$  was enhanced 21% in the elevated  $p\text{CO}_2$  plots (Tables 2 & 3, Fig. 3). These increases were highly significant ( $P < 0.001$ ). There was however, a significant interaction ( $P < 0.05$ ) with the date of sampling for  $A_m$  that could be explained by the loss of enhancement resulting from elevated  $p\text{CO}_2$  on 28 April (Table 3). On four dates (16 and 30 March, 9 and 28 April) the relative enhancement of leaf photosynthesis in the elevated  $p\text{CO}_2$  plots appeared to decline after midday. Parallel declines in stomatal conductance ( $g_s$ ) were apparent on these dates (Fig. 4).

Throughout,  $g_s$  was lower in the leaves grown in elevated  $p\text{CO}_2$  (Fig. 4). Dew formation prevented measurement of  $g_s$  until midmorning during February and March. Despite progressive increases in PPFD,  $T_{\text{air}}$  and  $D$  (Fig. 1), peak leaf  $g_s$  showed little change within treatments across



**Figure 2.** Diurnal courses of leaf net photosynthetic CO<sub>2</sub> uptake ( $A$ ) in spring wheat grown in elevated CO<sub>2</sub> partial pressure (FACE,  $\approx 55$  Pa; ■) and current ambient partial pressure of CO<sub>2</sub> (control  $\approx 37$  Pa; □), on the 8 d for which weather data are given in Fig. 1. Each illustrated point is the mean ( $\pm 1$  SD) of the replicate plots measured at that point in time ( $n = 4$ ). Each of the four individual plot values was the pooled average of five leaves sampled within the plot at that point in time. The eight diurnal courses illustrated are therefore from measurements of 2480 leaves over the course of the season.

the season. Midday values of conductance ranged from 0.47 to 0.68 mol m<sup>-2</sup> s<sup>-1</sup> for control leaves and from 0.31 to 0.51 mol m<sup>-2</sup> s<sup>-1</sup> for leaves grown in elevated  $p$ CO<sub>2</sub> (Fig. 4). For this well watered crop, the results suggest that  $g_s$  over the season is independent of  $D$ , which increased three-fold from a daily maximum value of 1.3 kPa on 12 February to 4.9 kPa on 28 April (Table 3). On average, the midday  $g_s$  in elevated  $p$ CO<sub>2</sub> was 64% of control  $g_s$ , although the difference in the early afternoon was often larger (Fig. 4). Despite these marked reductions in  $g_s$ , there was only a slight decrease of  $p_i/p_a$  ( $< 6\%$ ) in the elevated  $p$ CO<sub>2</sub> plots and this was not significant ( $P > 0.05$ ). The average  $p_i$  (data not shown) in leaves growing in elevated  $p$ CO<sub>2</sub> was 37% greater than in controls.

Averages of PPFD,  $T_{air}$  and  $D$  recorded in the gas-exchange systems were used with the mathematical model of Humphries & Long (1995) to analyse the

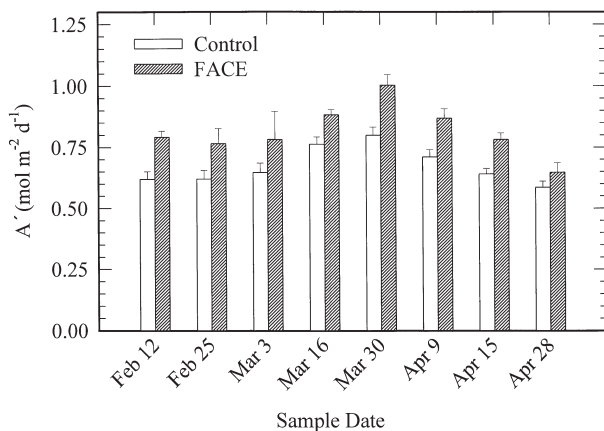
changes in  $A$  across the season and identify any acclimation affecting  $A$  *in situ*. The model provided a close prediction of the observed enhancement of midday  $A$  in elevated  $p$ CO<sub>2</sub> on all dates, except 16 March and 28 April when the predicted enhancement was substantially greater than the observed (Table 3). Model predictions were also compared with observed  $A$  throughout the diurnal courses of 16 March and 9 April (Fig. 5). On average, observed values were about 10% lower than model predictions in elevated  $p$ CO<sub>2</sub> plots. These lower-than-expected values were attributable to significant decreases in the observed, relative to expected, values in mid to late afternoon; at other times of the day there was close agreement between expectation and observation. The diurnal course of  $A$  plotted against PPFD for 16 March and 9 April shows hysteresis, which is more marked in the elevated  $p$ CO<sub>2</sub> treatment (Fig. 6).

Source of variation	SS	d.f.	MS	F(P)
Midday leaf CO <sub>2</sub> uptake rate ( $A_m$ )				
CO <sub>2</sub> treatment	550	1	550	63.0 ( $P < 0.001$ )
Date	718	7	103	11.7 ( $P < 0.001$ )
Interaction	152	7	22	2.5 ( $P < 0.05$ )
Within	419	48	9	
Total	1839	63		
Total daytime net leaf CO <sub>2</sub> uptake rate ( $A'$ )				
CO <sub>2</sub> treatment	0.323	1	0.323	100.3 ( $P < 0.001$ )
Date	0.447	7	0.064	19.8 ( $P < 0.001$ )
Interaction	0.024	7	0.003	1.1 ( $P > 0.05$ )
Within	0.155	48	0.003	
Total	0.948	63		

**Table 2.** Results of the analysis of variance examining the effects of growth  $p\text{CO}_2$  and date of measurement on pooled midday average leaf CO<sub>2</sub> uptake ( $A_m$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; LSD between means within each date: 4.9) and pooled daily total of net leaf CO<sub>2</sub> uptake ( $A'$ ,  $\text{mol m}^{-2} \text{d}^{-1}$ ; LSD between means within each date: 0.095)

**Table 3.** A summary of daily integral of solar radiation, maximum and minimum air temperatures ( $T_{\text{air}}$ ), maximum air saturation deficit ( $D$ ),  $A_m$ , and  $A'$  for each treatment for 8 d of the 1993 spring wheat growing season at Maricopa, Arizona. Model predicted values for  $A_m$  are included in parentheses. Significant differences between pairs of experimental means on the same date are assessed by the LSD at  $P = 0.05$  of the  $p\text{CO}_2$  effect and indicated by 'a'

Date	Solar rad'n ( $\text{MJ m}^{-2}$ )	$T_{\text{air}}$ max. ( $^{\circ}\text{C}$ )	$T_{\text{air}}$ min. ( $^{\circ}\text{C}$ )	$D$ max. (kPa)	Observed $A_m$ (predicted $A_m$ ) a, $P < 0.05$		$A'$ a, $P < 0.05$	
					Control	Elevated	Control	Elevated
12 February	16.0	20	4	1.3	24(26)	33 (34)a	0.62	0.79a
25 February	13.9	19	6	1.2	18(22)	25 (28)a	0.62	0.76a
3 March	19.8	22	4	1.6	21(25)	27 (31)a	0.65	0.78a
16 March	21.9	28	8	3.0	24(22)	28 (30)	0.76	0.88a
30 March	24.1	23	7	1.6	25(25)	30 (31)a	0.80	1.00a
9 April	25.8	30	6	3.6	20(21)	29 (30)a	0.71	0.87a
15 April	26.5	28	5	3.3	19(19)	27 (28)a	0.64	0.78a
28 April	27.8	35	17	4.9	17(15)	16 (25)	0.59	0.65
% increase over control (all dates)						28.1 (35.4)		20.8
% increase over control (12 February–15 April)						32.2 (31.8)		22.1

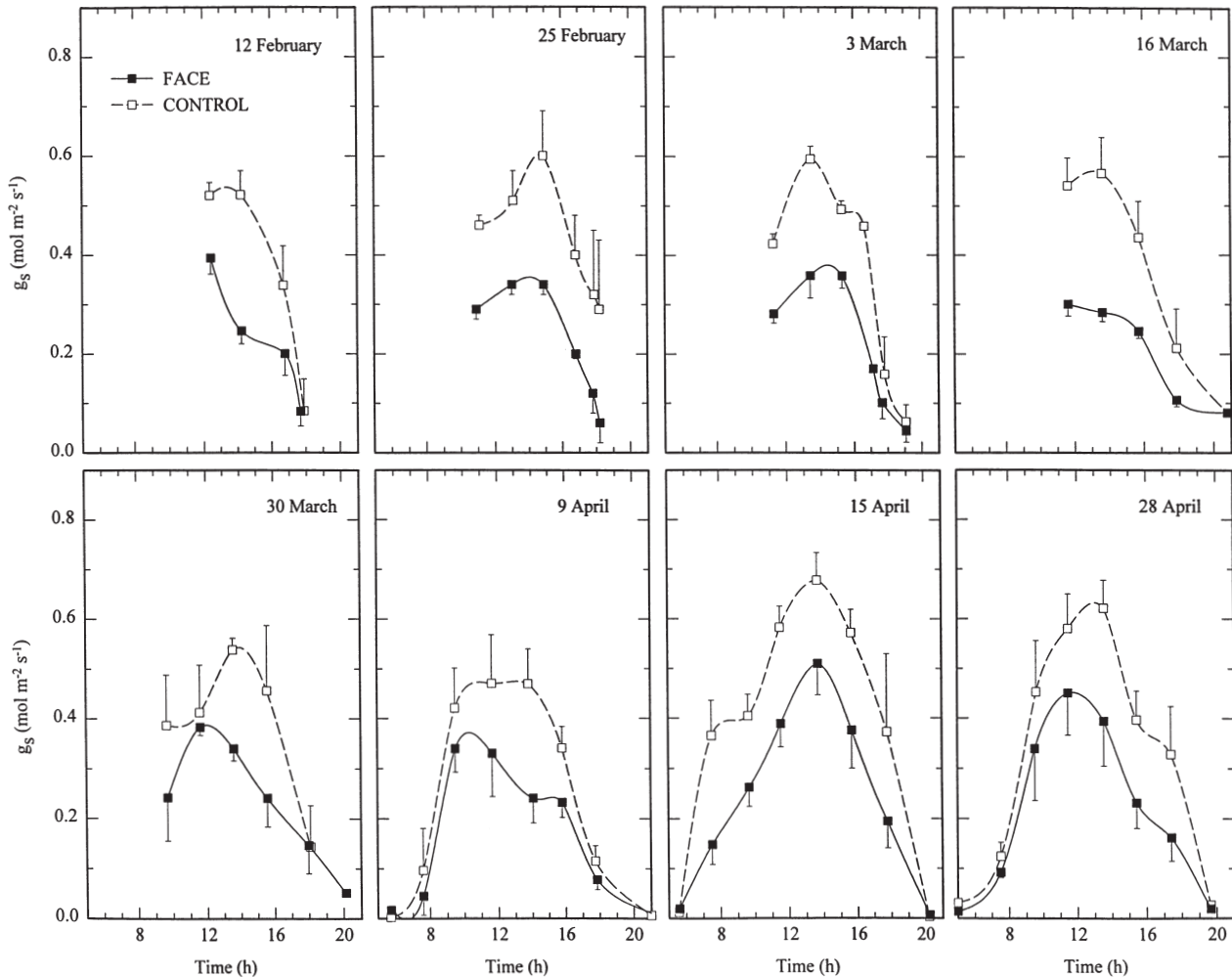


**Figure 3.** Total net uptake of CO<sub>2</sub> per unit leaf area ( $A'$ ) in each plot for the daylight hours estimated from the 8 d of data plotted in Fig. 2; bars indicate mean values (+ 1 SD;  $n = 4$ ) of the FACE and control treatments. These values are equal to the areas under the curves illustrated in Fig. 2. FACE = elevated CO<sub>2</sub> partial pressure of  $\approx 55$  Pa; control = current ambient CO<sub>2</sub> partial pressure of  $\approx 37$  Pa.

## DISCUSSION

The results of the present study show that the stimulation of photosynthesis in upper canopy leaves of a wheat crop grown under open-air CO<sub>2</sub> enrichment persists until completion of grain filling. In contrast to the weight of evidence derived from controlled environment and field enclosure studies, there is little indication of any decrease in the response of photosynthesis to elevated  $p\text{CO}_2$  across the 37–55 Pa range. For the period from tillering through soft dough (12 February–15 April) the predicted increase in midday assimilation at elevated  $p\text{CO}_2$  was 32% and almost identical to the observed average increase (Table 3).

Predictions assumed no difference or change in the maximum rates of electron transport ( $J_{\text{max}}$ ) or Rubisco-catalysed carboxylation ( $V_{\text{c,max}}$ ) between elevated  $p\text{CO}_2$  grown plants and controls, that is, no acclimation. Close agreement is not surprising for the control plants, given that the parameters of the model were set with this same plant material. However, the model also shows a good fit to the

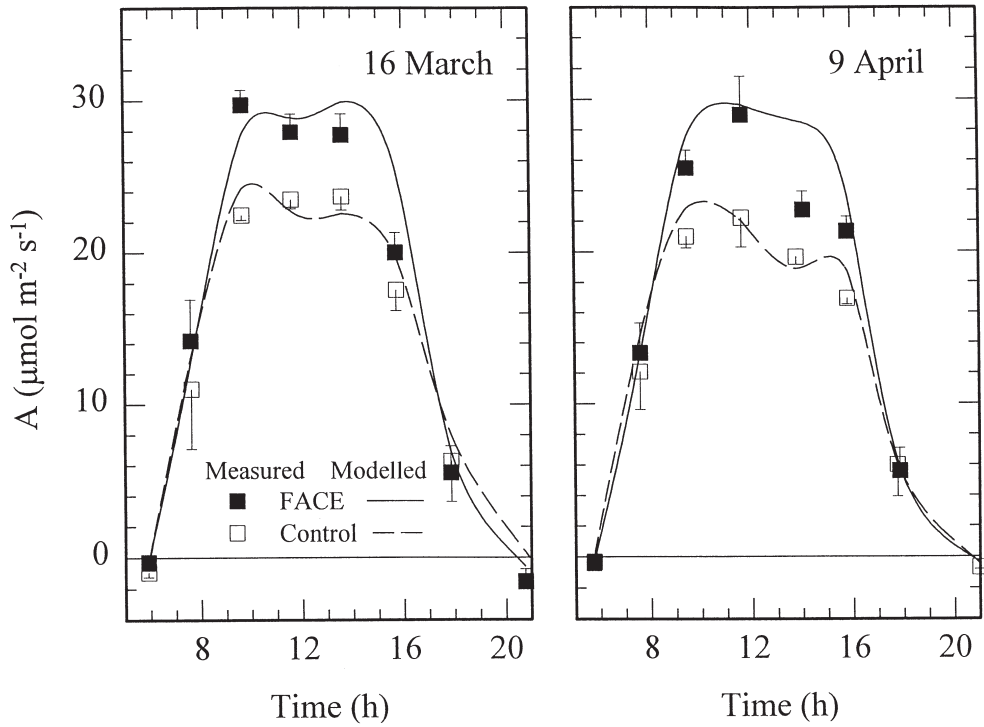


**Figure 4.** Diurnal courses of mean ( $\pm 1$  SD,  $n = 4$ ) leaf stomatal conductance to water vapour ( $g_s$ ) grown under elevated (FACE; ■) and control (□) CO<sub>2</sub> partial pressures. Measurements were simultaneous with, and sampled as described for, *A* in Fig. 2.

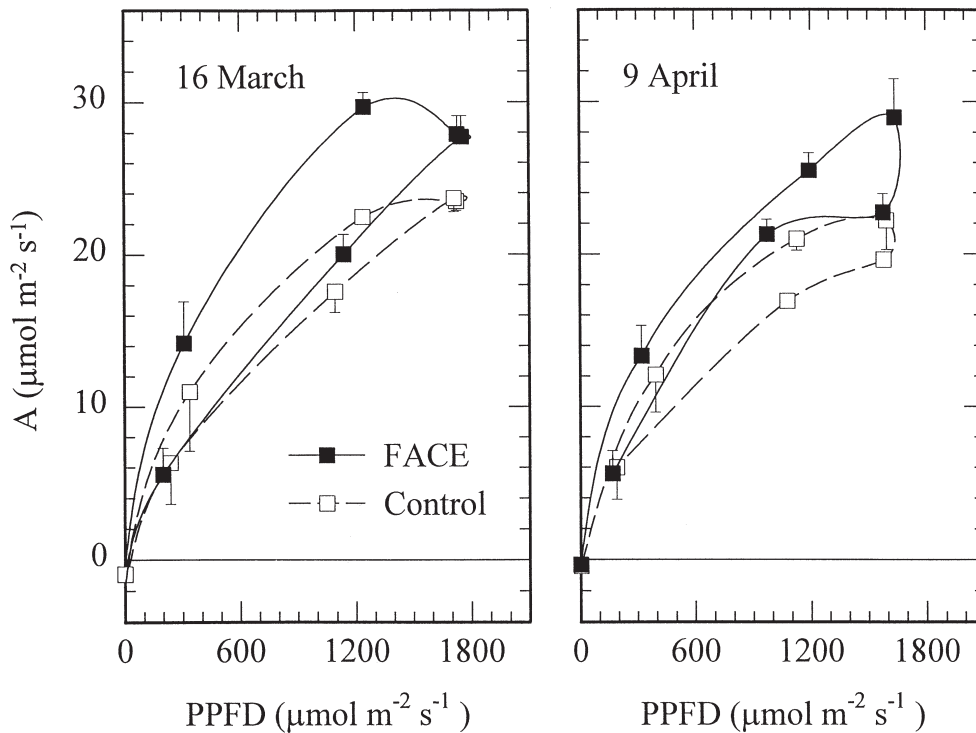
elevated  $p\text{CO}_2$  leaves. This suggests that the small, but significant, loss of Rubisco observed between anthesis and soft-dough stage in the leaves grown in elevated  $p\text{CO}_2$  (Nie *et al.* 1995a) did not affect CO<sub>2</sub> assimilation rates. This might be explained by the relatively high  $V_{c,\text{max}}$  that might place Rubisco in significant excess at elevated  $p\text{CO}_2$  (Drake *et al.* 1997). Thus a significant loss of Rubisco could occur before *A* was affected. However, the large loss of Rubisco induced by elevated  $p\text{CO}_2$  and smaller losses of other photosynthetic proteins observed after the soft-dough stage (Nie *et al.* 1995a) corresponded to the lower-than-expected photosynthetic rate and evidence of acclimation of 28 April. There was no evidence of any change in the magnitude of the decrease in stomatal conductance in elevated  $p\text{CO}_2$  relative to controls with time (Fig. 4). The season-long stimulation of photosynthesis in the upper canopy leaves, which are the major contributors to total crop photosynthesis and grain yield, is fully consistent with observed increases in dry-matter production and grain yield (Pinter *et al.* 1996). By the

stem-elongation stage (Table 1), root mass was 23% greater and shoot mass was 21% greater in elevated  $p\text{CO}_2$  (Wechsung *et al.* 1995). Final grain yield was 8% higher ( $P < 0.05$ ) (Pinter *et al.* 1996).

The findings agree with and extend those of Miglietta *et al.* (1996), which suggest that under free-air CO<sub>2</sub> enrichment and optimum nitrogen fertilization, the stimulation of photosynthesis by elevated  $p\text{CO}_2$  persists without any evidence of acclimation. This contrasts sharply with the results of other studies of wheat. Although differences from the results of controlled-environment studies might be explained by limitations on rooting volume and lighting, it is hard to explain differences from the findings of studies using open-top chambers in similar temperature regimens, where marked acclimation of wheat photosynthesis has been observed (Tuba *et al.* 1994; Sicher & Bunce 1997). In these two studies winter wheat cultivars were used. Barnes *et al.* (1995) found greater acclimation of photosynthesis to elevated  $p\text{CO}_2$  in winter wheats. The increase in light saturated *A*, as indicated by  $A_m$ , in our



**Figure 5.** The diurnal course of the mean ( $\pm 1$  SD) measured leaf  $\text{CO}_2$  uptake (symbols) and the predicted leaf  $\text{CO}_2$  uptake (lines) for 16 March and 9 April. Predicted rates were calculated from a mechanistic model of photosynthesis in which parameters were set with material from the control plots, using as inputs the record of photon flux, leaf temperature, air saturation deficit (Fig. 1) and ambient  $\text{CO}_2$  partial pressure recorded simultaneously with the measurements of  $A$  and  $g_s$ . FACE = elevated  $\text{CO}_2$  partial pressure of  $\approx 55$  Pa; control = current ambient  $\text{CO}_2$  partial pressure of  $\approx 37$  Pa.



**Figure 6.** The progression of mean net leaf photosynthesis ( $A$ ) in response to the diurnal course of incident photon flux density (PPFD) for 16 March and 9 April. Replotted from Fig. 2.

study in elevated  $p\text{CO}_2$  (Table 3) was considerably greater than in others (cf. Delgado *et al.* 1994; Tuba *et al.* 1994; Sicher & Bunce 1997). Elevated  $p\text{CO}_2$  increased  $A_m$  by 26%, averaged across the 5 d of measurements of flag leaves (calculated from Table 2). Sicher & Bunce (1997) similarly report the increase in light-saturated  $A$  over 5 d spread through the life of the flag leaf, but found only a 12% increase for plants grown at a  $p\text{CO}_2$  of 53 Pa, and close to the  $p\text{CO}_2$  of our experiment. In common with the present experiment, increase in  $A$  attributable to elevated  $p\text{CO}_2$  was lost in the final phase of flag-leaf photosynthesis. By contrast Miglietta *et al.* (1996) reported a 50% increase in light-saturated  $A$  for plants grown at  $p\text{CO}_2$  60 Pa, an even larger increase than seen in our study, even though  $p\text{CO}_2$  and the measurement temperatures were similar. As all these studies were conducted with a good supply of nutrients and irrigation, differences must result from climate prehistory, subtle differences in soil or cultivar or possibly the method of  $p\text{CO}_2$  elevation. While cultivar differences in photosynthetic response have been reported within a study, these appear small (Barnes *et al.* 1995). It is of interest that the largest increases in leaf photosynthesis for growth at  $p\text{CO}_2$  50–60 Pa come from the two FACE experiments.

The decrease of about 36% in midday  $g_s$  of the elevated  $p\text{CO}_2$  plants (Fig. 4) over the season corresponded within the same crop with significant decreases in canopy transpiration (Kimball *et al.* 1995), rates of soil water extraction (Hunsacker *et al.* 1996) and stem water flow (Senock *et al.* 1996) and an increase in daytime canopy surface temperature (Kimball *et al.* 1995). The persistent decrease in  $g_s$  occurred despite any significant change in stomatal numbers per unit leaf area (Estiarte *et al.* 1994). The increase in leaf assimilation and decrease in  $g_s$  is also consistent with about a 33% increase in water use efficiency, as defined by the final crop mass per unit mass of water use (Kimball *et al.* 1995; Hunsaker *et al.* 1996).

In contrast to all other dates, on 28 April there was no significant elevated  $p\text{CO}_2$  enhancement of  $A'$  (Table 3). However, the crop under elevated  $p\text{CO}_2$  showed more rapid phenological development. Using well defined phenological events, the crop under elevated  $p\text{CO}_2$  reached the stages of stem elongation  $\approx$  1.5 d, anthesis  $\approx$  2.5 d and completion of grain filling  $\approx$  7 d before controls (Kimball *et al.* 1995). Thus, on 28 April the crop in elevated  $p\text{CO}_2$  was about 7 d more advanced than the control crop, and the grains had reached 83% of their final weight compared with 64% in the controls. Therefore, the lower-than-expected rate of leaf assimilation (Table 3) might be attributable to earlier senescence in elevated  $p\text{CO}_2$ . This is consistent with the observation of marked decreases in steady-state mRNA transcript levels and proteins of the photosynthetic membrane in the leaves grown in elevated  $p\text{CO}_2$  relative to controls at this stage (Nie *et al.* 1995a,b). Average night-time temperatures were slightly higher ( $\approx$  0.5 °C) in the elevated  $p\text{CO}_2$  plots. Subsequent analysis in 1995–97, in which blowers were added to the control rings, suggests that this night-time temperature increase may be in part an artifact of the  $p\text{CO}_2$

elevation method (Kimball, unpublished results). It is possible that the air blown into the plots with the blower system, although at a minute fraction of total wind-speed, was sufficient to disrupt temperature inversions that formed on some evenings. Such an increase in night-time temperature would induce acceleration of development and decrease the time available for grain fill, which would most likely result in decreased final yields. As final yields in the elevated  $p\text{CO}_2$  plots were significantly higher than yields in the control plots it seems unlikely that this possible night-time temperature artifact could affect our conclusions. It should also be noted that the daytime increase in temperature in the elevated  $p\text{CO}_2$  plots persisted even after adding the blowers in the control rings (Kimball, unpublished results). These daytime increases in temperature may be attributed to reductions in canopy transpiration and in loss of latent heat (Kimball *et al.* 1995).

The enhancement of midday carbon assimilation in elevated  $p\text{CO}_2$  on 16 March was also less than predicted (Table 2), suggesting potential inhibition of photosynthesis. This decrease occurred when tillering had ceased but before anthesis. It was therefore possible that the crop had become partially sink-limited for this brief stage in its development. On both 16 March and 9 April, declines in photosynthesis were observed in the mid-afternoon in elevated  $p\text{CO}_2$ ; these declines were below the model predicted rates of photosynthesis for the PPFD,  $T_{\text{air}}$ ,  $C_a$  and  $D$  measured at the time (Fig. 5 & Fig. 6). This again suggests that there may be a transient partial inhibition of photosynthesis in elevated  $p\text{CO}_2$ . At this time of day, accumulation of carbohydrates would be greatest. At dawn on 16 March, in leaves in both treatments, the total non-structural carbohydrate (TNC) content was 60 mg g<sup>-1</sup>. By 1600 h on 16 March, measured leaf photosynthesis was 22% lower than predicted for elevated  $p\text{CO}_2$ ; this corresponded to an accumulation of TNC per unit of leaf dry mass of 266 mg g<sup>-1</sup> compared with 192 mg g<sup>-1</sup> in the controls, that is, a 39% difference (Nie *et al.* 1995b). This greater accumulation of TNC in elevated  $p\text{CO}_2$  could result from sequestration of cytosolic inorganic phosphate by sugar phosphates within the metabolic pathway leading to sucrose synthesis, or decreased activation of Rubisco leading to a temporary inhibition of photosynthesis (Sharkey, Socias & Loreto 1994). Although the bulk of the TNC accumulated was fructans, elevated  $p\text{CO}_2$  resulted in a rapid rise in starch contents in the afternoon to 28 mg g<sup>-1</sup> versus 12 mg g<sup>-1</sup> in controls, indicative of a limitation on export of carbohydrate from the chloroplast (Nie *et al.* 1995b). After anthesis, flag-leaf TNC contents were less than 20% of those before anthesis. Overall, the close agreement between predicted and observed increases in leaf photosynthesis suggest that elevated  $p\text{CO}_2$  rarely induced decreases in photosynthesis.

In attempting to predict the future production of wheat, with an increase in global atmospheric CO<sub>2</sub> concentration to 55 Pa, Rosenzweig & Parry (1994) assumed a 17% increase in light-saturated photosynthesis compared with the 28% increase observed in the present study for about the same increase in  $p\text{CO}_2$ . This first open-field study of

the photosynthesis of wheat under a  $p\text{CO}_2$  elevated to  $55 \mu\text{mol mol}^{-1}$  suggests a much greater increase when the changes in photosynthesis are assessed in the absence of possible artifacts imposed by controlled environments or by field enclosures. The average climate of southern Arizona is much warmer than that of the major belts of wheat production in North America, Western Europe and the former Soviet Union. However, the earlier sowing results in exposure of the crop to a similar range of temperatures and photon fluxes. Thus, the increases observed here have global relevance.

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