Elevated CO_2, drought and soil nitrogen effects on wheat grain quality

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Received: *8 August 2000*
Accepted: *16 November 2000*

Summary

- The likely consequences of future high levels of atmospheric CO_2 concentration on wheat (*Triticum aestivum* L.) grain nutritional and baking quality were determined.
- Two free-air CO_2 enrichment (FACE; 550 mmol mol^{-1}) experiments were conducted at ample (Wet) and limiting (Dry) levels of irrigation, and a further two experiments at ample (High-N) and limiting (Low-N) nitrogen concentrations. Harvested grain samples were subjected to a battery of nutritional and bread-making quality tests.
- The Dry treatment improved grain quality slightly (protein +2%; bread loaf volume +3%). By contrast, Low-N decreased quality drastically (protein –36%; loaf volume –26%). At ample water and N, FACE decreased quality slightly (protein –5%; loaf volume –2%) in the irrigation experiments and there was no change in the nitrogen experiments. At Low-N, FACE tended to make the deleterious effects of Low-N worse (protein –33% and –39%, at ambient CO_2 and FACE, respectively; loaf volume –22% and –29% at ambient CO_2 and FACE, respectively).
- The data suggest that future elevated CO_2 concentrations will exacerbate the deleterious effects of low soil nitrogen on grain quality, but with ample nitrogen fertilizer, the effects will be minor.

Key words: *Triticum aestivum* (wheat) grain, quality, nitrogen, Free air CO_2 enrichment (FACE), drought, water-stress, global change, protein.

Introduction

The CO_2 concentration of the atmosphere is increasing, and climate modelers have predicted a consequent global warming as well as changes in precipitation patterns. The report of the IPCC (Intergovernmental Panel on Climate Change, 1996) projects CO_2 increasing from present day concentrations of about 370 µmol mol^{-1} to about 500 µmol mol^{-1} by the end of this century if emissions are maintained at 1994 levels. They further project that the increase in CO_2 plus that of other radiatively active 'greenhouse' gases – methane, nitrous oxide, chlorofluorocarbons (CFCs), ozone – will cause an increase in global mean temperature of 0.9–3.5°C depending on future emission rates. Some regions might receive increases in precipitation, while others might receive less.

However, such elevated concentrations of CO_2 are also likely to stimulate photosynthesis and the growth of plants, including the yield of agricultural crops (Kimball, 1983, 1986). The stimulation of photosynthesis leads to greater production of carbohydrates, which can accumulate in the leaves (Hendrix, 1992) and possibly other organs. The higher concentration of carbohydrates, relative to that of proteins, suggests that the nutritional value of plants to animals and other organisms at other trophic levels would be decreased. Leaf-eating insects (and other herbivores) are likely to be affected (Akey & Kimball, 1989; Lincoln, 1993; Hesman, 2000), whereas seed-eating animals may not be (Akey *et al.*, 1988) if the quality of other organs is unaffected by elevated CO_2. Therefore, it is very important to ascertain whether or how much the quality of agricultural crops and of other plants – but especially of wheat

(*Triticum aestivum* L.) grain, the world's foremost food and feed crop – will be affected by higher atmospheric CO_2 concentrations in the future.

Of course, to humans, the bread-making quality of future wheat grain is also important, in addition to its nutritional value. Whereas soft wheat flour with lower protein concentration is used to make such foods as cookies, growers usually receive higher prices for hard wheat grain with higher protein concentrations and good ratios of gliadin and glutenin components, traits fundamental to making high-quality leavened breads (Stone & Savin, 1999).

The effects of environmental variables other than CO_2 on wheat grain protein concentration were studied intensively two decades ago. For example, Benzian & Lane (1979, 1981, 1986) assembled data from many experiments in England. For spring wheat, such as studied herein, the protein concentrations ranged from *c.* 9–16% with the optimum associated with maximum yield from *c.* 12–15%. About 75% of their values were above the minimum protein concentration for bread making (10% d. wt basis or 11.6% at 14% moisture). The percentages varied with variety, soil type, prior cropping history (especially legume vs nonlegume), and N fertilizer amount, as well as N application timing. However, below the optimum rate, the protein concentration was most sensitive to the N application rate, and they determined that about 56 kg ha^{-1} of applied N corresponded to an increase in grain protein concentration of 1% (absolute). They also examined weather factors, finding that a 1°C rise in temperature was associated with a 0.4% absolute increase in grain protein concentration, but they did not detect any effect of moisture stress under their English conditions. On the other hand, Barber & Jessop (1987) in Australia and Garrot *et al.* (1994) in Arizona studied irrigated wheat, and they both found that a water stress regime that reduced yields to two-thirds tended to increase grain protein slightly.

Then, within the past decade, several reports have appeared about the effects of elevated CO_2 concentration on wheat grain quality from various chamber-based CO_2 enrichment experiments, as reviewed recently by Lawlor & Mitchell (2000). Many of the experiments reported in the literature found little or no change in wheat grain nutritional or bread-making quality due to elevated levels of CO_2 (Havelka *et al.*, 1984; Williams *et al.*, 1995; Tester *et al.*, 1995; Rogers *et al.*, 1998; Reuveni & Bugbee, 1997; Hakala, 1998 (at higher temperature); Dijkstra *et al.*, 1999). On the other hand, a comparable number of such experiments have found decreases in the protein concentration, which reduced nutritional value and adversely affected baking quality (Conroy *et al.*, 1994 (–1 to –14% over a range of CO_2 and N levels); Thompson & Woodward, 1994 (–28% in monoculture and zero to –20% in competition with weeds); Manderscheid *et al.*, 1995 (–29%); Blumenthal *et al.*, 1996 (–14%); Monje & Bugbee, 1998 (–9%); Hakala, 1998 (–7% at ambient temperature). Therefore, whether or not elevated CO_2 concentration will affect wheat grain quality in the future is an unresolved question.

All of the CO_2 enrichment experiments cited above utilized chambers of various designs to confine the CO_2 around the plants. However, the environment inside chambers is usually not the same as outside, and plants generally grow differently inside (Kimball *et al.*, 1997). Therefore, we can not be confident the chamber-based data reliably represent what the responses of field-grown plants will be. About a decade ago, however, technology for free-air CO_2 enrichment (FACE) was developed (Hendrey, 1993). Although slight changes in the microclimate have been detected at night (Pinter *et al.*, 2000), the FACE technique, which eliminates walls, enables the growing of plants at elevated levels of CO_2 under conditions as representative of future fields as is possible today.

During the 1992–3 and 1993–4 growing seasons, wheat crops were grown using FACE at ample and limiting levels of soil water supply, and similar FACE experiments were conducted during 1995–6 and 1996–7 at ample and limiting levels of soil nitrogen. The wheat grain from these four experiments was sampled and subjected to a battery of tests to determine both nutritional and baking quality. The purpose of this paper is to examine the results from these many quality tests on wheat grain from robust field experiments in order to further elucidate how grain quality is likely to change due to the increasing atmospheric CO_2 concentration.

Materials and Methods

Two experiments were conducted during the 1992–3 and 1993–4 growing seasons to determine the interactive effects of elevated atmospheric CO_2 concentration and limited soil water supply on spring wheat (*Triticum aestivum* L. cv. Yecora Rojo) at the University of Arizona Maricopa Agricultural Center (MAC), Maricopa, Arizona, USA. Two additional experiments were similarly conducted to determine the interactive effects of elevated CO_2 and limited soil nitrogen during the 1995–6 and 1996–7 growing seasons. A field plot plan for the 1992–3 and 1993–4 experiments is presented by Wall & Kimball (1993), and for the 1995–6 and 1996–7 experiments by Kimball *et al.* (1999). Additional details about the methodology are presented by Kimball *et al.* (1995), Hunsaker *et al.* (1996), and Pinter *et al.* (1996, 2000).

CO_2 treatments

Briefly, elevated CO_2 levels were maintained 24 h d^{-1} all season long in four replicate 25-m-diameter plots using free-air CO_2 enrichment (FACE) technology (Hendrey, 1993). During the first two FACE × H_2O experiments, a constant set-point concentration of 550 µmol mol^{-1} CO_2 was used, whereas for the later FACE × N experiments, a constant elevation of 200 µmol mol^{-1} above ambient was imposed. There also were four plots at ambient CO_2 concentration in each experiment. For the two FACE × H_2O experiments these 'Ambient' plots had similar piping to that of the FACE plots, but there were no blowers installed. As a consequence, these Ambient plots with no forced air movement were 0.6–1.0°C cooler at night than the FACE plots (Pinter *et al.*, 2000). For the later FACE × N experiments, blowers were installed, and microclimatic differences between these 'Control' plots and the FACE plots due to differences in apparatus were minimal. The season-long daytime average CO_2 concentrations were 550, 550, 548, and 559 µmol mol^{-1} CO_2 in the FACE plots and 370, 370, 363, and 370, in the Ambient/Control plots for 1992–3, 1993–4, 1995–6, and 1996–7, respectively.

Irrigation treatments

Using a strip-split-plot design, the four FACE and four Ambient plots were split into halves with each half receiving either an ample (Wet, 100% replacement of potential evapotranspiration) or a limiting (Dry, 50% of Wet) supply of irrigation water during the 1992–3 and 1993–4 experiments via a subsurface drip irrigation system (Hunsaker *et al.*, 1996). The amounts of irrigation plus rain were 676 and 681 mm applied to the Wet plots for each of the two seasons, respectively (rain was 76 and 61 mm). The amounts of irrigation plus rain applied to the Dry plots were 351 and 318 mm. An ample amount of nitrogen fertilizer was applied to all plots (277 and 261 kg N ha^{-1}).

Nitrogen treatments

The same strip-split-plot design was used in 1995–6 and 1996–7, except N instead of water was the limiting factor (Kimball *et al.*, 1999). Amounts of N applied to the ample (High-N) plots were 350 kg N ha^{-1} for both seasons, and to the limited (Low-N) plots were 70 and 15 kg N ha^{-1} for 1995–6 and 1996–7, respectively. During 1996–7, an unfortunate mix-up of the fertilizer applications occurred in Replicate 3, and data from these plots have been excluded from all presentations in this paper. Ample irrigation amounts were applied in these FACE × N experiments.

Crop culture

Certified Yecora Rojo wheat seed was planted at mid-December in all seasons in east–west rows that were spaced 0.25 m apart (parallel to the drip irrigation tubing) (Kimball *et al.*, 1999). Fifty percent emergence of seedlings was observed about 1 January in all seasons, and FACE treatments commenced at that time. Air temperatures (2-m height) typically ranged from −5 to 42°C. Growing-degree-days amounted to about 2000, except for the Low-N treatments which matured earlier (Table 1 of Kimball *et al.*, 1999). Final harvests of grain occurred at the end of May for each season. The grain used in these quality tests came from the 18.1-m^2 'no-traffic' subplot areas that were reserved for noninvasive measurements and the final harvests (Wall & Kimball, 1993). Representative kilogram subsamples of grain from each plot each year were packaged and sent to the Western Wheat Quality Laboratory, USDA-ARS, Pullman, WA, USA for quality analyses.

Grain quality analyses

Upon receipt at the Western Wheat Quality Laboratory, all grain samples were frozen at −20°C for 2 d to kill any insects, then cleaned on a Carter dockage tester (Simon-Carter Co., Minneapolis, MN, USA), measured for test weight (Method 55–10; AACC, 2000), and scoured in a Forester and Son Cyclone Grain Scourer (Model 6, Forster and Son, Ada, OK, USA). Grain and flour moisture were determined according to Method 44–16 (AACC, 2000) (40 min for meal, 20 min for flour, both at 130°C). A subsample was ground in a UDY Cyclone (UDY Corp., Boulder, CO, USA) mill to pass a 0.5-mm screen. This ground meal was used for near infrared reflectance spectroscopy (NIR) grain hardness (Method 39–70 A; AACC, 2000) using a Technicon (Hoganas, Sweden) IA450 near infrared reflectance spectrometer, and grain protein (Dumas combustion method) (Method 46–30; AACC, 2000) using a Leco (St. Joseph, MI, USA) model FP-428. Grain protein is reported on a 12% moisture basis. Single kernel traits (hardness, moisture, weight and size) were obtained on a 300-kernel aliquot with a Perten (Perten Instruments North America, Springfield, IL, USA) model 4100 Single Kernel Characterization System.

Before milling, grain was tempered to 14.5% moisture content and held overnight. Milling was conducted on a modified Quadrumat (Brabender) system (Jeffers & Rubenthaler, 1979). Break flour (the amount of flour obtained early in the milling process from the break rollers. It is a measure of how easily the grain can be milled to flour, with higher values desired.) and straight-grade flour (the total amount of flour obtained) were expressed as per cent of total products. Flour ash was determined by Method 08–01 (AACC, 2000), and low values are desired. Milling score is a composite score that includes flour yield, break flour yield, and flour ash, with high values desired. Mixograph analysis was conducted on a 10-g sample at optimum water absorption (Method 54–40 A; AACC, 2000). (The mixing time requirement is a reliable index of loaf volume potential or protein quality, with medium to long mixing times associated with good loaf volumes (Finney *et al.*, 1987).) Ash and Mixograph absorption are expressed on a 14% flour moisture basis.

Bread baking used a straight-dough 100-g flour system at optimum water absorption and mixing time, 90-min fermentation and 75-ppm ascorbic acid (Method 10–10B; AACC, 2000). Determination of optimum water absorption (desirable to have high water absorption (Finney *et al.*, 1987)) and mixing time were judged by an experienced baker; internal crumb grain score was assigned on a scale of 1 (best) to 10 (unacceptable) by a three-member panel. Absorption is on a 14% flour moisture basis.

Protein-N yields

The total amounts of nitrogen in the grain protein harvested from the field (protein-N) were calculated from the protein concentrations times the grain yields in kg ha^{-1}, as reported by Pinter *et al.* (1997). A factor of 5.80 kg protein per kg N (Yamaguchi, 1992) was used to convert protein to nitrogen.

Statistical analysis

The data were analysed as a strip-split-plot design using the SAS 'Mixed' Procedure (Littell *et al.*, 1996) for the ANOVAs. The 1992–3 and 1993–4 FACE × H_2O experiments were analysed separately from the 1995–6 and 1996–7 FACE × N experiments. Year was handled as a repeated measure for both sets of experiments.

Results

Irrigation had relatively minor effects on grain quality (Table 1; 5th and 6th bars in panels of Figs 1, 2). For many of the tests the effects were small and not statistically significant (grain hardness (Fig. 1b), grain moisture (Fig. 1d), single kernel weight and size (Figs 1f,g), flour and break flour yields (Figs 1j,k), flour ash (Fig. 1l), milling score (Fig. 2a), mixograph absorption (Fig. 2b), bread crumb grain score (Fig. 2g)). Even when they were highly significant statistically, the changes due to the Dry treatment still were relatively small (test weight (–1%; Fig. 1a), single kernel hardness (–10% at Ambient and –2% at FACE; Fig. 1c), single kernel moisture (+1%; Fig. 1e), grain protein concentration (+2%; Fig. 1h), flour protein concentration (+4%; Fig. 1i), optimum mixing time for bread dough (+5%; Fig. 2e), and bread loaf volume (+3%; Fig. 2d), where all of these percentages are relative changes averaged over the CO_2 treatments, unless otherwise stated). Thus, the overall effect of drought in these experiments was a slight improvement in grain quality.

By contrast to irrigation, the Low-N nitrogen fertilizer treatment caused highly significant changes relative to High-N (Table 1, last two bars in the panels of Figs 1, 2), which were relatively large for many of the tests (test weight (+2%; Fig. 1a), grain hardness (–15%; Fig. 1b), single kernel hardness (–5%; Fig. 1c), grain protein (–36%; Fig. 1h), flour protein (–39%; Fig. 1i), flour yield (–2%; Fig. 1j), break flour yield (−9%; Fig. 1k), flour ash (+29%; Fig. 1L), milling score (−6%; Fig. 2a), mixograph and bake water absorption (−8%; Figs 2b,c), optimum mixing time for bread dough (+48%; Fig. 2e), bread loaf volume (–26%; Fig. 2d), and bread crumb grain score (+188%; Fig. 2g), where all of these percentages are relative changes averaged over the CO_2 treatments). Thus, Low-N, which had caused serious reductions in yield (Pinter *et al.*, 1997), also caused very serious reductions in both nutritional and baking quality of the wheat grain.

The effects of elevated CO_2 concentration (FACE; left 4 bars in the panels of Figs 1, 2) were often statistically significant but very much smaller than those due to Low-N (right 2 bars in Figs 1, 2), and there were several cases of significant interactions with both irrigation and nitrogen (Table 1, Figs 1, 2). First, at ample irrigation (Wet; 2nd bar in Figs 1, 2) and ample nitrogen (High-N; 4th bar in Figs 1, 2), there were changes in test weight (–1%; Fig. 1a), single kernel hardness (−9%, Wet only, Fig. 1c), grain and flour protein (–5% in Wet but 0% in High-N, Figs 2h,i), mixograph and bake water absorption (0%, only changed with Dry or Low-N, Figs 2b,c), optimum mixing time for bread dough (+6%, Fig. 2e), and bread loaf volume (–2% in Wet and 0% at High-N, Fig. 2d). The fact that the grain and flour protein concentrations and the bread loaf volume were unaffected by elevated CO_2 at High-N (with ample water) but that they decreased slightly under Wet (at ample N) suggests that perhaps our nitrogen application was not as ample as we intended in the FACE × H_2O experiments. Recall that in the FACE × H_2O experiments, the 'Ample' amount of N applied was about 270 kg ha^{-1}, whereas for the FACE × N experiments, the High-N treatment received 350 kg N ha^{-1}. On the other hand, these results suggest that the slight deterioration in nutritional and baking quality caused by elevated CO_2 may be overcome by applying additional fertilizer.

We have shown that the Dry treatment tended to cause slight improvements in protein concentration and bread loaf volume (Figs 1h,i,d) while the FACE treatment tended to cause slight decreases. Consequently, the effects of FACE were smaller under the Dry treatment than they were under the Wet.

Conversely, under the Low-N treatment, with respect to Control, FACE exacerbated the deleterious effects of inadequate nitrogen on nutritional and baking quality. For example, grain protein decreased 33% at Ambient CO_2 and 39% under FACE (Fig. 1h), and loaf volume similarly decreased 22% at ambient and 29% under FACE (Fig. 2d).

Under Dry, the FACE treatment increased the yield of protein-N harvested by 18% relative to Ambient (1st bar, Fig. 2f), in spite of the 4% decrease in protein concentration in the grain (Fig. 1h). Under Wet, the protein-N yield increase due to FACE was 4% (2nd bar, Fig. 2f), in spite of the 5% decrease in protein concentration (Fig. 1h). There were equal yields of protein-N harvested from Control and FACE under Low-N (3rd bar, Fig. 2f), in spite of the 11% reduction in protein concentration under FACE (Fig. 1h). With High-N, there was no effect of FACE on protein concentration (Fig. 1h), so a 16% increase in grain yield with respect to Control (Pinter *et al.*, 1997) also resulted in a 16% increase protein-N yield (4th bar, Fig. 2f).

Discussion

The limited water (Dry), limited nitrogen (Low-N), and elevated CO_2 (FACE) treatments all affected the growth and grain yield of the wheat plants compared with those at ambient CO_2 (Ambient or Control) and ample water (Wet)

Table 1 Significance of elevated CO_2 (C), irrigation (I, i.e. water supply), nitrogen (N), Year (Y), and their interactions on various measures of wheat (*Triticum aestivum* L.) grain quality from the 1992–3 and 1993–4 FACE × H_2O experiments and from the 1995–6 and 1996–7 FACE × N experiments, as well as yields of protein-N harvested from the plots

		Nonstress Ambient or Control		Significance					
Parameter	I or N	Mean	SE	C	I or N	C*I or C*N	Y	C*Y	I*Y or N*Y
Test weight (kg m^{-3})	I	819.0	1.4	**	***	***	***	*	ns
	N	811.4	2.6	ns	***	ns	***	ns	ns
Grain hardness (NIR value, dimensionless)	I	80.88	1.70	ns	ns	ns	*	ns	**
	N	95.27	3.07	ns	***	ns	***	ns	***
Single kernel hardness (dimensionless)	I	69.88	0.83	***	**	**	only in 1993–94		
	N	57.27	1.72	ns	*	ns	***	ns	***
Wheat grain moisture (% by weight)	I	9.56	0.01	ns	ns	ns	***	ns	ns
Single kernel moisture (% by weight)	I	11.30	0.04	ns	***	ns	only in 1993–94		
Single kernel weight (mg)	I	44.85	0.46	ns	ns	ns	only in 1993–94		
	N	39.05	1.37	ns	ns	ns	**	ns	ns
Single kernel size (mm)	I	3.09	0.02	ns	ns	ns	only in 1993–94		
	N	2.55	0.08	ns	ns	ns	**	**	ns
Protein concentration of grain (% by weight at 12% moisture)	I	14.98	0.09	***	**	**	***	ns	***
	N	14.95	0.16	***	***	***	ns	*	ns
Flour protein concentration (% by weight at 14% moisture)	I	13.15	0.07	***	**	ns	***	**	***
	N	13.61	0.13	***	***	***	ns	**	ns
Flour yield (% by weight of total products)	I	70.49	0.36	ns	ns	ns	***	ns	ns
	N	68.85	0.32	ns	***	ns	ns	ns	**
Break flour yield (% by weight of total products)	I	38.63	0.59	ns	ns	**	***	**	ns
	N	34.35	0.46	ns	***	*	***	**	***
Flour ash (% by weight at 14% moisture)	I	0.350	0.007	ns	ns	**	***	ns	**
	N	0.353	0.006	ns	***	**	ns	ns	ns
Milling score	I	87.74	0.46	ns	ns	*		ns	ns
	N	85.84	0.52	ns		*	ns	ns	
Mixograph absorption (% by weight corrected to 14% moisture)	I	64.18	0.37	**	ns	*	***	ns	*
	N	62.47	0.25	***	***	***	*	ns	ns
Bake water absorption (% by weight corrected to 14% moisture)	I	67.12	0.23	**	*	ns	***	ns	ns
	N	64.94	0.29	***	***	**	ns	ns	ns
Optimum mixing time for bread dough (min)	I	3.60	0.07	***	***	ns	***	ns	ns
	N	3.50	0.20	**	**	ns	***	*	*
Bread loaf volume (cm^3)	I	985	9	***	***	ns	ns	ns	***
	N	992	14	**	***	**	ns	ns	ns
Bread crumb grain score (1-excellent, 9-unsatisfactory)	I	3.12	0.14	ns	ns	ns	**	ns	ns
	N	2.55	0.42	ns	***	***	ns	ns	ns
Protein-N harvested from the field in the wheat grain (kg N ha^{-1})	I	232	6	**	***	**	***	ns	ns
	N	197	8	*	***	**	**	ns	*

I or N in the second column indicates which set of the experiments generated the data in that particular row. Also shown are the absolute means and SE for the nonstress Ambient or Control treatments (i.e. Ambient-Wet or Control-High-N). From these absolute values and the relative responses presented in Figs 1 and 2, the absolute values for the other treatments can be calculated. Probability levels are indicated by ns, *, **, and *** for 'not significant', 0.1, 0.05, and 0.01, respectively. Near infrared reflectance spectroscopy (NIR).

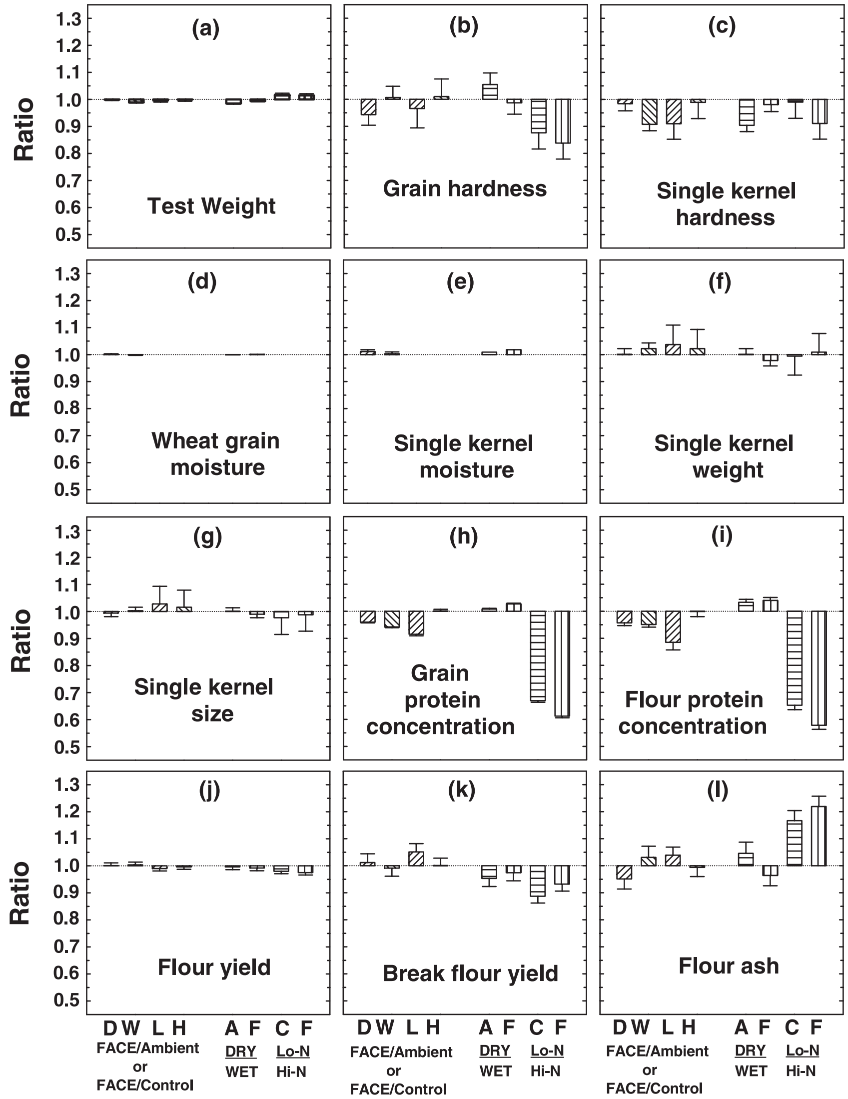

Fig. 1. Relative response ratios of wheat (*Triticum aestivum* L.) grain test weight (a), grain hardness (b), single kernel hardness (c), whole grain moisture (d), single kernel moisture (e), single kernel weight (f), single kernel size (g), grain protein concentration (h), flour protein concentration (i), flour yield (j), break flour yield (k), and flour ash (l) from free-air CO_2 enrichment (FACE) experiments. Wet and Dry irrigation levels were imposed in experiments conducted in 1992–3 and 1993–4, and Ambient daytime CO_2 levels were 370 µmol mol^{-1}, while CO_2 levels were controlled at 550 µmol mol^{-1} in the FACE plots. Low and High nitrogen fertilizer levels were applied in experiments conducted in 1995–6 and 1996–7, and Control daytime CO_2 levels were again about 370 µmol mol^{-1}, while CO_2 levels were controlled at 200 µmol mol^{-1} above ambient in the FACE plots. The left two bars in each panel are the FACE/Ambient response ratios under the Dry (D) and Wet (W) treatments, respectively. The 3rd and 4th bars are the FACE/Control ratios under the Low-N (L) and High-N (H) treatments, respectively. The 5th and 6th bars are the Dry/Wet response ratios under Ambient and FACE CO_2 treatments, respectively. The right two bars are the Low-N/High-N response ratios under Control and FACE CO_2 treatments, respectively. Bars indicate standard errors of the ratios, which were calculated from the standard errors of the individual means using the formula, $\Delta r = (|D\Delta N| + |N\Delta D|)D^{-2}$, where Δ indicates the standard error, D is the Ambient or Control mean value in the denominator, N is the FACE mean value in the numerator, and the vertical bars denote absolute values.

and nitrogen (High-N) (Pinter *et al.*, 1997). Dry and Low-N reduced yields by *c.* 33 and 21%, respectively. Under ample water and nitrogen, FACE increased yields about 16%. Under the Dry irrigation regime, FACE stimulated yields even more (*c.* 23%); whereas under Low-N, the stimulation due to FACE was smaller (*c.* 9%). Thus, with such effects occurring in the overall grain productivity of the wheat, some effects on grain quality might be expected.

Overall, the quality of grain produced under ample water and N and under drought was high (15.0% grain protein, Table 1). Smith & Gooding (1999), for example, state that 11.3% grain protein concentration (adjusted to 12% moisture) is the threshold commonly used by UK millers. Only the grain produced in our Low-N treatment was below this minimum acceptable value (10.00 and 9.15% ± 0.16% under Control and FACE CO_2 treatments, respectively).

Although the FACE treatment caused decreases in grain protein concentration relative to Ambient or Control, except for High-N (Fig. 1h), the increased grain yields due to FACE (Pinter *et al.*, 1997) resulted in greater harvests of protein-N when the N supply was high or ample. Thus, the overall production of protein-N was increased by elevated CO_2 except at Low-N (Fig. 2f, albeit at somewhat lower concentrations than produced in the Ambient or Control plots).

During the first two FACE × H_2O experiments, we had Ambient plots with no blowers, while the FACE plots had blowers. Then during the second two FACE × N experiments, we had Control and FACE plots all with blowers. However, in addition, during the 1995–6 growing season, two Ambient rings without blowers were established besides the four Control rings with blowers, as described by Pinter *et al.* (2000). Grain samples from these Ambient plots were subjected to the same battery of quality tests as those from the Control plots. The grain protein, bread loaf volume, and other parameters at both high and low levels of N were almost identical between the Ambient and Control plots (data not shown). Therefore,

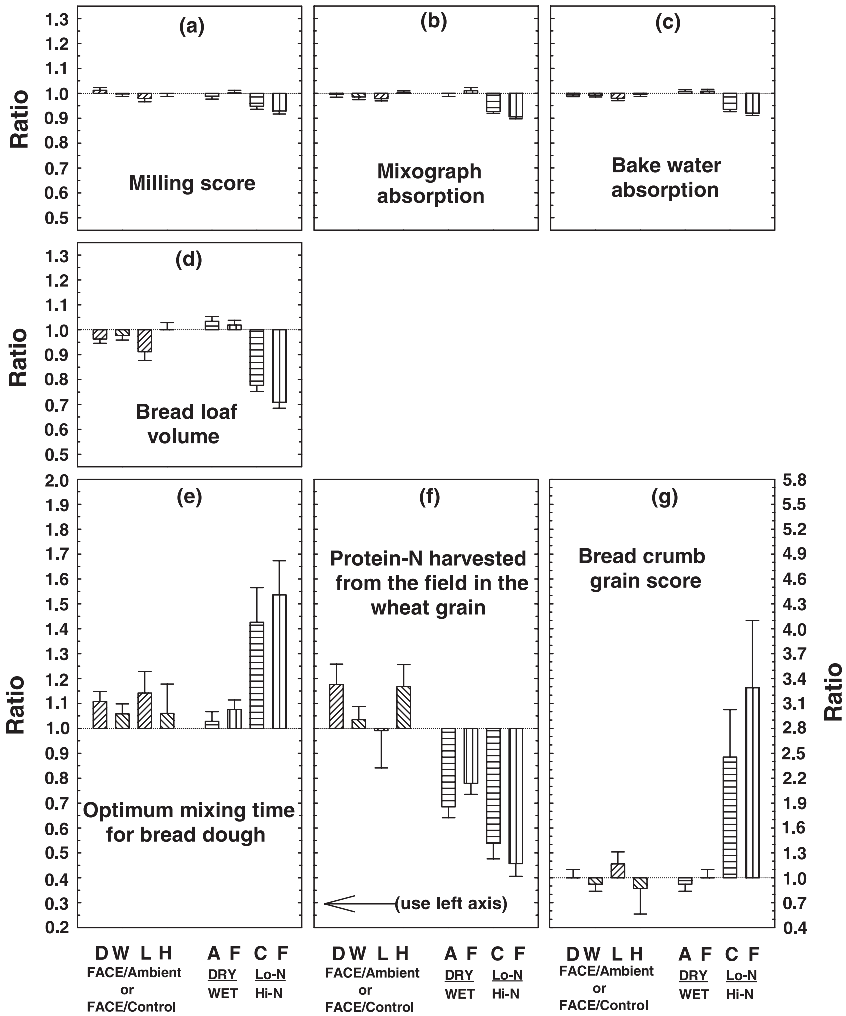

Fig. 2. Relative response ratios of wheat grain milling score (a), mixograph absorption (b), bake water absorption (c), bread loaf volume (d), optimum mixing time for bread dough (e), protein-N harvest from the field in the grain (f), and bread crumb grain score (g) from free-air CO_2 enrichment (FACE) experiments. The left two bars in each panel are the FACE/Ambient response ratios under the Dry (D) and Wet (W) treatments, respectively. The 3rd and 4th bars are the FACE/Control ratios under the Low-N (L) and High-N (H) treatments, respectively. The 5th and 6th bars are the Dry/Wet response ratios under Ambient and FACE CO_2 treatments, respectively. The right two bars are the Low-N/High-N response ratios under Control and FACE CO_2 treatments, respectively.

we conclude that the lack of blowers in the FACE × H_2O experiments did not significantly affect grain quality.

The pattern of grain quality results is surprisingly similar to that of leaf N concentrations measured during the course of the same four FACE experiments by Sinclair *et al.* (2000). They found that drought had little effect on leaf N, as did elevated CO_2 under optimal conditions. However, the Low-N treatment caused a large decrease in leaf N compared with High-N, which was exacerbated by elevated CO_2. Thompson *et al.* (1997) measured the protein concentration of the grain from the 1995–6 experiment, and although not statistically significant, the trend was for FACE to decrease the concentration compared with Control. Their samples were smaller than those used herein. F. Porteous *et al.* (unpublished) similarly measured the N concentration of the whole shoots of the wheat plants sampled at the end of the 1996–7 experiment. Although CO_2 effects were not statistically significant, the trend again was for FACE to exacerbate the deleterious effects of the significant Low-N treatment. They did not detect any significant treatment effects on the N concentration of the roots or of the grain, but their sample sizes also were much smaller than those used herein.

Our results are mostly in agreement with prior results reported in the literature, as reviewed in the Introduction of this paper. Like Barber & Jessop (1987) in Australia and Garrot *et al.* (1994) in Arizona, who both studied irrigated wheat, we found a slight improvement in grain quality with water stress. For about a 300-kg N ha^{-1} reduction in applied N, we found about a 5% absolute decrease in protein concentration, or 1% per 60 kg N ha^{-1}, which is close to the 1% per 56 kg N ha^{-1} determined by Benzian & Lane (1981) from many experiments in England. We mentioned that many of the prior experiments found little or no change in wheat grain nutritional or bread-making quality due to elevated levels of CO_2. On the other hand, a comparable number of such experiments did find decreases in the protein concentration, which reduced nutritional value and adversely affected baking quality. The data from these FACE experiments are basically consistent with these prior CO_2-enrichment data, suggesting that under conditions of optimum water and nitrogen, elevated

CO_2 will likely cause a small decrease in grain quality. However, quality is very sensitive to nitrogen supply, and if nitrogen were limiting even somewhat in the prior experiments (as may have been in our FACE × H_2O experiments), elevated CO_2 may cause a relatively larger decrease in grain quality.

It is difficult to assess how widely these results are applicable across the plant kingdom, across C_3 annual grasses, or even across varieties within the wheat species. Cotrufo *et al.* (1998) reviewed about 75 reports of the effects of elevated CO_2 on plant tissue N concentration. However, the research has mostly focused on leaves, and they list no data at all for grain or seeds or nuts, which is surprising considering how important seeds are to dispersal of plant species and to the diet of many animals. It is possible that some of the inconsistency among the wheat quality experiments mentioned previously has been due to differences in response among varieties. Benzian & Lane (1979, 1981, 1986) show substantial differences among wheat varieties with respect to absolute grain protein concentrations, but relative changes with respect to soil nitrogen supply were similar. Manderscheid *et al.* (1995) and Blumenthal *et al.* (1996) both included two varieties of wheat in their experiments. Manderscheid *et al.* (1995) found similar reductions in grain N concentration to elevated CO_2 between varieties, whereas Blumenthal *et al.* (1996) report a significant interaction with genotype. While these two studies found mean reductions in grain N concentration, we must remember several others found no significant effect of elevated CO_2 on grain quality. Considering the class of C_3 grasses, Manderscheid *et al.* (1995) also studied two varieties of barley, finding that one had reduced N content (but a smaller reduction than that of their wheat) while the other's did not change significantly. Similar variability exists among rice (*Oryza sativa* L.) experiments, with Ziska *et al.* (1997) reporting unspecified reductions of grain protein at elevated CO_2, while Seneweera *et al.* (1996) and Seneweera & Conroy (1997) found reductions of about 0.3% (absolute) in grain N concentration. Variability across other plant types may be similar. In a FACE experiment, which was very similar those reported herein, with cotton (*Gossypium hirsutum* L.), a C_3 woody perennial (cultivated as an annual for insect control), Prior *et al.* (1998) found absolute reductions in seed N concentration of *c.* 0.4% (7% relative). Thus, it appears that intra- and interspecific variations in effects of elevated CO_2 on grain quality exist. However, the results reported herein are certainly within the range of those in the literature, and they likely are representative of additional genotypes within wheat and other C_3 grasses and somewhat across the plant kingdom.

In conclusion, the data from these experiments suggest that adequate fertilizer is necessary to attain good quality grain and that, with ample fertilizer, the deleterious effects of elevated CO_2 will be minor. On the other hand, crops grown with limiting levels of N (such as is often the case in developing countries, or for other plants in unmanaged natural ecosystems) probably now have poorer quality grain than they could have, and future high CO_2 concentrations are likely to make the quality poorer yet.

Acknowledgements

This research was supported by the Agricultural Research Service, U.S. Department of Agriculture, especially the U.S. Water Conservation Laboratory, Phoenix, AZ. It was also supported by Grant #DE-FG03–95ER-62072 from the U.S. Department of Energy Terrestrial Carbon Processes Research Program to the University of Arizona, Tucson and Maricopa, Arizona (S. Leavitt was principal investigator). We also acknowledge the helpful cooperation of Dr Roy Rauschkolb (deceased) and his staff at the Maricopa Agricultural Center. The FACE apparatus was furnished by Brookhaven National Laboratory, and we are grateful to Mr Keith Lewin, Dr John Nagy, and Dr George Hendrey for assisting in its installation and consulting about its use. This work contributes to the Global Change Terrestrial Ecosystem (GCTE) Core Research Programme, which is part of the International Geosphere-Biosphere Programme (IGBP). We gratefully acknowledge the technical assistance of M. Baker, O. Cole, M. Gerle, S. Gerszewski, D. Johnson, C. O'Brien, L. Olivieri, J. Olivieri, D. Pabian, R. Osterlind, R. Rokey, R. Seay, L. Smith, K. Smith, R. Suich, and K. West.

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