

**PLANT GROWTH AND WATER USE AS AFFECTED BY ELEVATED CO₂
AND OTHER ENVIRONMENTAL VARIABLES**

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**PLANT GROWTH AND WATER USE AS AFFECTED BY ELEVATED CO₂
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MISSION

To predict the effects of elevated CO₂ and climate change on the photosynthesis, growth, yield, and water use of crops under optimal and limiting levels of water and fertility.

THE FREE-AIR CO₂ ENRICHMENT (FACE) PROJECT: PROGRESS AND PLANS

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PROBLEM: The CO₂ concentration of the atmosphere is increasing and expected to double sometime during the next century. Climate modelers have predicted that the increase in CO₂ will cause the Earth to warm and precipitation patterns to be altered. This project seeks to determine the effects of such an increase in CO₂ and any concomitant climate change on the future productivity, physiology, and water use of crops.

APPROACH: Numerous CO₂ enrichment studies in greenhouses and growth chambers have suggested that growth of most plants should increase about 30% on the average with a projected doubling of the atmospheric CO₂ concentration. However, the applicability of such work to the growth of plants outdoors under less ideal conditions has been seriously questioned. The only approach that can produce an environment today as representative as possible of future fields is the free-air CO₂ enrichment (FACE) approach. Therefore, the FACE Project was initiated, and experiments were conducted on cotton from 1989-1991 (Hendrey, 1993; Dugas and Pinter, 1994). Then, from December 1993 through May 1994 two FACE experiments were conducted on wheat at ample and limiting levels of water supply, with about 50 scientists from 25 different research organizations in eight countries participating. About 42 papers have been published (e.g., Kimball et al., 1995, 1999; Pinter et al., 1996) or are in press from these experiments, and more are being prepared.

However, one of the greatest uncertainties in determining the impact of global change on agricultural productivity, as well as on natural ecosystems, is the response of plants to elevated CO₂ when levels of soil nitrogen are low. Therefore, we conducted two additional FACE wheat experiments at ample and limiting supplies of soil nitrogen from December 1995 - May 1996 and December 1996 - May 1997. Funded by the Department of Energy through a grant to the University of Arizona, U.S. Water Conservation Laboratory (USWCL) personnel were major collaborators on the project and provided management support. In addition, soil cores and leaf samples were obtained and were stored frozen for later analyses of root biomass and soil nitrogen, photosynthetic proteins, and carbohydrates. Thanks to a grant from a NSF/DOE/NASA/USDA program (TECO II) plus ARS Temporary Global Change Funds, personnel to do these analyses were hired, and many analyses were conducted during this past year. Much data from many kinds of measurements made during these latter experiments have been analyzed during the past year, manuscripts are being prepared, and papers are starting to appear in print (e.g., Kimball et al., 1999).

Much of the CO₂ enrichment research that has been conducted in the past has been with C₃ plants and relatively little with C₄ crops such as corn, sugarcane, or sorghum. The neglect of C₄s was because their photosynthetic process was known to respond relatively less to elevated CO₂. However, their stomata do partially close in elevated CO₂, thereby suggesting the possibility of some water conservation. Therefore, with grants (one to the USWCL and one to the University of Arizona) from the NASA/NSF/DOE/USDA/EPA (TECO III) Program, we conducted an initial

FACE experiment on sorghum from mid-July through mid-December 1998. Then we conducted a second replicate experiment from mid-June through October 1999. Our hypothesis was that there will be only a small enhancement of growth due to the FACE treatment when the plants have ample water; but, under water-stressed conditions, there will be a substantial growth enhancement resulting from the water conservation due to the partial stomatal closure.

Similar to the previous experiments, measurements included leaf area, plant height, aboveground biomass plus roots that remained when the plants were pulled, morphological development, canopy temperature, reflectance, chlorophyll, light use efficiency, energy balance, evapotranspiration, soil and plant elemental analyses, soil water content, photosynthesis, stomatal conductance, grain quality, video observations of roots from minirhizotron tubes, soil CO₂ and N₂O fluxes, and changes in soil C storage from soil and plant C isotopes. Some soil cores for roots also have been obtained. As before, all of the data will be assembled in a standard format for validation of plant growth models.

FINDINGS: Analyses of the data from the FACE wheat experiments are nearing completion. Briefly, the results indicate that under the high nitrogen treatment, wheat grain yields were increased about 15% by FACE at 200 μmol/mol above ambient. At low nitrogen, elevated CO₂ increased yields by about 12% in 1996 and only 5% in 1997. The low nitrogen treatment reduced yields about 20% at both levels of CO₂.

Despite some problems, the 1998 and 1999 FACE sorghum experiments were successfully conducted. A problem encountered both years is that relatively large irrigations were required to get good distribution uniformity because the Trix clay loam soil in the field cracks severely when it dries. Therefore, besides the initial irrigation at planting time, only one additional irrigation was applied to the Dry plots. Bird and insect damage were minimal both years. In 1998, there was some frost damage before the final harvest, and on September 19, 1999, there was a hail storm that tattered the upper leaves, but the heads appeared to survive all right.

Preliminary results from 1998 showed that the effects of elevated CO₂ were minimal on the biomass and grain yield in the plots with ample water. However, under water-stress conditions there were significant stimulations of growth (+13%) and yield (+17%), consistent with the CO₂-induced partial stomatal closure and resultant water conservation.

INTERPRETATION: The data from the FACE wheat experiments suggest that with ample water, wheat production is likely to increase 10-15% by an increase in atmospheric CO₂ levels to 200 μmol/mol above current levels (about 370 μmol/mol). Moreover, in contrast to many chamber studies, our results suggest that the yield increases will occur even at low levels of soil nitrogen characteristic of the agriculture in developing countries and most natural ecosystems. Irrigation requirements may be unchanged or slightly reduced for future wheat production, provided climate changes are minimal.

The preliminary FACE sorghum data suggest that there will be little effect of higher atmospheric CO₂ concentrations on future sorghum productivity when there is ample water. Under water-stress conditions, which are typical of much of the rain-fed areas where sorghum is grown in the U.S. and in Africa and other developing countries, the future higher levels of CO₂ are likely to increase productivity by 15% or so.

FUTURE PLANS: Analyses and reporting of the results from the FACE wheat and especially from the sorghum experiments will continue. This is the last partial year of funding from the TECO III program, so the FACE project will have to terminate unless additional funding can be obtained. If an appropriate request for proposals is made by a funding agency, a proposal will be prepared. Neither the choice of crop nor the experiment design has been decided.

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WATER RELATIONS OF GRAIN SORGHUM GROWN IN FREE-AIR CO₂ ENRICHMENT (FACE) WITH VARIABLE SOIL MOISTURE REGIMES

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PROBLEM: Based on reports by the IPCC (Intergovernmental Panel on Climate Change, 1996) atmospheric CO₂ is rising. Because elevated CO₂ is known to reduce stomatal conductance (g_s) in plants, it may decrease the transpiration rate and the increase net assimilation rate (Garcia et al. 1998; Wall et al., 2000a). An increase in the pool of total non-structural carbon supply may result in an increase in the translocation of carbon below ground for the development of a more robust root system. As observed in wheat, a more robust root system for absorbing water and a greater resistance to water loss by stomata might enable a sorghum plant grown under elevated CO₂ and limited water supply to avoid drought by conserving water (Wall et al., 2000b) and increasing drought tolerance by an enhancement in its osmoregulatory mechanism (Wall et al., 2000c). Consequently, a sorghum plant grown under elevated CO₂ and limited water supply is likely to have less negative leaf water potential (Ψ_T). Based on these observations, a need exists to determine how the interactive effect of elevated CO₂ and water stress will effect Ψ_T for sorghum grown in an open field. Hence, a reasonable hypothesis to test is that any enhancement in water relations through a combination of a reduction in g_s , improvement in osmoregulation, and an increase in water uptake capacity of roots due to elevated CO₂ will result in less negative Ψ_T for sorghum leaves throughout the ontogeny of the crop.

APPROACH: In this study we characterized and qualified values of g_s and Ψ_T for field-grown sorghum grown in air enriched with CO₂ and in ambient air under water-stressed and well-watered soil moisture regimes. During the 1998 and 1999 growing seasons, two experiments were conducted to investigate the interactive effects of elevated CO₂ and limited soil moisture on a sorghum (*Sorghum bicolor* L. Moench cv. Dekalb Hybrid DK54) crop grown in an open field at The University of Arizona's Maricopa Agricultural Center, located 50 km south of Phoenix, Arizona (33.1 N, 112.0 °W). Seeds were sown into flat beds in north-south rows ~ 0.76 m apart on July 15-16, 1998 and June 14-15, 1999; seeding rates were 10.9 kg ha⁻¹ (~40 mm apart for 33 seeds m⁻²; plant density of 22 plants m⁻²) during 1998 and 10.0 kg ha⁻¹ (~41 mm apart for 32 seeds m⁻²; plant density of 21 plants m⁻²) during 1999. Regardless of year, a final plant population of about 90,000 plants ha⁻¹ was obtained. Fertilizer amounts were applied so that nutrients were non-limiting. Plants were exposed to an elevated CO₂ treatment of ~200 μmol mol⁻¹ above ambient (ca. 370 μmol mol⁻¹) for 24 hrs using the FACE approach (Hendrey, 1993). The main CO₂ plots were split. Flood irrigation created a water (H₂O) treatment with each half of the circular plots receiving either ample irrigation regime (Wet, well-watered) or a water-stress treatment with only two irrigation events. The CO₂ and H₂O levels gave four treatment combinations of Control-Dry (CD), Control-Wet (CW), FACE-Dry (FD), and FACE-Wet (FW) replicated four times. Several portable closed-exchange (transient) systems with a 250 cm³ transparent assimilation chamber were used to make in-situ measurements of g_s at mid-morning (MM; 2.5 h prior to solar noon), midday (MD; solar noon), and mid-afternoon (MA; 2.5 h after solar noon). During similar time periods, values of Ψ_T were obtained from excised uppermost

fully-expanded sunlit leaves using a pressure chamber.

FINDINGS: At the stem-elongation growth stage on day of year (DOY) 252, g_s was independent of time of day (Fig. 1). A significant $\text{CO}_2 \times \text{H}_2\text{O}$ interaction occurred because g_s was lower due to CO_2 under Wet, but there was no effect of CO_2 on this particular day under Dry. As evidenced in the Wet treatment, CO_2 reduced g_s , but the water conserving effect of the CO_2 -based reduction in g_s in the Dry plots may have delayed reductions in g_s due to water-stress. Consequently, g_s was actually slightly higher under elevated CO_2 for the Dry plots for a few days (data not shown). Nevertheless, in agreement with other FACE trials on wheat (Wall et al., 2000a,b), FACE consistently reduced g_s in sorghum.

The CO_2 -based reductions in g_s for both Wet and Dry treatments caused less negative Ψ_T throughout the ontogeny of the crop (Fig. 2). But, on a relative basis, the water-conserving effect of elevated CO_2 was more pronounced in Dry compared with Wet, and in dehydration cycle II compared with I (significant $\text{CO}_2 \times \text{H}_2\text{O}$ interaction effects for Ψ_T during dehydration cycle II). Apparently, plants had become preconditioned to drought during dehydration cycle I, and CO_2 affected this effect in a positive manner because in dehydration cycle II the water conserving effect of elevated CO_2 was more significant. A reduction in the internal water deficits in sorghum leaves grown under elevated CO_2 is best explained by the direct effect of CO_2 in reducing g_s (Fig. 1), which conserved water throughout the ontogeny of the crop.

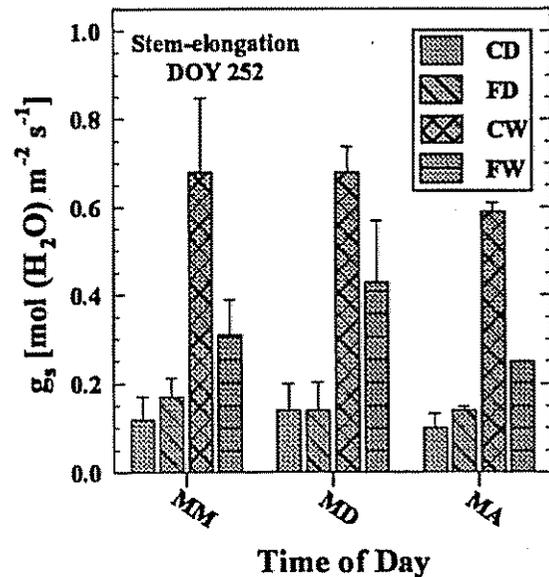


Figure 1. Leaf stomatal conductance (g_s) of uppermost sunlit sorghum leaves at the stem-elongation growth stage during 1998. The CO_2 treatments were Control [ambient $370 \mu\text{mol (CO}_2\text{) mol}^{-1}$] and FACE [ambient +200 $\mu\text{mol (CO}_2\text{) mol}^{-1}$ for 24 hs. d^{-1}], and the H_2O treatments were either ample or only two irrigation events for well-watered (Wet) and water stress (Dry) treatments, respectively. The combination of CO_2 and H_2O treatments resulted in Control-Dry (CD), FACE-Dry (FD), Control-Wet (CW), and FACE-Wet (FW) treatments. Measurements were taken at mid-morning (MM, 2.5 h prior to solar noon), midday (MD, solar noon), and mid-afternoon (MA, 2.5 h after solar noon). Vertical bars around each datum represent one standard error of the replication means.

INTERPRETATION: In a future high CO₂ world, the pore size of stomata for a sorghum leaf will decrease which will decrease g_s and increase the resistance of water vapor flux from the sub-stomatal cavity to the atmosphere (Fig. 1). For comparable atmospheric and soil moisture conditions, the transpiration rate per unit leaf area of a sorghum leaf grown under elevated CO₂ will be lower than that for a leaf growing at present-day ambient CO₂ (~370 μmol mol⁻¹) levels. Consequently, a leaf growing under elevated CO₂ should have the capacity to maintain higher internal water content throughout the day than a leaf growing at present-day ambient CO₂ levels. A higher internal water content will result in less negative values of Ψ_T at MM, MD, and MA (MD given in Fig. 2) throughout the ontogeny of the crop. The water conserving effect of elevated CO₂ will delay the onset of drought symptoms, thereby enabling stomata to remain open for longer into the day-lit period, even as the drought condition becomes more severe. In the absence of any adverse effects of a concomitant rise in global temperature resulting from the rise in atmospheric CO₂, improved water relations for a herbaceous, warm-season, perennial, C4 grain crop, i.e. sorghum, are anticipated in the future as the CO₂ concentration of the atmosphere continues to rise.

FUTURE PLANS: Our intention is to focus on data summary, analysis, interpretation, and documentation of results from previous FACE experiments on wheat and sorghum. We also will actively plan our next FACE experiment and attempt to obtain the necessary funding.

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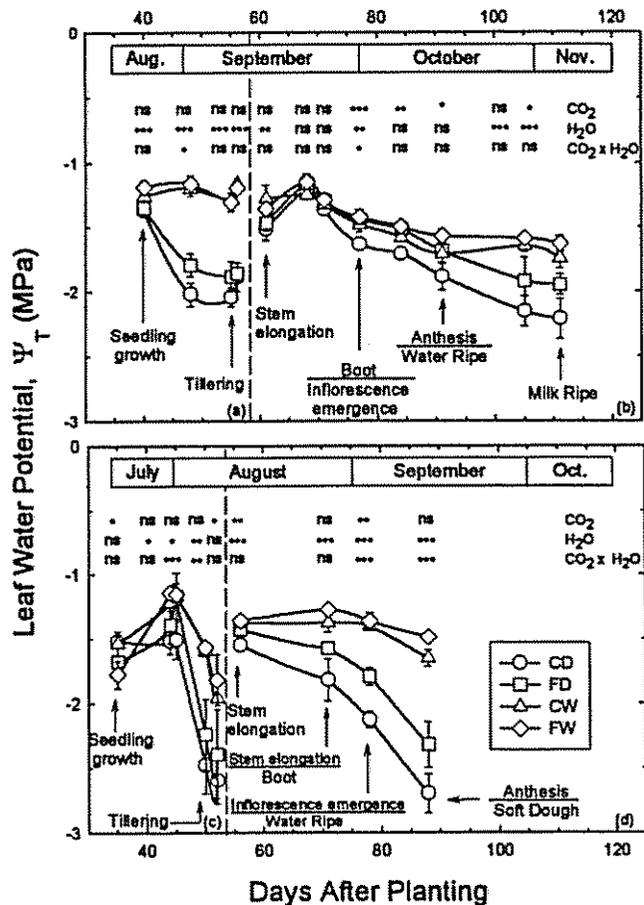


Figure 2. Midday total leaf water potential (Ψ_T) during 2 soil dehydration/rehydration cycles [cycle I given in panels a and c, and cycle II given in panels b and d, during 1998 and 1999, respectively]. The CO₂ treatments were Control [ambient 370 μmol (CO₂) mol⁻¹] and FACE [ambient +200 μmol (CO₂) mol⁻¹ for 24 hrs d⁻¹], and the H₂O treatments were either ample or only two irrigation events for well-watered (Wet) and water stress (Dry) treatments, respectively. Legend same as Fig. 1. Vertical bars around each datum represent one standard error of the replication means. Results of ANOVA for CO₂, H₂O and CO₂xH₂O interaction effects given for each growth stage above each datum as ***, **, *, and NS for p < 0.01, p < 0.05, p < 0.10, and not significant, respectively.

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FACE 1998-99: SOIL MOISTURE AND EVAPOTRANSPIRATION IN SORGHUM UNDER ELEVATED CO₂ AND WATER STRESS CONDITIONS

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PROBLEM: Deforestation and increased combustion of fossil fuels due to global industrialization are causing increases in atmospheric CO₂ concentrations. Increases in CO₂ concentration affect plant growth. Previous FACE experiments investigated the impact of a 200 $\mu\text{mol mol}^{-1}$ CO₂ increase on cotton and wheat, which utilize the C3 photosynthetic pathway. The 1998-99 FACE experiment investigated this futuristic atmospheric environment on sorghum, which is a C4 plant.

APPROACH: A Free-Air CO₂ Enrichment (FACE) system consisting of four 25-m diameter rings was used to increase the background CO₂ over field-grown sorghum by 200 $\mu\text{mol mol}^{-1}$. Four additional rings with identical air flow but at ambient CO₂ served as experimental controls. Vertical 2.5-m-tall CO₂ vent pipes were spaced 2 m apart along the perimeter of each ring. Wind direction, wind speed, and CO₂ concentrations were measured for each FACE ring. These data were used in a computer controlled system that delivered CO₂-enriched air to the upwind side of each FACE ring. The system maintained CO₂ concentrations at 200 $\mu\text{mol mol}^{-1}$ above ambient, 24 hours a day, throughout the growing season.

Half of each ring received ample flood irrigation (Wet) while the other half was water stressed (Dry) in a split-strip plot design. Thus, there were two levels of CO₂ and two levels of soil water supply, each replicated four times creating sixteen semicircular plots.

Soil moisture in terms of percent water by volume, or volumetric theta ($\text{m}^3 \text{H}_2\text{O} / \text{m}^3 \text{soil}$), were determined through use of a Cambell Pacific nuclear Hydroprobe. A two-inch-diameter neutron access tube was inserted vertically into the ground in each of the sixteen plots. Measurements were taken at each foot to the six-foot depth during the 1998 season and to the ten-foot depth during the 1999 season. Additional measurements were taken during the 1999 season, including a profile measurement where 120 measurements were taken over a ten-foot profile.

FINDINGS: Significant differences in soil water were detected between Wet and Dry plots during dry-down periods (Fig. 1). There were also substantial differences in soil water between the CO₂ treatments. In addition, a high degree of variability was detected among plots, and by depth. The majority of this variability could be attributed to variable soil texture. Although there was a high degree of relative variability in total water by soil volume, the portion of water available to plants, between field capacity and permanent wilting point, spanned from 1-8% of soil volume with an average of 4%, over a ten-foot profile.

Data for Wet plots in 1998, over six feet of depth (Fig. 1, panel 1), indicate a soil texture difference between the average FACE-Wet plot (more sand) and the average Control-Wet plot (more clay). This resulted in a consistent 1% volumetric water content difference between the two treatments. The soil texture difference between FACE-Dry and Control-Dry plots was smaller. Additionally, two distinct

dry-down periods for the Dry plots were apparent, first DOY 228-254 and second, DOY 275-330. A small amount of rainfall (5 mm) occurred on DOY 331.

Soil water content data for the 1999 season, across ten feet of depth (Fig. 1 panel 2), show a consistent 1% difference in soil water similar to 1998 (Fig. 1, panel 1), again suggesting that FACE-Wet contained slightly more sand on average. There was no significant difference in soil water content between the CO₂ treatments under the Dry irrigation treatment. Also shown were three dry-down periods involving the Dry treatments, DOY 200-218, DOY 228-258, and DOY 268-290. Rainfall totaling 56 mm occurred between DOY 263-267.

Evapotranspiration (ET) was calculated using the volumetric dry-down of stored soil water between irrigations. ET was extrapolated during days exhibiting increased volumetric water (due to irrigation or rain water application) by averaging the estimated ET before and after the volumetric increase.

Figure 2, panel 1, depicts mean cumulative evapotranspiration (ET) for the 1998 season calculated over six feet of depth. A difference in cumulative ET occurred between Wet and Dry treatments in early September DOY 240. FACE-Wet and Control-Wet plots show different cumulative ET by mid September. This trend continued until the end of the season where FACE-Wet evapotranspired 161 mm less water than Control-Wet. FACE-Dry and Control-Dry plots did not show significant differences in cumulative ET. This is consistent with previous FACE experiments (Hunsaker et al., 1996).

Figure 2, panel 2, shows ET data for the 1999 season calculated over six feet of depth. Trends are similar to the 1998 season results except that FACE-Dry and Control-Dry treatments do show a difference in cumulative ET by mid August, and by the end of the season FACE-Dry evapotranspired 102 mm of water less than Control-Dry. One reason why the FACE effect is visible in Dry treatments during the 1999 season and not in the 1998 season is that Dry treatments in 1999 were more water stressed than in 1998 due to an earlier planting date and decreased amount of applied irrigation. FACE-Wet evapotranspired 159 mm less water than Control-Wet.

Figure 3, panel 1, shows the soil water profile of plot FACE-Wet Replication-1 on two different days. DOY 236 occurred after an irrigation, while DOY 295 took place near the end of the season in a dry period. Fluctuations in volumetric soil moisture through the ten-foot profile are indicative of a varying soil texture. A "surface effect" created by the scattering of neutrons to the atmosphere is evident in the first nine inches of depth. Estimations of available water and bulk density were derived from physical sampling before the season. Soil horizon labels and relative soil texture designations are provided. There was significant drying of the whole ten-foot profile between the two days.

Figure 3, panel 2, depicts the soil water profile of plot FACE-Dry Replication-1 on the same two days as in Figure 3, panel 1. As with Figure 3, panel 1, the neutron scattering "surface effect" is evident and soil horizon and general relative texture designations are given. Of special interest is that most of the soil dry-down occurred between 0 - 5.5 feet of depth, which suggests that there was a reduced rooting zone in the Dry plots (0 - 5.5 feet).

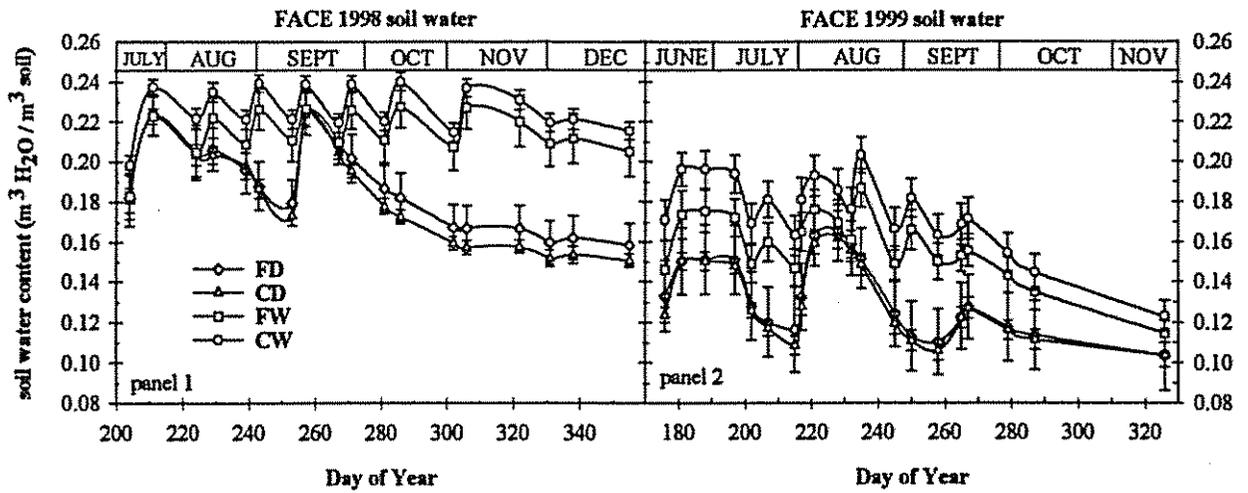


Figure 1. Soil water content means averaged over four replicates and six feet of depth for 1998 (left panel) and over ten feet of depth for 1999 (right panel) versus day of year, where FD = FACE-Dry, FW = FACE-Wet, CD = Control-Dry and CW = Control-Wet. Error bars show standard error calculated for each day between replications.

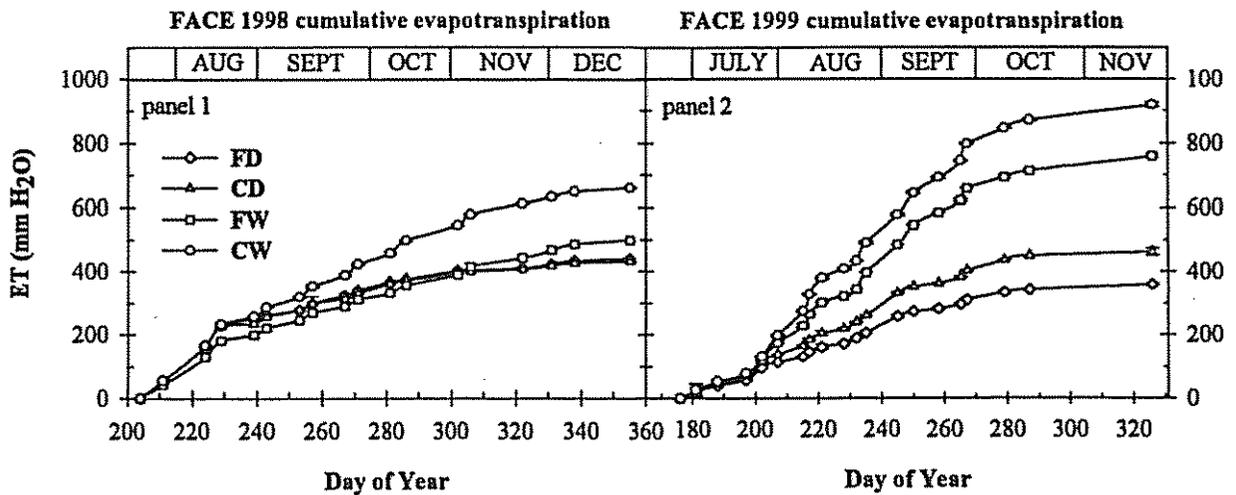


Figure 2. Cumulative evapotranspiration (ET) versus day of year, calculated across six feet of depth for the 1998 and 1999 seasons, where the treatment designations and errors are the same as Figure 1.

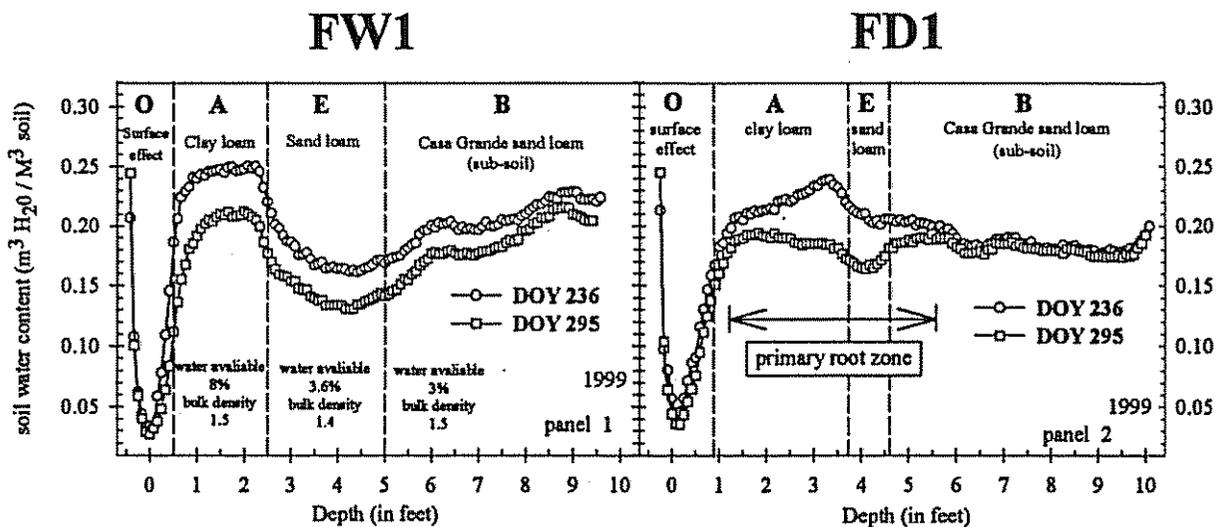


Figure 3. Soil water content in the FACE-Wet Replication-1 plot (FW1) left panel and in the FACE-Dry Replication-1 plot (FD1) right panel, versus depth, taken on contrasting (wet / dry) days with soil horizon, texture, available water and bulk density information.

INTERPRETATION: Measurements with neutron scattering equipment confirmed that a wide separation in the soil water content was achieved between the ample (Wet) and water-stress (Dry) irrigation treatments, as planned. However, within an irrigation treatment, consistent differences in water content were observed that appeared to be due as much to field variability in soil texture, i.e., water holding capacity, as to the CO₂ treatments. Nevertheless, when cumulative evapotranspiration (ET) was computed from the changes in water content during drying within each plot, a consistent, statistically significant pattern emerged. The average cumulative ET for 1998 and 1999 showed sorghum plants in the FACE-Dry plots used 10% less water than those in Control-Dry plots. Likewise, in the amply irrigated (Wet) plots, elevated CO₂ from the FACE treatment reduced seasonal ET by about 20.9%, which suggests that irrigation requirements for sorghum will be smaller in the future high-CO₂ world, provided that global climate changes are minimal.

FUTURE PLANS: Measurements of the soil moisture release characteristic, saturated K⁺, bulk density, porosity, and soil texture are to be determined from the plot locations.

COOPERATORS: See list given by Kimball et al. (this volume).

**ENERGY BALANCE AND EVAPOTRANSPIRATION OF SORGHUM:
EFFECTS OF FREE-AIR CO₂ ENRICHMENT (FACE)
AND SOIL WATER SUPPLY**

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PROBLEM: The CO₂ concentration of the atmosphere is increasing and is expected to double sometime during the next century. Climate modelers have predicted that the increase in CO₂ will cause the Earth to warm and precipitation patterns to be altered. Such increases in CO₂ and possible climate change could affect the hydrologic cycle and future water resources. One component of the hydrologic cycle that could be affected is evapotranspiration (*ET*), which could be altered because of the direct effects of CO₂ on stomatal conductance and on plant growth. Therefore, one important objective of the Free-Air CO₂ Enrichment (FACE) Project (Kimball et al., this volume) is to evaluate the effects of elevated CO₂ on the *ET* of sorghum and other crops.

APPROACH: We conducted two FACE experiments on sorghum from mid-July to mid-December 1998 and again from mid-June to the end of October 1999 (Kimball et al., this volume).

Briefly, the FACE apparatus consists of the following: Four toroidal plenum rings of 25 m diameter constructed from 12" irrigation pipe were placed in a sorghum field at Maricopa, Arizona, shortly after planting. The rings had 2.5-m-high vertical pipes with individual valves spaced every 2 m around the periphery. Air enriched with CO₂ was blown into the rings, and it exited through holes at various elevations in the vertical pipes. Wind direction, wind speed, and CO₂ concentration were measured at the center of each ring. A computer control system used wind direction information to turn on only those vertical pipes upwind of the plots so that the CO₂-enriched air flowed across the plots no matter which way the wind blew. The system used the wind speed and CO₂ concentration information to adjust the CO₂ flow rates to maintain desired CO₂ concentrations at the centers of the rings. The FACE CO₂ concentration was elevated by 200 ppm CO₂ above ambient (about 360 ppm in daytime) 24 hr/day all season long. Four matching Control rings with blowers to provide air flow but no added CO₂ were also installed in the field. Some additional measurements were made in "mid-field" areas between the FACE and Control plots where neither CO₂ nor air flow were altered.

In addition to the CO₂ treatments, varying soil water supply was also a factor. Using a split-plot design, the main circular CO₂ plots were divided into semi-circular halves, with each half receiving an ample irrigation regime (Wet) or else receiving a water-stress treatment (Dry). Using flood irrigation, the Wet plots were irrigated on roughly a two-week schedule, whereas the Dry plots were irrigated only twice (shortly after planting and at mid-season).

The determination of the effects of elevated CO₂ on *ET* by traditional chambers is fraught with uncertainty because the chamber walls that constrain the CO₂ also affect the wind flow and the exchange of water vapor. Therefore, as done previously in the FACE cotton and wheat experiments (Kimball et al., 1994, 1995, 1999), a residual energy balance approach was adopted whereby *ET* was

calculated as the difference between net radiation, R_n , soil surface heat flux, G_0 , and sensible heat flux, H :

$$\lambda ET = R_n - G_0 - H$$

R_n was measured with net radiometers and G_0 with soil heat flux plates. H was determined by measuring the temperature difference between the crop surface and the air and dividing the temperature difference by an aerodynamic resistance calculated from a measurement of wind speed. Air temperatures were measured with aspirated psychrometers, and crop surface temperatures were measured with infrared thermometers (IRTs) mounted above each plot. Fifteen-minute averages were recorded on a datalogging system. The net radiometers and IRTs were switched weekly between the FACE and Control plots. Moreover, the instruments were carefully calibrated before and after each experiment.

FINDINGS: The micrometeorological data have not yet been analyzed, so no report of the effects of the FACE treatment on the ET of sorghum can be made. In the prior FACE cotton experiment, the cotton had a large growth response (40% increase) to the elevated CO_2 , but no effect on ET was detectable (Kimball et al., 1994). In contrast, with wheat which had a modest growth response (about 20%), the FACE treatment decreased, ET , by an average 6.7% ($\pm 1.2\%$) for the four seasons under Wet, high-nitrogen conditions. Under low nitrogen, the reduction in ET was 19.5% (Kimball et al., 1999).

The sorghum growth response to elevated CO_2 was insignificant under the Wet conditions (Kimball et al., this volume), so we hypothesize that there was a reduction in ET . Then under the Dry conditions, the lower rate of ET following an irrigation enabled the FACE plants to photosynthesize and grow longer into a drought cycle, which resulted in greater total growth and yield under the FACE treatment compared to the Control plots.

INTERPRETATION: It appears from the prior FACE cotton experiments that cotton irrigation requirements will not change, whereas for wheat they may become somewhat lower in the future high- CO_2 world (provided that any global warming is small). We can make no definitive statement about sorghum yet.

FUTURE PLANS: Complete the analysis of the micrometeorological data from the two FACE sorghum experiments and write the corresponding manuscript.

COOPERATORS: See Kimball et al., "The Free-Air CO_2 Enrichment (FACE) Project: Progress and Plans" (this volume).

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FACE 1999: CHANGES IN PHOTOSYNTHETIC APPARATUS OF SORGHUM IN RESPONSE TO CO₂-ENRICHMENT AND WATER STRESS

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BACKGROUND: With the expected doubling of atmospheric levels of CO₂ sometime in the 21st century, it is important to understand how this change will affect our way of life and, more specifically, how it will affect plant life. The Free Atmospheric CO₂ Enrichment (FACE) facility at the University of Arizona Maricopa Agricultural Research Center is helping to determine how plants respond and acclimate to long-term exposure to elevated levels of CO₂ in the field. A compilation of the Maricopa FACE data from 1995 and 1996 showed that elevated CO₂ caused final grain yields of spring wheat, a cool-season crop, to rise by an average of 16% under non-limiting water and nutrient conditions (Pinter et al., 1997). However, under lower levels of nitrogen, CO₂ enrichment increased yields by 8%. In order to predict the responses of wheat to further increases in atmospheric CO₂, we investigated the responses of photosynthetic parameters and proteins of spring wheat to elevated CO₂.

Plants acclimate to changes in CO₂ concentration through changes in the amounts and activities of enzymes required to reestablish a balance within the photosynthetic apparatus. An earlier report (Adam et al., 1997) presented data from a gas-exchange technique in which photosynthesis (A) is measured at a range of intracellular CO₂ concentrations (C_i), providing information on changes within the photosynthetic apparatus. Since Rubisco (the enzyme catalyzing the initial reaction of photosynthesis in spring wheat) capacity is limiting at low values of C_i, the slope of the A-C_i relationship at those low values of C_i can be used to assess changes in the ability of Rubisco to fix CO₂. This slope, called the "carboxylation efficiency", can be used as an indicator of down-regulation in which the amount of Rubisco is decreased in response to the greater concentration of atmospheric CO₂. Because Rubisco can fix either CO₂ or O₂, increases in the relative proportion of CO₂ in the atmosphere could be expected to help the plant fix more CO₂ or to reduce (or down-regulate) the amount of Rubisco in order to fix the same amount of CO₂. If down-regulation does occur, we could expect an upper limit to the yield increases commonly seen under CO₂ enrichment. The carboxylation efficiency of spring wheat was affected by growth in elevated CO₂. Follow-up work to support the carboxylation efficiency data was presented by Adam et al. (1998). By assaying the Rubisco activity and content of the leaves which were used for the A-C_i gas-exchange analysis, we provided further support for down-regulation. The Rubisco activity and content data supported the interpretations of the carboxylation efficiency data, indicating that down-regulation of the photosynthetic apparatus in response to elevated CO₂ did occur. However, the responses of Rubisco activity of wheat to CO₂ enrichment and N fertilization were dependent on growth stage and the position of the leaf within the canopy.

The work with spring wheat, a so-called C₃ plant, indicated that, in order fully to assess the response of crop plants to elevated CO₂, various growth stages and a canopy profile must be measured. Similar experiments were conducted in 1998 and 1999 on sorghum, a warm-season crop with a different, so-called C₄, carbon-trapping mechanism. The carbon-trapping enzyme of sorghum is PEPCase which, unlike Rubisco, fixes only CO₂ and not O₂. The product of the reaction catalyzed by PEPCase is then shuttled to Rubisco and into the Calvin cycle. Because PEPCase has the effect

of concentrating CO₂ in the leaf, then plants with this pathway have not been expected to respond greatly to increases in atmospheric CO₂ concentrations. The objective of these experiments was to determine the effect of growth in elevated CO₂ on the gas exchange parameters and photosynthetic proteins of sorghum.

APPROACH: *Sorghum bicolor* (L.) Moench (cv. Dekalb 54) was planted in an open field at The University of Arizona Maricopa Agricultural Research Center, located 50 km south of Phoenix, Arizona (33.1 °N, 112.0 °W). Sorghum was planted on July 13 and 14, 1998, and again on June 14 and 15, 1999, (Kimball et al., 1999, this volume). Fifty percent emergence occurred July 30, 1998, and July 1, 1999. Following sowing, a FACE apparatus was erected on site to enrich the CO₂ concentration of the ambient air (ca. 370 μmol mol⁻¹) to ca. 570 μmol mol⁻¹. Water was applied as a split plot factor using flood irrigation such that "Wet" plots received ample water while "Dry" plots received only two irrigations and were severely stressed. All plots received 278.7 kg ha⁻¹ N.

For the first sampling date (the 4th and 5th leaf stage), gas exchange analyses were conducted on the uppermost fully-expanded leaf (referred to as flag leaf) and on the flag minus one leaf. Thereafter, measurements were made on the flag leaf and the flag minus two leaf. Photosynthesis rates were measured over a range of intracellular CO₂ levels, generating an A-Ci curve. At the end of each curve, the leaf was frozen as quickly as possible with a liquid nitrogen-cooled clamp and stored in liquid nitrogen. Activity of Rubisco, PEPCase, and PpdK will be assayed from leaves collected from both years.

FINDINGS: The A-Ci gas exchange measurements made on sorghum during the 1998 season showed a strong reduction in the initial slope of the A-Ci curve due to CO₂ enrichment in the early part of the season (Fig 1). The effect of the CO₂ on the flag leaf was not influenced by irrigation level. However, the effect of the CO₂ on the flag minus two leaf was more consistent in the dry treatment.

INTERPRETATION: The reductions in the carboxylation efficiency of sorghum due to CO₂ enrichment were surprising and seemed to depend on leaf age or growth stage. However, biochemical assays on the frozen leaf pieces must be conducted before conclusions may be drawn as to whether down-regulation did occur.

FUTURE PLANS: Biochemical assays will be conducted on both the 1998 and 1999 samples to determine activities and relative quantities of key enzymes of the sorghum photosynthetic pathway. The assay results will be compared to the gas exchange data in order to determine whether down-regulation is occurring or if there is some other mechanism involved.

COOPERATORS: We wish to acknowledge the collaborative efforts of Andrew Webber of Arizona State University, Tempe AZ, for helpful advice and the use of his laboratory; Steve Leavitt, Alan Matthias, and Tom Thompson of the University of Arizona, Tucson AZ; Bob Roth and Dave Langston from the Maricopa Agricultural Center, Maricopa AZ; Keith Lewin, John Nagy, and George Hendrey of Brookhaven National Laboratory, Uptown NY; and George Koch of Northern Arizona University, Flagstaff AZ. We also thank Jonathan Triggs and Jose Olivieri for technical assistance.

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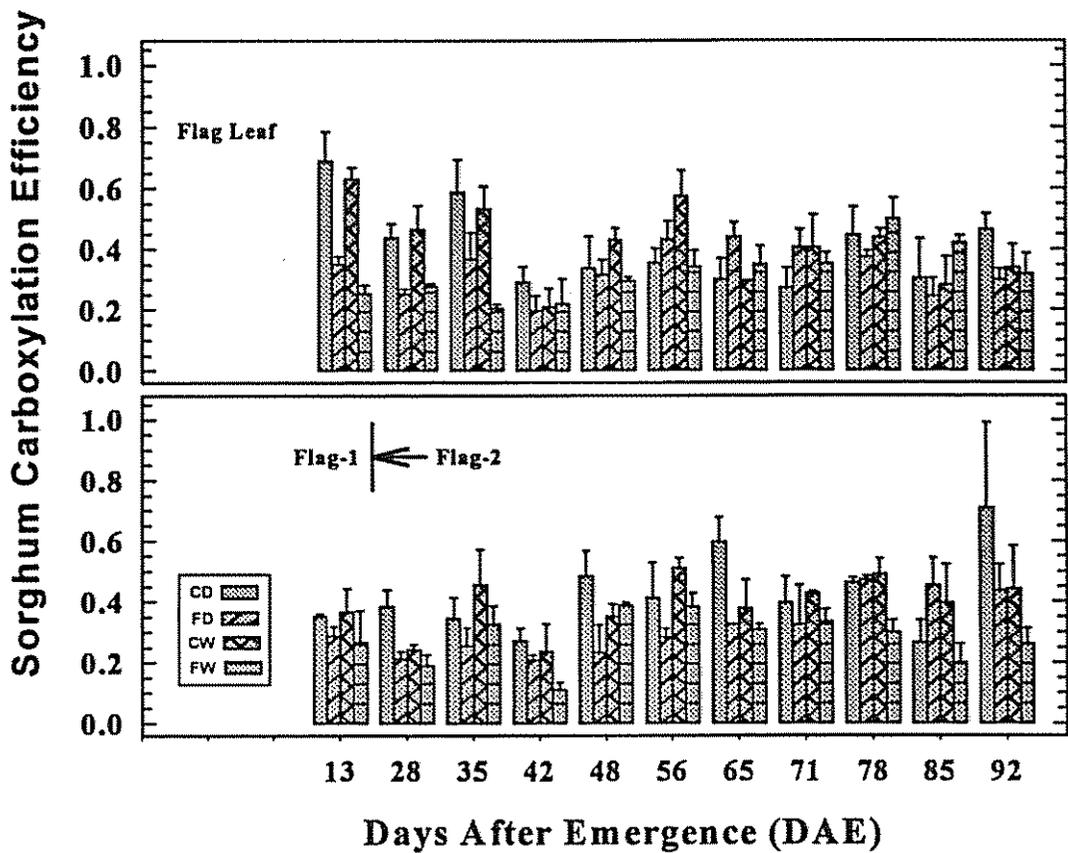


Figure 1. Carboxylation efficiency of the uppermost, fully-expanded (flag) leaf and a lower (flag-1 or flag-2) leaf of sorghum throughout the 1998 season. Treatments include: CD or Control-Dry (normal CO₂ and water stress); FD or FACE-Dry (enriched CO₂ and water stress); CW or Control-Wet (normal CO₂ and ample water); and FW or FACE-Wet (enriched CO₂ and ample water).

THE ENHANCEMENT OF PHOTOSYNTHESIS DURING THE 1999 FACE SORGHUM EXPERIMENT.

A.B. Cousins, Graduate Student; N.R. Adam and G.W. Wall, Plant Physiologists;
and A.N. Webber, Professor

PROBLEM: Natural processes of the earth, coupled with the onset of the industrial revolution and the exponential growth of the human population, are causing rapid changes to our environment at unprecedented rates. By the end of the next century, anthropogenic activities are predicted to cause the earth's atmospheric carbon dioxide (Ca) concentration to double to ca. $700 \mu\text{mol mol}^{-1}$ (McElroy, 1994). Researchers have studied effects of elevated Ca on many aspects of plant development, metabolic regulation and net photosynthetic productivity (see recent reviews Stitt, 1991; Bowes, 1991). Increasing Ca is predicted to cause a significant response in photosynthesis of terrestrial plants, in particular the assimilation of carbon by Ribulose biphosphate carboxylate/oxygenase (Rubisco) and Phosphoenol Pyruvate carboxylate (PEPC) (Edwards and Walker, 1983).

C4 plants, such as sorghum, have the ability to concentrate CO_2 at the site of Rubisco to concentrations 10-20 times that of atmospheric levels. Coordination and compartmentalization of specific biochemical and anatomical features are required to maintain and utilize this CO_2 pump. In mesophyll cells (MSC) CO_2 , in the form of HCO_3^- , is fixed by PEPC into Oxalacetic Acid (OAA), which is converted to malate (MA) and passively transported into bundle sheath cells (BSC). Within BSC, MA is decarboxylated releasing CO_2 which enters the photosynthetic carbon reduction (PCR) cycle. Two adenosine-triphosphate (ATP) molecules are required to regenerate Phosphoenol Pyruvate (PEP) from pyruvate produced from the NADP-malic enzyme catalyzed MA decarboxylation reaction. The basal energy requirement of the NADPH-type C4 mechanism is 5 ATP and 2 NADPH per CO_2 fixed. However, the quantum requirement of C4 plants varies depending on the extent of CO_2 leakage from the BSC, overcycling of the C4 pump, and the contribution of the Q-cycle to the production of the proton motive force. Calculations of the resistance to CO_2 diffusion from BSC to intercellular space r_c ($\text{m}^2 \text{ s mol}^{-1}$) and the total resistance to diffusion of CO_2 from air to chloroplast of BSC r_t ($\text{m}^2 \text{ s mol}^{-1}$) have been made from measurements of photosynthesis, photorespiration, and O_2 isotope exchange measurements (He and Edwards, 1996). Data analyzed from Dai *et al.* (1995) shows the lowest r_c values in *Z. mays* came from young and senescing tissues. This implies that as the value of r_c decreases, the ability of CO_2 to diffuse between the BSC and the intercellular spaces of the mesophyll cells increases.

Due to the ability of C4 plants to concentrate CO_2 they are not expected to show an increased growth response to elevated Ca levels. However, several studies have shown that growth of C4 plants under elevated atmospheric CO_2 concentrations, even under well watered conditions, stimulates an increase in biomass production. One possible explanation for the growth stimulation of C4 plants at high CO_2 is that the immature C4 pathway in young leaves has C3-like characteristics, and consequently photosynthesis is responsive to increasing CO_2 supply above the current ambient concentrations. Under current atmospheric CO_2 concentrations, the carboxylation reaction in C3 plants is inhibited by the oxygenation reaction, which reduces the net uptake of carbon. A reduction in O_2 partial pressure increases the efficiency of the carboxylation reaction and stimulates the net rate of carbon assimilation. In *Zea mays*, the development of the C4 photosynthetic apparatus occurs early in leaf development. As young leaves emerge into full sunlight from the surrounding whorls formed by older

leaves, the expression and compartmentalization of the C4 photosynthetic apparatus is nearly complete. A similar pattern of development of the C4 photosynthetic pathway is observed in sorghum (Cousins, unpublished observations).

In this study we monitored the effects of Free Air Carbon-Dioxide Enrichment (FACE) conditions on the photosynthetic performance of *Sorghum bicolor* DK54. Following the ontological plant development throughout the growth season of the FACE sorghum project, the uppermost fully-expanded leaves were sampled at various days after planting (DAP). We used the measurements of chlorophyll-A fluorescence and carbon assimilation to address energy utilization, quantum requirement, oxygen sensitivity and net photosynthesis (A^*) enhancement of FACE-grown plants.

APPROACH: Field grown *Sorghum bicolor* (DK54) was exposed to ambient ($\sim 370 \mu\text{mol mol}^{-1}$) and FACE (ambient +200 $\mu\text{mol mol}^{-1}$) CO_2 levels as described by Kimball *et al.* "Progress and Plans for the FACE Project" and Pinter *et al.* "Daytime CO_2 and Nighttime Blower Effects on Canopy Temperatures and Frost Damage during the 1998 FACE Sorghum Experiment" in the 1998 USWCL Annual Research Report.

Plant material was sampled prior to 7:30 am and stored at 10°C in darkness until measurements were made as described by Adam *et al.* (submitted). Leaf samples to be measured were placed into a 6400-06 PAM2000 Adapter cuvette (LiCor, Inc., Lincoln NE), which fits the fiber-optic probe of the pulse modulated fluorometer (PAM 2000, Walz, Effeltrich, Germany) above the leaf at a 60 degree angle. Plants were dark-adapted for a minimum of one hour and simultaneous measurements of chlorophyll A fluorescence and gas exchange were made to determine dark respiration rates (R_D), F_O and F_M values. Subsequently, the cuvette was illuminated with $\sim 800 \mu\text{mol photon m}^{-2}\text{s}^{-1}$ by a 400 W halogen lamp, and leaf temperature was maintained at $30 \pm 1^\circ\text{C}$. Leaf samples were acclimated for approximately one hour until steady state photosynthesis and chlorophyll-A fluorescence were attained. The quantum yield of PSII (ϕPSII), determined by $\phi\text{PSII} = (F_M - F_S)/F_M$, and carbon assimilation (A , $\mu\text{mol CO}_2\text{m}^{-2}\text{s}^{-1}$) were simultaneously determined at $[\text{Ca}]$ values of 75, 200, 370, 570 and $700 \mu\text{mol mol}^{-1}$ in air containing 21% or 2% oxygen.

FINDINGS: The photosynthetic ($\mu\text{mol CO}_2 \text{m}^{-2}\text{s}^{-1}$) response (A) and dark respiration rates of *S. bicolor* measured at growth CO_2 concentrations for ambient and FACE conditions were measured throughout the season, Table 1. The percent stimulation of A for FACE grown plants is shown in Table 1. At 6 days after planting (DAP), the second leaf was the uppermost fully expanded and showed the largest percent increase, 37% ($p=0.027207$). Less pronounced increases in assimilation occurred later in the growth season at 28 ($p=0.017191$), 36 ($p=0.017521$) & 60 ($p=0.02224$) DAP. Carbon assimilation of fully expanded leaves of C4 plants have been reported to be saturated at current atmospheric CO_2 concentrations. To investigate further the enhancement of carbon assimilation by FACE, condition A was measured at growth CO_2 concentrations in air containing oxygen concentrations of both 21% and 2%, Figure 1a & b. A 16% ($p=0.094363$) increase in stimulation of A by 2% O_2 for ambient grown plants occurred in the second leaves at 6 DAP.

The stimulation of A at 2% O_2 observed at growth $[\text{CO}_2]$ was not observed at lower CO_2 concentrations. This may be due to the requirement of C3 cycle activity on the supply of NADPH from C4 cycle activity. The quantum efficiency of CO_2 fixation for leaves 3 and 5 are approximately 12 and 13 at higher $[\text{Ca}]$ but increased slightly as the $[\text{Ca}]$ decreased (data not shown). In the second

leaves, the $\phi\text{PSII}/\phi\text{CO}_2$ greatly exceeded the theoretical minimum energy requirement at low [Ca] but the ratio dropped close to the minimum value of 8 as [Ca] increases. At low [Ca] the large energy requirement per CO_2 fixed may be due to the overcycling of the C4 pump and leakage of CO_2 from within the BSC. Increasing the [Ca] reduces overcycling and leakage of CO_2 from the BSC and inhibits photorespiration, causing a reduction in the $\phi\text{PSII}/\phi\text{CO}_2$ ratio.

INTERRETATIONS: Carbon assimilation, when measured at growth [Ca], in young *S. bicolor* leaves was enhanced by FACE conditions. Partial stimulation of A in young plants was due to oxygen sensitivity as shown by the increase in A at 2% O_2 . Additionally, elevated [Ca] enhanced energy use efficiency possibly by decreasing overcycling of the C4 pump and reducing the amount of CO_2 leaking from the BSC. These results suggest that the stimulation of C4 photosynthesis under elevated CO_2 is due to the enhancement of A and energy use efficiency early in the development of the plant. Further investigations are needed to understand leakiness and overcycling of leaves in young sorghum plants.

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Table 1. Gross assimilation, A, and respiration rates, R_D ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), of the uppermost fully expanded leaves from ambient and FACE treatments at various days after planting. Measurements were made at ambient and FACE growth CO_2 concentrations, $370 \mu\text{mol mol}^{-1}$ and $570 \mu\text{mol mol}^{-1}$ respectively. $n = 3$ (SE).

DAP	Gross Photosynthesis (A) $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$		% difference	R_D $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$	
	Ambient	FACE		Ambient	FACE
6	23.33(+2.05)	31.90(+2.61)	36.7	-0.80(+0.28)	-1.86(+1.01)
9	27.86(+2.73)	28.00(+1.66)	0.5	-2.30(+0.13)	-2.49(+0.61)
19	29.40(+2.15)	26.33(+1.29)	-10.4	-1.19(+0.29)	-0.61(+0.21)
23	22.23(+1.51)	25.80(+1.35)	16.1	-2.39(+0.39)	-2.28(+0.17)
38	26.46(+ 1.31)	29.91(+1.54)	13.0	-0.93(+0.27)	-1.10(+0.28)
60	25.91(+ 0.29)	29.85(+0.93)	15.2	-1.00(+0.26)	-0.76(+0.23)

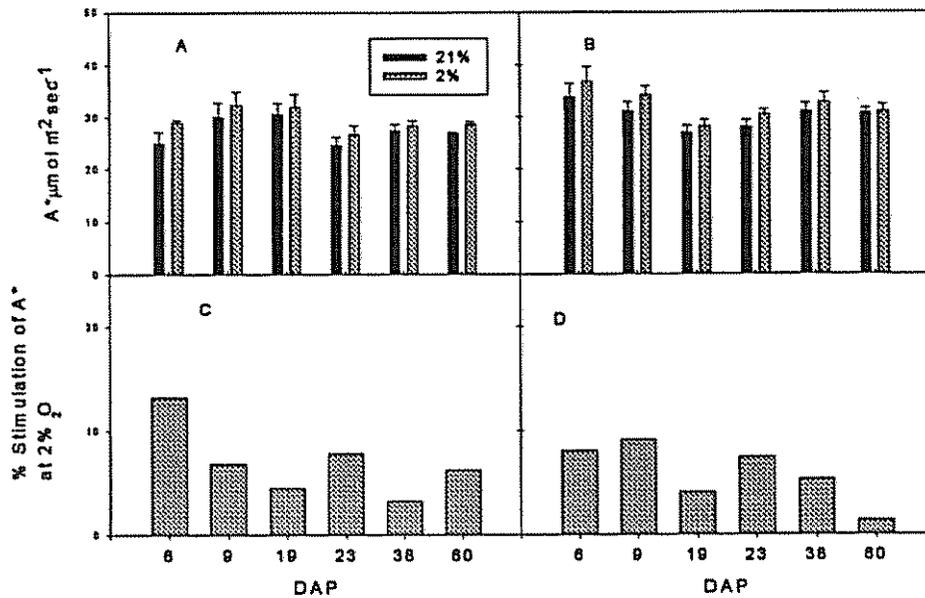


Figure 1. The response of A^* to changes in ambient O_2 concentrations. Measurements were carried out at ambient growth [Ca] values of 370ppb (A) and FACE-grown conditions of 570ppb (B). The percent enhancement of A^* by 2% oxygen for ambient and FACE conditions, (C) and (D).

EFFECTS OF WATER STRESS AND CO₂ ON SORGHUM CANOPY ARCHITECTURE AND GAS EXCHANGE: A RATIONALE FOR STUDY AND PROGRESS REPORT

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G.W. Wall, Plant Physiologist; P.J. Pinter, Jr., Research Biologist;
B.A. Kimball, Research Leader; and R.L. LaMorte, Civil Engineer

PROBLEM: The Intergovernmental Panel on Climate Change (IPCC) reports that global CO₂ levels will rise from the current ambient level of 370 $\mu\text{mol mol}^{-1}$ to over 500 $\mu\text{mol mol}^{-1}$ by the end of the 21st century (IPCC, 1995). Of primary concern to the human population is the impact that rising global CO₂ concentrations will have on agriculture. The U.S. Water Conservation Laboratory (USWCL) Environmental and Plant Dynamics (EPD) Group has been investigating the impact of increased CO₂ and water stress on various C₃ agricultural crops for the past 8 years through the use of a Free-Air CO₂ Enrichment apparatus (FACE) (Hendrey, 1993; Hendrey and Kimball, 1994). These FACE experiments used small (1m²) pop-on chambers to measure the rates of net photosynthesis and canopy conductance, thereby providing "snapshots" of crop physiology (Kimball et al., 1995) Results from these investigations have enabled members of the EPD Group to conclude that canopy photosynthesis and water use efficiency are improved in C₃ plants, such as wheat and cotton, when subjected to CO₂-enriched environments (Kimball et al., 1995).

The primary carboxylating enzyme for carbon assimilation in C₃ plants is ribulose-1,5-bisphosphate oxygenase/carboxylase (Rubisco), though phosphoenolpyruvate carboxykinase (PEPC) is present (approximately 20:1 ratio of Rubisco to PEPC). Rubisco may account for as much as 50% of all soluble leaf protein. Rubisco is not saturated at normal atmospheric CO₂ concentration. Thus, as CO₂ is increased, the relative saturation of Rubisco increases and the overall photosynthesis rate increases.

A second type of carbon assimilation, the C₄ pathway, is used by plants such as sorghum, maize, and sugarcane. This pathway relies on PEPC as the primary carboxylating enzyme. Unlike a C₃ plant, special Kranz anatomy compartmentalizes the carbon assimilation pathway into the bundle sheath cells and the mesophyll. In essence, compartmentalization and PEPC act to serve as a CO₂ concentrating mechanism.

The FACE 1998-2000 investigation sought to understand if improved plant growth and yield observed for C₃ crops would be similar for C₄ sorghum when grown in a CO₂-enrichment x water stress experiment. For well-watered conditions, it is unlikely that sorghum will respond to CO₂ enrichment, because the CO₂ concentrating mechanism keeps PEPC near saturation regardless of ambient CO₂ concentration. However, it is suspected that drought stress may cause minute changes in the cellular structure of Kranz anatomy allowing CO₂ to "leak" in and out of the bundle sheath cells. Bundle sheath cell leakiness would allow for a greater response to CO₂ for sorghum grown under water-stressed conditions than ample-watered conditions. In addition, previous FACE experiments on C₃ species have demonstrated that elevated CO₂ causes partial stomatal closure thereby improving plant water use efficiency and reducing soil water depletion. It is possible that sorghum may react in a similar manner during early growth as the C₄ mechanism develops and later due to bundle sheath cell leakiness.

APPROACH: Four 25-m-diameter rings were placed in the field and used continually to enrich the CO₂ concentration of the air to 200 μmol mol⁻¹ above ambient. Four identical rings served as controls. Ample water was applied to one half of each ring, while the other half was subjected to water stress (strip-split plot design). “Flow-through” chambers were placed in each treatment of replicates 3 and 4 and were used to collect canopy carbon exchange data for a period of 10 days. At the end of this time period the chambers were moved to a new location within the treatment. Measurements of canopy greenness, plant area index (PAI), mean leaf tip angle distribution (LTA), and solar radiation (PAR) were made on a weekly basis. Resulting data are in the process of being analyzed.

FINDINGS: As of the writing of this report, data collection and analysis were still under way; however, a sample of each data type collected has been provided in the following figures.

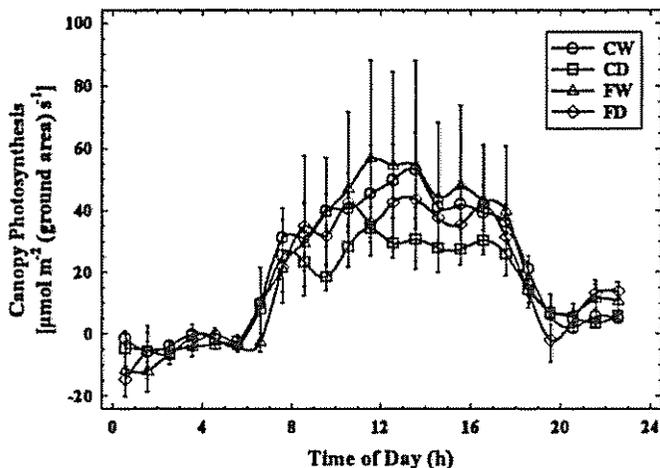


Figure 1. Sorghum canopy photosynthesis rate for July 24, 1999 (DOY 203). Data are plotted as hourly averages across 2 replicates. Error bars represent 1 standard error of the mean.

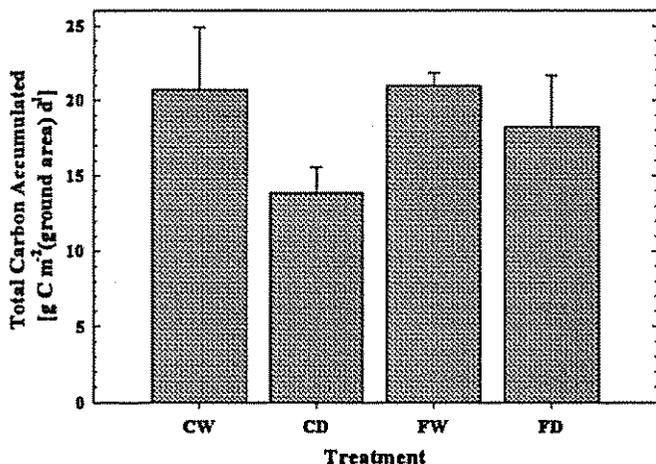


Figure 2. Daily total carbon accumulation. The areas under each curve presented in Figure 1 were integrated to provide the total amount of carbon accumulated by the plant during that day (DOY 203). Error bars represent 1 standard error of the mean.

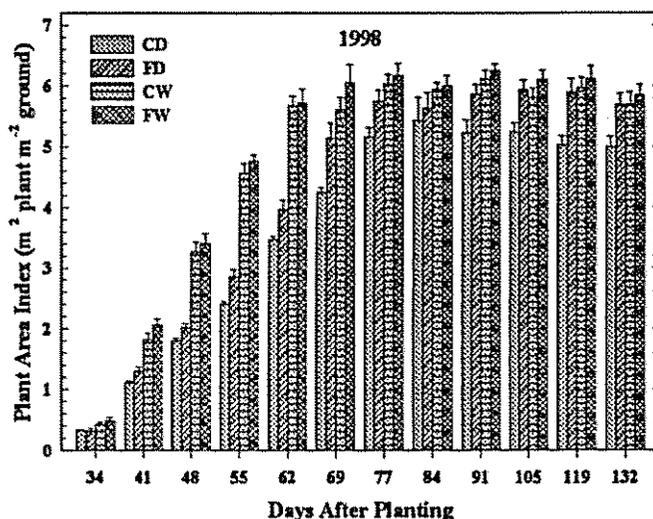


Figure 3. Plant area index for 1998. Plant area index is a derived factor relating the surface area of standing plant occupying a given ground area.

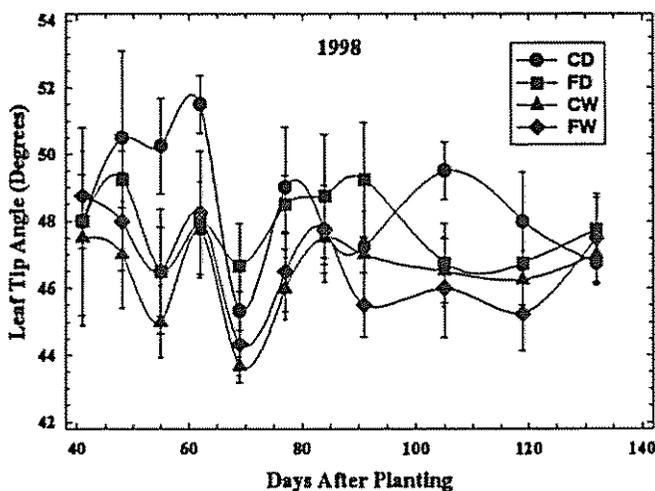


Figure 4. Mean leaf tip angle distribution during 1998. 90 degrees represents a horizontal leaf surface.

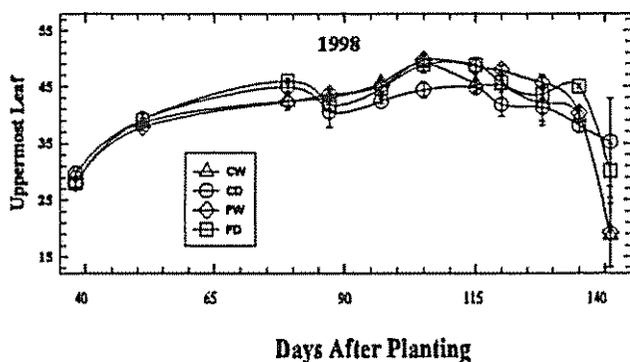


Figure 5. Uppermost fully expanded leaf greenness during 1998, as measured using a portable leaf chlorophyll meter. Similar measurements were made on leaves lower in the canopy as well.

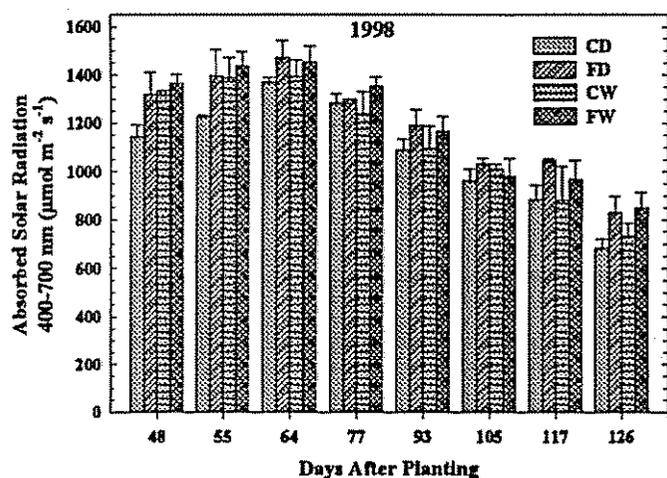


Figure 6. Total absorbed solar radiation during 1998. This data was collected in such a manner that it can be broken down into 0.20 - m increments throughout the growing season to allow for the calculation of radiation use efficiency at different canopy heights

INTERPRETATION: Initial analysis suggests that CO_2 affected whole canopy photosynthesis rates, architecture, and light capture for plants grown under water-stressed conditions more than those given ample water. The degree to which each parameter was affected is indeterminate at this stage of the analysis.

FUTURE PLANS: We will continue with data analysis; the results will be used in a manuscript for publication in a peer-reviewed journal and as a chapter in my doctoral dissertation. Data collected during this experiment also will be used as a validation data set for computer modeling applications pertaining to plant growth and global climate change. An extended stay at the University of Alberta to work with Dr. Robert Grant on the latter topic is currently planned for January-April 2000.

COOPERATORS: see Kimball et al., this report.

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NDVI, fAPAR, AND PLANT AREA INDEX IN THE 1999 FACE SORGHUM EXPERIMENT

P.J. Pinter, Jr., Research Biologist; B.A. Kimball, Supervisory Soil Scientist; R.R. Rokey and S.M. Gerszewski, Biological Technicians; T.J. Brooks, Research Technician; R.L. LaMorte, Civil Engineer; and G.W. Wall and N.R. Adam, Plant Physiologists

PROBLEM: Anticipated increases in atmospheric carbon dioxide (CO₂) and changes in global climate will have important consequences for production agriculture and the world's food supply. Evidence from Arizona Free Air Carbon dioxide Enrichment (FACE) experiments and similar studies at other FACE sites around the world have revealed positive effects of elevated CO₂ on the productivity of C₃ plants under natural field conditions. During 1998 and 1999 we used FACE to study the interactive effects of supra-ambient concentrations of CO₂ and reduced water supply on the agronomy and final yields of grain sorghum, a globally important C₄ crop species. This report discusses preliminary results from that experiment using several non-destructive methods to infer canopy development and light absorption.

APPROACH: Sorghum (*Sorghum bicolor* L. Dekalb Hybrid DK54) was sown (10.0 kg ha⁻¹; ~318,000 seeds ha⁻¹) in north-south rows spaced ~0.76 m apart at The University of Arizona Maricopa Agricultural Center (MAC) on June 14 and 15, 1999. Emergence of seedlings occurred on or about July 2, 1999; density was estimated at 26.0 plants m⁻². Treatments were similar to those imposed in 1998. Plants were exposed to ambient (Control, ~370 μmol mol⁻¹) and enriched (FACE, +200 μmol mol⁻¹ above ambient) CO₂ levels; treatments were replicated four times. CO₂ treatment plots were split in half to test the effect of different flood irrigation regimes on sorghum response to CO₂. The final harvest of plots in the FACE arrays took place on October 26, 1999. Additional experimental details can be found in Kimball et al., "The Free-air CO₂ Enrichment (FACE) Project: Progress and Plains" pages 58-60 in this volume.

NDVI. Canopy reflectance factors were obtained 45 times between June 24 and October 22, 1999, using a handheld radiometer (Exotech, Inc., Gaithersburg, MD) equipped with 15° fov optics. Twenty-four measurements were taken along a 7 m transect on the north edge of the no-traffic, final harvest area in each treatment combination and replicate. Data were obtained at a time corresponding to a nominal solar zenith angle of 45°. Red (0.63 to 0.69 μm) and near-infrared (NIR, 0.78 to 0.89 μm) reflectance factors were used to compute the Normalized Difference Vegetation Index [NDVI = (NIR-red)/(NIR+red)].

fAPAR. Incident (I), transmitted (T), and reflected (R) light in photosynthetically active wavelengths (PAR, 0.4 to 0.7 μm) were measured just prior to midday (1100-1215h MST) on 7 dates during the season using an Accupar sensor (Decagon Instruments, Inc., Pullman, WA). Measurements were taken above and below the plant canopy in six adjacent rows along the north edge of the final harvest area. The 80 cm-long sensor was oriented perpendicular to plant rows. Data were recorded separately for each 5 cm segment of the sensor. Reflected PAR also was obtained over a bare soil plot (RPAR_s). The fractional amount of PAR (fAPAR_c) was computed using a light balance equation:

$$fAPAR_c = 1 - \left(\frac{TPAR_c}{IPAR} \right) - \left(\frac{RPAR_c}{IPAR} \right) + \left(\frac{TPAR_c}{IPAR} \right) * \left(\frac{RPAR_s}{IPAR} \right)$$

where the subscripts c and s refer to the sorghum canopy and a bare soil plot, respectively.

PAI. A Plant Canopy Analyzer (LAI-2000, LICOR Inc., Lincoln, Nebraska) was used to obtain data on plant area index (PAI). Data were collected shortly after dawn at 1-3 week intervals during the season (7 dates). The sensor was deployed between six adjacent rows on the north edge of the final harvest area. The sensor and canopy were shaded from direct beam solar radiation with a manually positioned, opaque panel measuring 1 by 1 m and held at a distance of 5 to 10 meters from the optics. The PAI parameter was determined from a total of 3 measurements above the canopy and 18 below the canopy in each plot. A radiative transfer algorithm computes PAI from canopy light interception at 5 different angles of incidence on the fish-eye like sensor (148° field-of-view).

FINDINGS: NDVI. The seasonal NDVI data are shown in figure 1. The upper panel shows the mean ± 1 standard error of each treatment combination (abbreviations refer to: CD, Control Dry; CW, Control Wet; FD, FACE Dry; FW, FACE Wet). The bottom panel shows the mean of each treatment relative to the value observed in the Control Wet treatment. The NDVI trajectory revealed an early season phase where the signal was dominated by reflectance of bare soil and modulated by seedlings that were slowly increasing in size. This was followed by a rapid increase in NDVI as the plants entered an exponential growth phase and rapidly attained full canopy cover. Water stress between day of year 190 and 225 resulted in lower NDVI values for the dry treatments. The NDVI for the wet treatments attained a plateau for about 3 weeks before panicle emergence (~DOY 235), then declined gradually due to spectral properties of developing heads and senescing leaves. The NDVI decline was slightly faster in FACE Wet compared with Control Wet, and more pronounced in the Dry compared with the Wet treatments.

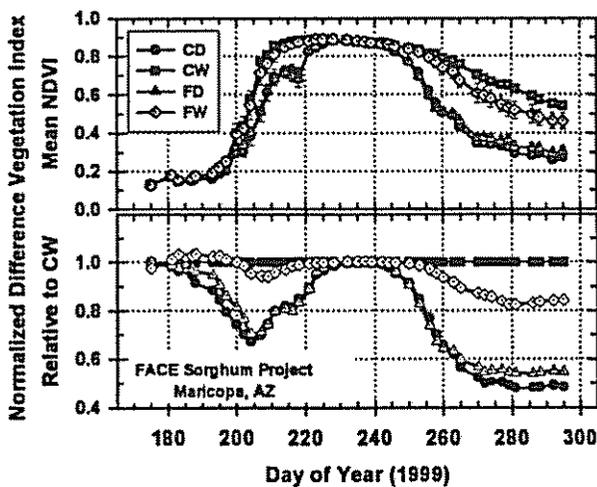


Figure 1. NDVI measured in sorghum in 1999.

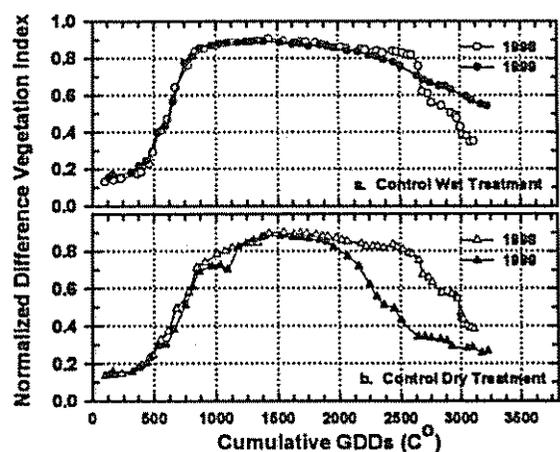


Figure 2. NDVI vs cumulative growing degree days for the Control CO₂ treatments in 1998 and 1999.

Seasonal NDVI trajectories can be superimposed to examine year-to-year differences in canopy response to treatments or variation in climate. In Figure 2, we graph NDVI against cumulative growing degree days (GDDs) calculated from the AZMET weather station at MAC. Figure 2a reveals early season similarities in the Control Wet treatments for both 1998 and 1999 despite a one-month difference in planting date. Additionally, the NDVI data from 1999 shows what we believe is a typical end-of-season senescence pattern, while that from 1998 displays a non-typical, protracted green canopy duration caused by suboptimal late-season temperatures followed by a two-step decline in green canopy after frost events (Pinter et al, 1998 USWCL Annual Research Report). The early- and late-season water stress in

the dry treatment was much more severe in 1999 than in 1998 as shown by lower NDVI from ~900 to 1100 GDDs and after 1900 GDDs.

fAPAR_c Data on canopy PAR absorption were first obtained with the Accupar sensor beginning in mid-August (DOY 201), about 3 weeks after plant emergence (Fig. 3). From that point in the season until the plants completely covered the soil in mid-August, we observed rapid increases in midday fAPAR_c. Considering the differences in planting dates and thermal time between 1998 and 1999, the fAPAR_c data were qualitatively similar in both years. Significant fAPAR differences were noted between irrigation treatments both early and late in the season while there was little effect that could be attributed to the CO₂ treatments. The fAPAR_c for the wet irrigation treatments remained >90% from before heading until the end of September (early- to mid-grain fill). Towards the end of the season, the dry treatments absorbed about 10-15% less PAR than their wet counterparts.

PAI The LAI-2000 measurements of PAI also were similar to those seen in 1998. Significant

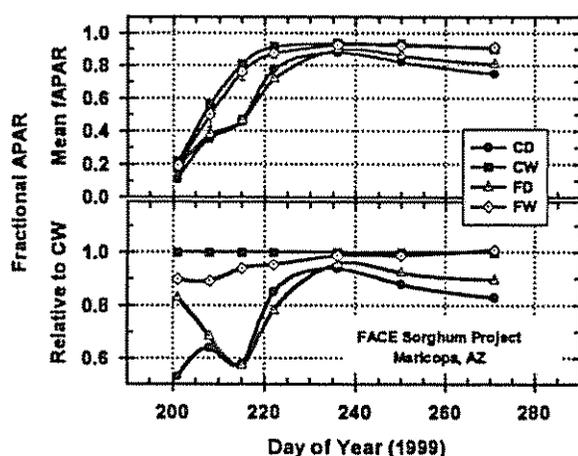


Figure 3. Fraction of absorbed photosynthetically active radiation (fractional APAR) during 1999.

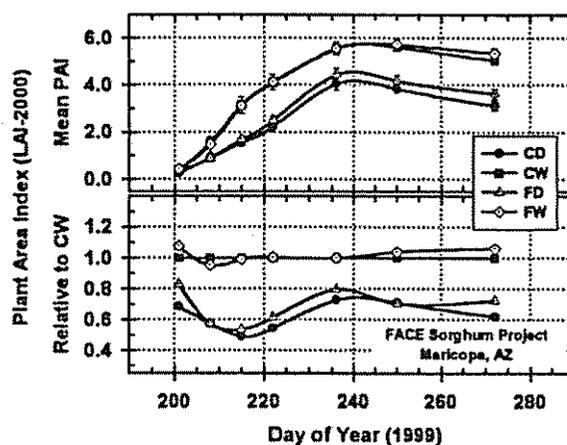


Figure 4. Plant area index measured with the LAI 2000 during 1999.

differences were observed between irrigation treatments in the rate and extent of plant canopy development (Fig. 4). There was a steady increase in PAI in the wet treatments up until early-September, and then it leveled off at about 5.5 to 6.0 units both in elevated and ambient CO₂ treatments. PAI in the dry treatments also increased in a regular fashion, albeit at a noticeably slower rate. At anthesis (early- to mid- September), PAI in the dry treatments was 70 to 80% of that observed in the wet treatments. Elevated CO₂ had little effect on PAI in either the wet or dry irrigation treatments. Unlike the NDVI, which saturated for several weeks around anthesis, PAI retained the capability clearly to distinguish between the water stressed treatments throughout the season.

INTERPRETATION: The NDVI, PAI, and fAPAR_c measurements were useful for contrasting sorghum response to experimental treatments and for comparing overall canopy behavior in the 1998 and 1999 FACE experiments. The early- and mid-season canopy responses under well-watered conditions were qualitatively similar both years despite the one-month difference in planting dates. On an absolute basis, however, the maximum PAI attained under the wet treatment in 1999 was about 8% lower than the maximum measured in 1998 (5.7 versus 6.2 units, respectively), an observation that may help explain the difference in grain yields between the two years. Each of the approaches discussed in this report also showed that the dry irrigation treatment reduced canopy development and persistence

significantly compared with the wet treatment. The data further confirmed that the water stress imposed on the dry treatments in 1999 was more severe than in the previous year and caused a significantly shorter green canopy duration.

The NDVI data revealed some interesting differences between 1998 and 1999 in the way the crop responded to elevated CO₂. For example, in 1998 we observed that extra CO₂ stimulated early season plant growth and increased the amount of PAR that was captured by the sorghum canopy for potential use in photosynthesis. In 1999, however, the wet treatments showed almost no effect of elevated CO₂ early in the season, and a slightly increased rate of senescence towards the end of the season. In both years however, sorghum exposed to elevated CO₂ and water stress displayed higher NDVI values than their Control counterparts. The NDVI data obtained during the final portion of the growing season revealed substantial differences between the two experimental years during the grain filling period. In 1998, the canopy stayed green later into the season. In fact, leaf senescence was not noticeable until frost actually killed the tissue. The 1999 NDVI trajectory showed a gradual decline in green canopy during grain fill, a response which may be more typical for this cultivar when end-of-season temperatures are more favorable.

In general, we found each of these non-invasive approaches very useful for monitoring the sorghum canopy. Depending on the size of the plants and stage of growth, each varied in its suitability for detecting treatment differences. Besides being much more convenient to measure, the NDVI proved superior to direct fAPAR_c or PAI measurements for several reasons. First, NDVI could be measured early in the season when plants were small and the physical size of the Accupar or LAI 2000 sensors precluded their use. NDVI also showed large differences between treatments late in the season, when canopies were senescing and the two other approaches were not able to distinguish between green and senescent plant tissues. On the other hand, during the middle of the season when canopy had reached full development and NDVI saturated, the PAI measurements with the LAI 2000 sensor remained sensitive to differences between the treatments. Finally, the Accupar sensor provided fundamental information on spatial variation of fAPAR_c, a biophysical parameter required to calibrate the NDVI and was useful as an input for plant growth models.

FUTURE PLANS: Analysis of these data are continuing. Comparisons will be made between data from these non-invasive techniques and conventional agronomic measures of plant growth. Methods will be sought to extend the Accupar-derived fAPAR_c versus NDVI relationship beyond anthesis so that NDVI can be transformed into a biophysical parameter having biological significance for the entire growing season. NDVI also will be used to confirm whether the experimental treatments or the blower apparatus itself has an influence on end-of-season rates of canopy senescence.

COOPERATORS: Collaborators include M. Ottman, A. Matthias, S. Leavitt, D. Williams and T. Thompson from The University of Arizona, Tucson AZ; B. Roth and J. Chernicky from MAC, Maricopa AZ; and K. Lewin, J. Nagy, and G. Hendrey from Brookhaven National Laboratory, Upton NY. We also wish to thank J. Triggs and P. Bierly for technical assistance in the field. See Kimball et al. "Progress and Plans for the FACE Project" in this volume for a more complete listing of cooperators.

CO₂ ENRICHMENT OF TREES

S.B. Idso, Research Physicist; and B.A. Kimball, Supervisory Soil Scientist

PROBLEM: The continuing rise in the CO₂ content of Earth's atmosphere is believed by many people to be the most significant ecological problem ever faced by humanity, primarily because of the widespread assumption that it will lead to catastrophic global warming via intensification of the planet's natural greenhouse effect. There are also, however, many *beneficial* effects of elevated atmospheric CO₂ concentrations that are experienced by Earth's plant life; and some of them, such as the ability of elevated CO₂ to enhance plant growth rates, actually impact the global warming problem. Earth's trees, for example, account for two-thirds of the planet's photosynthesis and are the primary players in the global cycling of carbon, removing CO₂ from the air, and sequestering its carbon in their tissues and, ultimately, the soil. Consequently, we seek to determine the direct effects of atmospheric CO₂ enrichment on the growth and development of trees, concentrating specifically on the long-term aspects of this phenomenon; for until someone conducts an experiment that is measured in *decades*, we will never know what the long-term impact of the ongoing rise in the air's CO₂ content will be on the planet's most powerful contemporary carbon sink.

APPROACH: In July 1987, eight 30-cm-tall sour orange tree (*Citrus aurantium* L.) seedlings were planted directly into the ground at Phoenix, Arizona. Four identically-vented, open-top, clear-plastic-wall chambers were then constructed around the young trees, which were grouped in pairs. CO₂ enrichment—to 300 ppmv (parts per million by volume) above ambient—was begun in November 1987 in two of these chambers and, other than for brief maintenance and construction periods, has continued unabated since that time. Except for this differential CO₂ enrichment of the chamber air, all of the trees have been treated identically, being irrigated and fertilized as deemed appropriate for normal growth (Idso and Kimball, 1997).

As in all prior years, we continue to measure the circumferences of the trunks of the trees at the midpoint of each month; and from these data we calculate – on the basis of relationships developed specifically for our trees (Idso and Kimball, 1992) – monthly values of total trunk plus branch volume. Then, from wood density (dry mass per fresh volume) measurements we have made over the past several years, we calculate monthly values of the total dry weight of the trunk and branch tissue of each tree. Results for December, January, February, and March – the winter period of virtually no trunk expansion – are then averaged to give a mean value for the year, from which the preceding year's mean value is subtracted to yield the current year's production of trunk and branch biomass.

We likewise continue our yearly fruit measurements, counting the number of fruit to reach maturity on each tree, weighing each such fruit individually, and determining the percent dry weight of one hundred ripe fruit from each tree, which allows us to calculate the total dry weight of fruit produced in each of the CO₂ treatments.

The last major component of aboveground biomass that we regularly assess is leaf tissue. From previously derived relationships (Idso and Kimball, 1992), we evaluate the number of new leaves produced each year from our trunk circumference measurements. And from bi-monthly assessments of leaf dry weight similar to those of Idso et al. (1993), we calculate the total dry weight of leaves produced on the trees each year. These results, added to the trunk and branch dry weights and fruit

dry weights, then give us the total aboveground dry weight production per year for all of the trees in the two CO₂ treatments.

When viewed in their entirety, the results continue to be encouraging. They indicate that the trees of both CO₂ treatments may be close to achieving a stage of maturity characterized by a near-steady-state of yearly aboveground biomass production (Fig. 1). For the last four years of the experiment, for example, the value of total aboveground productivity in the CO₂-enriched trees was 107, 90, 95, and 116 kg/tree; while for the ambient-treatment trees it was 62, 51, 57 and 61 kg/tree, producing a four-year-mean CO₂-enriched/ambient-treatment ratio of 1.77.

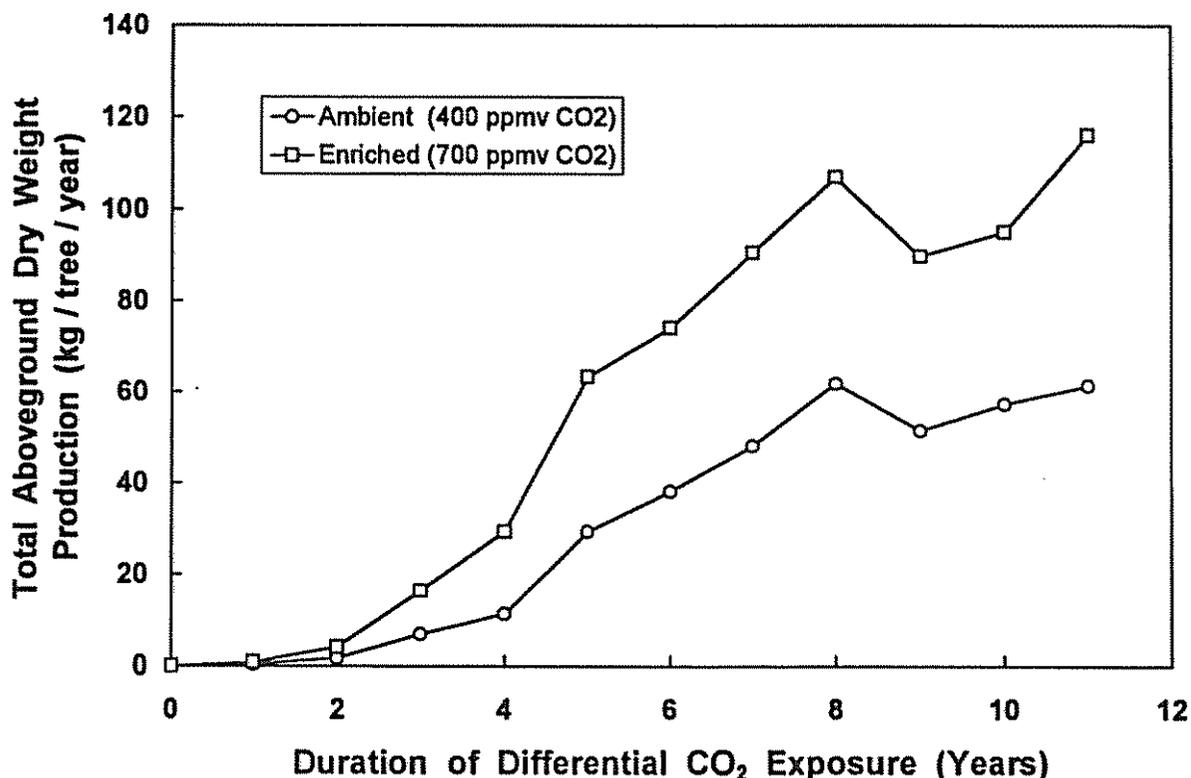


Figure 1. Yearly total aboveground biomass production in the ambient and CO₂-enriched sour orange trees as a function of time since the start of the experiment.

Fruit production has been a little more erratic; nevertheless, it too appears to be approaching an asymptotic upper limit (Fig. 2). For the last four years, for example, harvested fruit biomass has been 47, 38, 38, and 58 kg/tree in the CO₂-enriched trees; while in the ambient-treatment trees it has been 25, 13, 23, and 31 kg/tree, producing a four-year-mean CO₂-enriched/ambient-treatment fruit production ratio of 1.97. What happens from this point on could thus be of greater significance than all that has preceded it, for we are clearly close to determining the true long-term *equilibrium* response of the trees to atmospheric CO₂ enrichment.

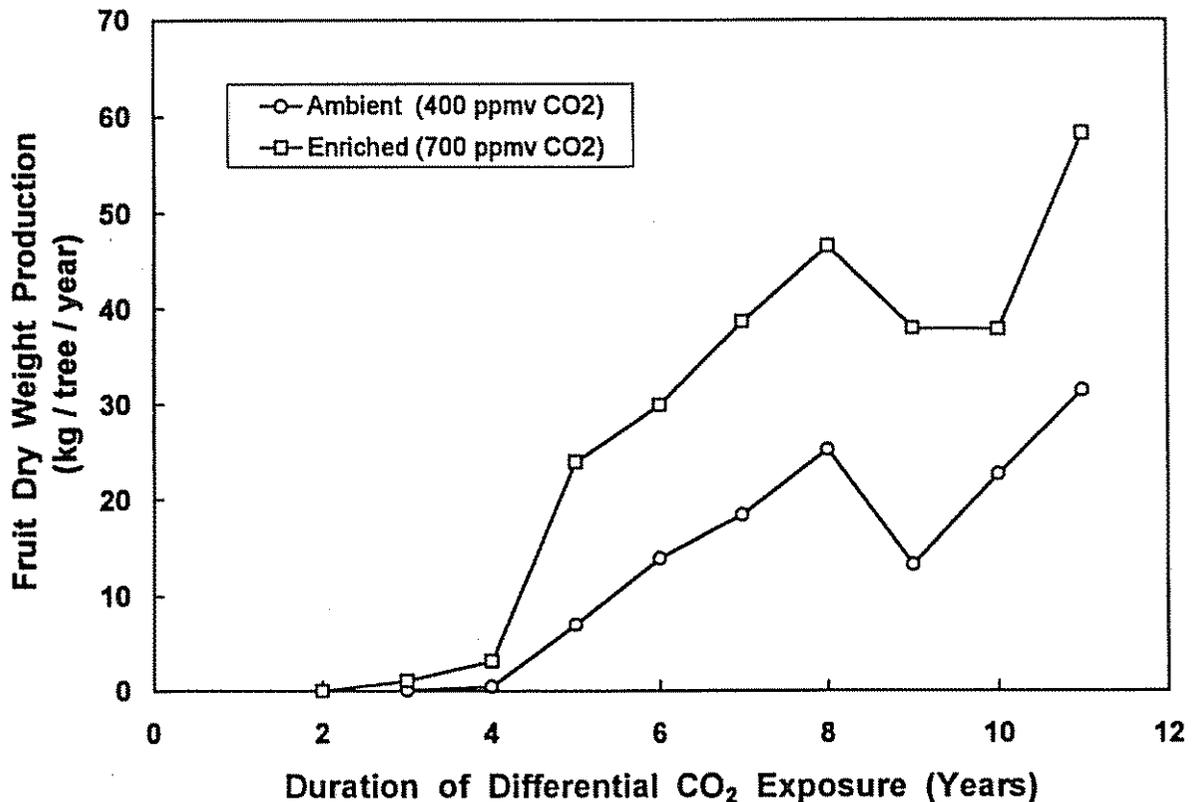


Figure 2. Yearly fruit dry weight production in the ambient and CO₂-enriched sour orange trees as a function of time since the start of the experiment.

In another intriguing development, we have discovered that in the spring of each year the CO₂-enriched trees experience an enormous growth enhancement. This initial stimulation begins immediately upon bud-burst, and three-and-a-half weeks later the new branches of the CO₂-enriched trees are typically more than four times more massive than those of the ambient-treatment trees. Furthermore, because there are more branches on the CO₂-enriched trees, their *total* new-branch biomass is generally over six times larger than that of the trees growing in ambient air.

Once achieved, peak CO₂-induced new-branch growth stimulation persists for about two weeks, whereupon the large initial biomass enhancement begins to subside. Then a decline sets in, and the CO₂-enriched/ambient-treatment new-branch biomass ratio of the trees ultimately levels out at a value commensurate with the long-term total aboveground productivity ratio of the CO₂-enriched and ambient-treatment trees; i.e., at a value of approximately 1.77.

It is possible that this phenomenon may be partially responsible for the ever-earlier spring “greening” of the Northern Hemisphere over the past few decades, which has been observed in long-term satellite studies of surface albedo, as well as in the increasingly earlier occurrence of the spring drawdown of the air’s CO₂ content, which is evident in long-term studies of the atmosphere’s seasonal CO₂ cycle (Idso *et al.*, in press).

INTERPRETATION: The stakes in this study are high, as no one has ever maintained an experiment such as ours for a long enough time to determine the long-term consequences of atmospheric CO₂ enrichment for long-lived woody plants. Indeed, the answer to this question is one of the critical elements that is needed to reveal the ultimate fate of the CO₂ that the people of the world yearly emit to the atmosphere. Will the trees of the planet be sufficiently stimulated by the ongoing rise in the air's CO₂ content to remove enough of it from the atmosphere to prevent a significant CO₂-induced warming of the globe? Our study provides important insight into this question and may help our government and others craft appropriate policies to meet this global environmental challenge. In the meantime, our findings continue to demonstrate that carbon dioxide is an effective aerial fertilizer, significantly increasing the size, growth rate, and fruit production of sour orange trees exposed to 75% more CO₂ than is normally in the air.

FUTURE PLANS: We hope to continue the sour orange tree experiment for as long as it takes to determine whether or not the trees will truly achieve steady-state yearly growth rates that produce a CO₂-induced productivity enhancement that can reasonably be expected to remain essentially constant over the remaining years of the trees' life span. We also are deeply involved in further studies of the ultra-enhanced spring branch growth we have observed in the CO₂-enriched trees.

COOPERATORS: J.H. Hooper and H.-S. Park, Arizona State University, Department of Plant Biology, Tempe, Arizona; R.C. Balling, Jr., Arizona State University, Department of Geography, Tempe, Arizona; C.E. Idso and K.E. Idso, Center for the Study of Carbon Dioxide and Global Change; U.S. Department of Energy, Atmospheric and Climate Research Division, Office of Health and Environmental Research.

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SIMPLE TECHNIQUES FOR CONDUCTING CO₂ ENRICHMENT AND DEPLETION EXPERIMENTS ON AQUATIC AND TERRESTRIAL PLANTS: THE "POOR MAN'S BIOSPHERE"

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PROBLEM: In order to act in the best interests of the biosphere in the face of the rising CO₂ content of earth's atmosphere, we need to determine the effects of atmospheric CO₂ enrichment on the growth habits of as many different plants as possible, both singly and in combination with competing plants and animals. Also needed is a knowledge of how the ongoing rise in the air's CO₂ content may interact with environmental changes such as global warming, more frequent and intense drought, and intensified soil, water, and air pollution, so we can determine if the deleterious effects of these latter phenomena will be ameliorated or exacerbated by the concurrent rise in atmospheric CO₂. Consequently, in an attempt to expand our research capabilities in this important area of science and to interest more young people in pursuing careers therein, this project has as its goal the development of a number of simple and inexpensive experimental techniques that will enable almost anyone to conduct significant research on a variety of questions related to the role of atmospheric CO₂ variability in ongoing and predicted global environmental change.

APPROACH: Over the first three years of the project, a set of guidelines was developed for using inexpensive and readily available materials to construct experimental growth chambers or "Poor Man's Biospheres," wherein CO₂ enrichment and depletion studies of both aquatic and terrestrial plants could be conducted (Idso, 1997). In their most basic form, these enclosures consist of no more than simple aquariums covered by thin sheets of clear polyethylene that are taped to their upper edges to isolate their internal air spaces from the room or outside air. Several low-cost, low-technology ways of creating a wide range of atmospheric CO₂ concentrations within these enclosures also were developed. Some of the CO₂ enrichment techniques utilize the CO₂ that is continuously evolved by the oxidation of organic matter found in common commercial potting soils, while others rely on the CO₂ that is exhaled by the experimenter. When CO₂ depletion is desired, the growth of the experimental plants themselves can be relied upon to lower the CO₂ contents of the biospheres' internal atmospheres, as can the photosynthetic activity of ancillary algal populations that often occur in watery habitats and that can be induced to grow in terrestrial environments as well. For all of these different situations, a set of simple procedures for measuring biospheric airspace CO₂ concentration has been developed. This technique utilizes any of a number of simple colorimetric CO₂ test kits that are sold in tropical fish stores throughout the world and that can be readily obtained by ordering over the internet (Idso, 1997).

To obtain hands-on experience in the technology transfer aspect of the Poor Man's Biosphere Program, outreach activities were initiated three years ago with five eighth-grade biology classes at McKemy Middle School in Tempe, Arizona, and with a fifth-grade class at the Salt River Elementary School of the Salt River Pima-Maricopa Indian Community. Students at both schools investigated the effects of atmospheric CO₂ enrichment and depletion on a common terrestrial plant, Devil's Ivy or Golden Pothos (*Scindapsus aureus*), and a common emergent aquatic plant, Yellow Water Weed (*Ludwigia peltoides*), under two different light intensities. New sets of students at McKemy Middle School repeated the Pothos experiment two years ago with some slight variations. Also, two honors biology classes at Tempe High School conducted a massive twice-replicated study of the growth

response of a submerged aquatic macrophyte, Corkscrew Vallisneria (*Vallisneria spiralis*), to three levels of atmospheric CO₂ (ambient, half-ambient, and twice-ambient) at three different water temperatures and two different light intensities, winning two \$10,000 first-place awards in a state environmental science curriculum contest.

This past year, as part of its environmental science education activities, the Center for the Study of Carbon Dioxide and Global Change conducted an ambitious program of employing the poor man's biosphere technique in a set of experiments that it described on its website (www.co2science.org) and updated on a weekly basis. Complete descriptions of these studies are now archived there for science teachers throughout the world to access and utilize in their classrooms.

In a further extension of the poor man's biosphere approach to conducting CO₂ enrichment and depletion experiments, the technique was successfully used over the past two years in a basic science study designed to explore the effects of atmospheric CO₂ enrichment and depletion on the production and distribution of biomass in sour orange trees grown from seed in nutrient-poor sand. This experiment, which lasted for 19 months, also included the study of tissue nitrogen concentrations and leaf chlorophyll concentrations. It revealed that even under conditions of extreme nutrient deficiency, there was still a sizable growth response to CO₂. Relative to biomass at 336 ppmv, for example, biomass at 1257 ppmv was over twice as great in leaves, four times greater in trunks and lateral roots, and nearly six times greater in tap roots. Nevertheless, these responses were only about half as great as those observed in sour orange trees of the same age growing under non-limiting conditions of nutrient availability.

FINDINGS: The simple experimental techniques developed in the initial years of the program have been found to work satisfactorily in actual classroom environments at elementary, middle, and high school levels. They now have been demonstrated to have a place in basic research programs as well. In addition, they have found a home on the internet, where anyone with a computer and internet access can go to learn of them.

INTERPRETATION: As the technology transfer aspects of the program are still ongoing, final conclusions have not yet been reached. However, all indications are that the poor man's biosphere approach to atmospheric CO₂ enrichment and depletion experiments has the potential to become a key element of environmental science education curricula in the years ahead. It is also beginning to show promise as a basic research tool.

FUTURE PLANS: A major article describing the use of the poor man's biosphere technique in a basic research program has been prepared (Idso and Adamsen, 2000). A second major journal article describing the use of the Poor Man's Biosphere Program in elementary, middle, and high schools will be prepared in the near future. Efforts to bring the program to state and national audiences of science teachers will continue via the internet in cooperation with educational organizations that have the capacity to provide such services.

COOPERATORS: Center for the Study of Carbon Dioxide and Global Change (C.D. Idso, K.E. Idso); McKerny Middle School, Tempe Elementary School District (M. Davis); Salt River Elementary School, Salt River Pima-Maricopa Indian Community (K.E. Idso); Tempe High School, Tempe Union High School District (S. Greenhaugh).