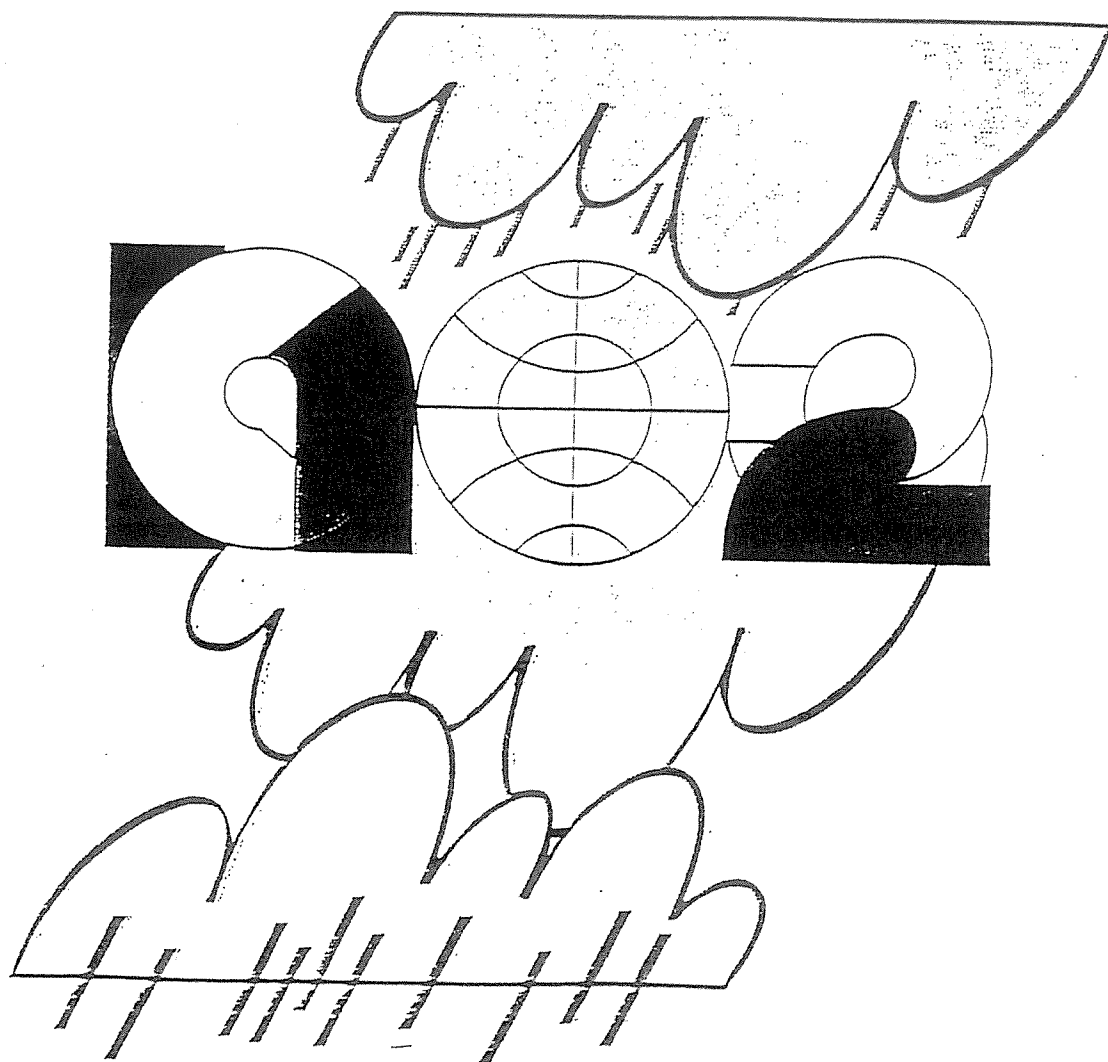


# Design and Execution of Experiments on CO<sub>2</sub> Enrichment

Edited by E.D. SCHULZE and H.A. MOONEY



COMMISSION OF THE EUROPEAN COMMUNITIES

# DESIGN AND EXECUTION OF EXPERIMENTS ON CO<sub>2</sub> ENRICHMENT

edited by

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Proceedings of a Workshop held at Weidenberg, Germany,  
October 26 to 30, 1992

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## 24. BIOLOGICAL DATABASES DERIVED FROM FREE AIR CARBON DIOXIDE ENRICHMENT EXPERIMENTS

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### 24.1 Introduction

The use of the Free-Air- $\text{CO}_2$ -Enrichment (FACE) technology to evaluate the effects of full-season  $\text{CO}_2$  enrichment on terrestrial vegetation has proven to be successful. The completion of a five year cooperative research project on cotton (*Gossypium hirsutum* L.) has given some valuable insights into both the engineering component of maintaining reliable  $\text{CO}_2$  control around a desired setpoint and the biological aspects of how full-season  $\text{CO}_2$  enrichment influences cotton morphology, growth and development, physiology, biochemistry, water use and productivity (Hendrey, 1993; Hendrey and Kimball, 1993). Ancillary measurements, such as determination of carbon sequestering in soil organic matter have also been taken (Leavitt, 1993). These studies included scientists from the United States Department of Agriculture-Agricultural Research Service, Brookhaven National Laboratory and several Universities, both domestic and foreign. A new FACE project is scheduled for the 1992-93 through 1995-96 growing season on wheat (*Triticum aestivum* L.)

The overall objectives of the FACE program are 1) to evaluate the effects of increasing  $\text{CO}_2$  concentration of ambient air on plants and ecosystem, and 2) to contribute to an evaluation of terrestrial plant feedback regulation of the rate of change of  $\text{CO}_2$  in the atmosphere (Hendrey and Kimball, 1993).

### 24.2 Engineering vs. Biological Databases

A historical perspective on the use of FACE technology to investigate the influence of  $\text{CO}_2$  enrichment on terrestrial vegetation has been discussed by Allen (1993). In short, a pilot study was conducted with a two-array FACE system in Yazoo City, Mississippi, U.S.A. during the 1987 cotton season. A second year was spent at the same location during the 1988 season perfecting and expanding the FACE system from a two- to a four-array system (Fig. 1a,b). The area where  $\text{CO}_2$  concentration was considered to be well-controlled during these two years had a 6.5-m radius which provided a biological activity area of 132.7 m<sup>2</sup> (Fig. 1c). In these early stages of FACE, at Yazoo City, the engineering aspects of the project took precedence over the biological components. The main focus was to perfect the  $\text{CO}_2$  delivery system (Lewin et al., 1993a,b; Lipfert et al., 1993). Adjustments in engineering features and software control algorithms were made which improved the temporal and spacial distribution of  $\text{CO}_2$  as a function of height and distance from the center of the FACE ring. Both accuracy and reliability of the system were improved, thus providing uniformity of the biological response across the designated activity area. During 1988, the first season-long field trial to collect detailed agronomic data occurred.

In 1989 the FACE project was moved to the University of Arizona's Maricopa Agricultural Center (MAC), Maricopa, Arizona, U.S.A., which is about 50 km south of Phoenix.

A uniform drip irrigation system supplied adequate soil moisture regimes and nutrients throughout the growing season. The drip tapes were buried 0.18-m below the cropping row with emitters spaced at 0.4-m intervals. Cotton, cultivar DPL-77, was planted at 1.016-m row spacing. All agronomic practices were in accordance with local cultural production methods. The same experimental protocol was adapted for subsequent cropping seasons to enable comparisons across years. In 1989, the FACE plots contained a plenum, vertical vent pipes,

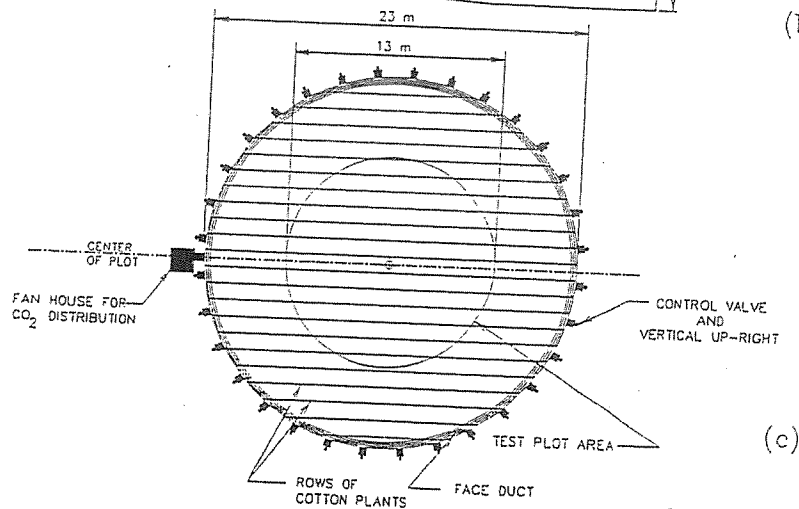
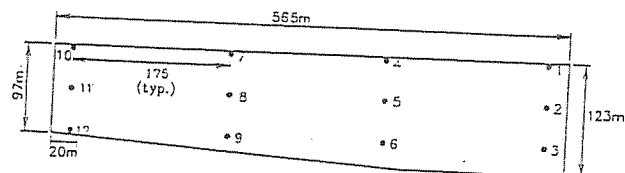
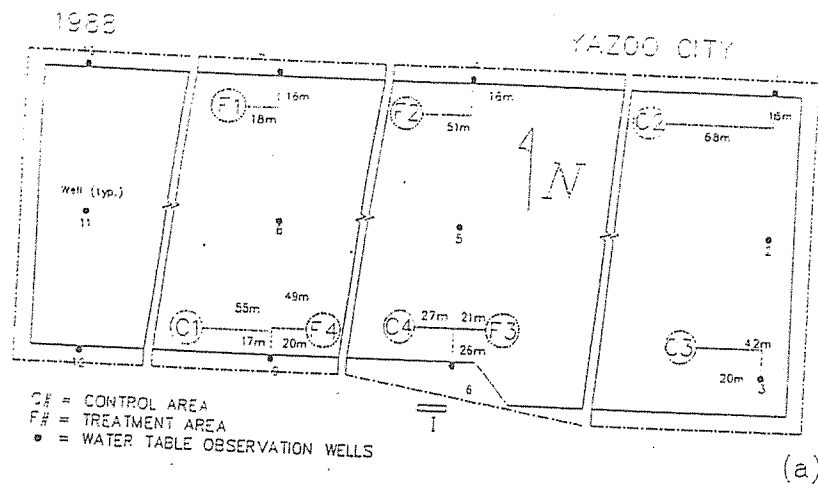


Fig. 1: (a) Field plan and overview of the plot location for 1988 FACE cotton experiment in Yazoo City, Mississippi. Roman numerals designate the location of the instrument trailers (I). Locations of the FACE rings are noted by circles in field plot layout, (b) scale drawing of the field site, and (c) enlargement of a FACE ring illustrating the biological activity area.

and a blower assembly for CO<sub>2</sub> delivery and dispersment, while the CONTROL plots were simply an adjacent portion of the field which contained no FACE apparatus. The dimensions of the FACE array and size of the corresponding activity area were similar to those used in

Yazoo City. Each treatment combination was replicated four times. A more detailed description of the 1989 experiment is given by Mauney et al.(1993a).

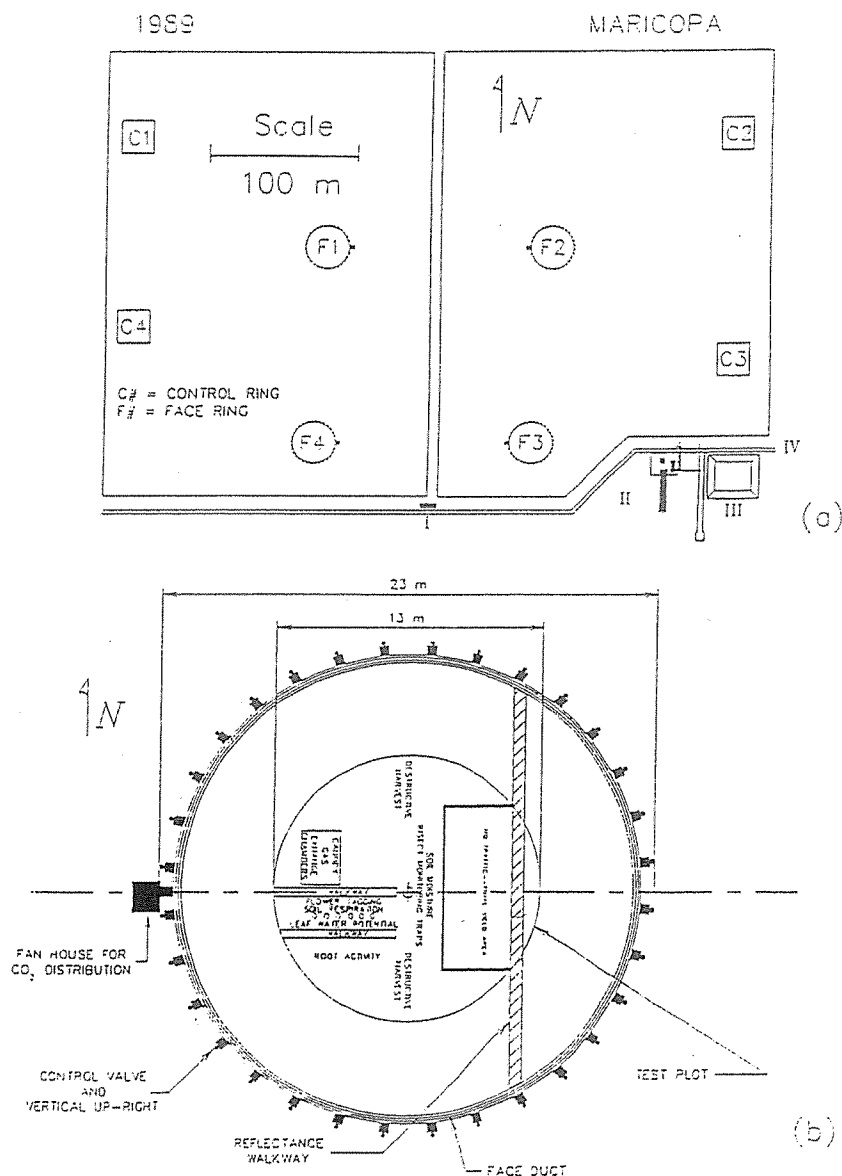


Fig. 2: (a) Field plan and overview of the plot location for the 1989 FACE cotton experiment in Maricopa, Arizona. Roman numerals designate the location of the instrument trailers (I), CO<sub>2</sub> storage tank and evaporator unit (II), evaporator pond (III), and irrigation canal used to supply water to evaporator pond (IV). (b) location of the assigned biological activity areas in a FACE ring.

After a successful experiment in 1989 at MAC, it was evident that the system was reliable and that it could adequately control  $\text{CO}_2$  concentrations in an open cotton field for season-long periods of time. The work through 1989 has been reported in a book edited by Hendrey (1993). This book contains nine chapters describing design and operational performance features of the FACE system; seven chapters presenting the growth, yield, and physiology of cotton in response to  $\text{CO}_2$  enrichment; one chapter comparing costs comparison among FACE, Open-Top-Chamber (OCT), and Soil-Plant-Atmosphere-Research (SPAR) facilities; and lastly, a lengthy appendix section tabulating meteorological, soil, cultural practices, and cotton growth and yield data.

In 1990 and 1991 the FACE plots were split, and a water-stress variable was introduced by restricting the irrigation water in half of each FACE plot to 2/3 that of the well-watered amount in the other half. Treatments, therefore, consisted of CONTROL & FACE, WET & DRY. For the 1990 and 1991 seasons plenums with vertical vent pipes, but excluding the blower unit, were constructed in the CONTROL plots. It was observed during the 1989 season that the plenum assembly influenced the microclimate within and surrounding the FACE plenum, particularly during early seedling development. Furthermore, the white color of the plenums might have attracted insects as evidenced by increased insect populations in the FACE rings when compared to the CONTROL plots without plenums in 1989. In order to determine if  $\text{CO}_2$  enrichment had an impact on insect populations, the CONTROL rings had to be similar to those used in the FACE ones.

Based on an analysis of the spatial  $\text{CO}_2$  concentration data,  $\text{CO}_2$  consumption (Nagy et al., 1993a,b) and the plant response data from 1989, for 1990 the radius of useable activity area was increased to 9.0-m. This alteration provided  $254.5 \text{ m}^2$  for FACE rings. A 2-m horizontal extension was added to each vertical vent pipe for a single FACE ring, F3 (Fig. 3a), in order to test whether the usable area could be made even larger. This structural

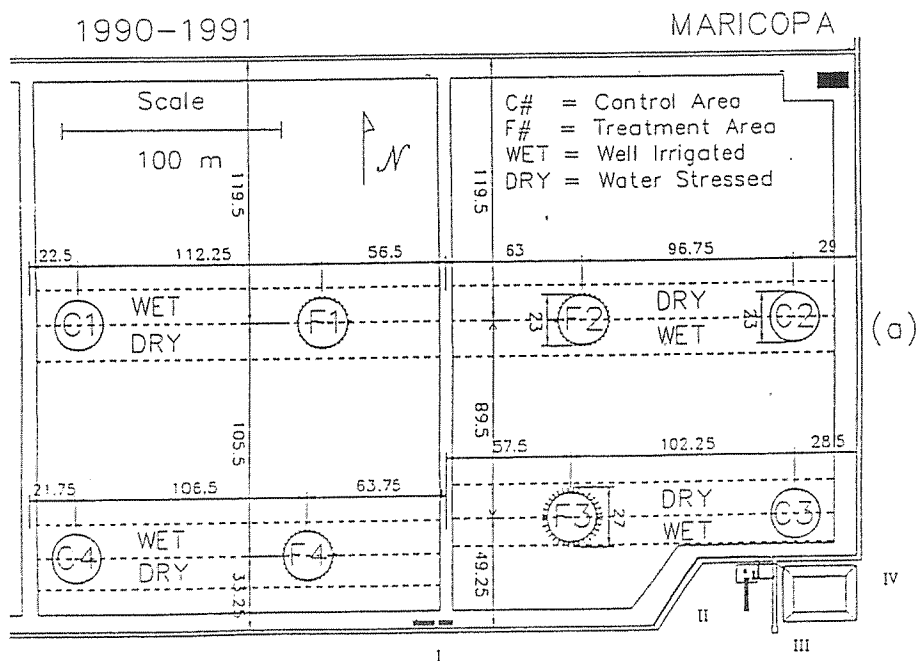


Fig. 3a: For explanations see next page

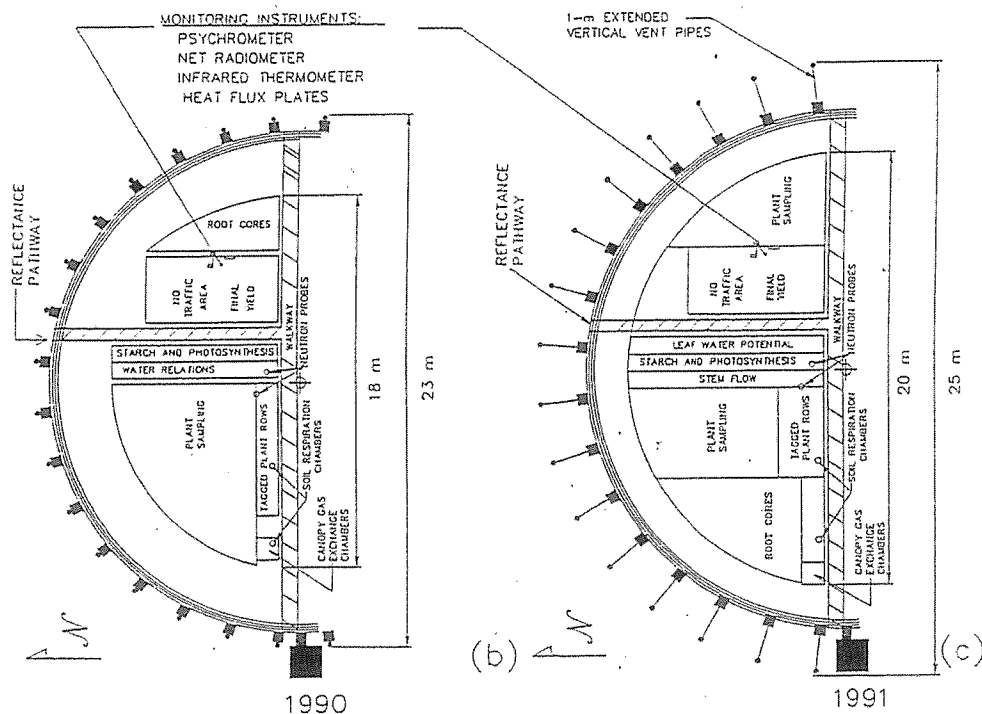


Fig. 3 : (a) Field plan and overview of the plot location for the 1990 and 1991 FACE cotton experiment in Maricopa, Arizona. Roman numerals designate the location of the instrument trailers (I), CO<sub>2</sub> storage tank and evaporator unit (II), evaporator pond (III), and irrigation canal used to supply water to evaporator pond (IV). (b) location of the assigned biological activity areas within an irrigation sub-plot of a FACE ring for the 1990, and (c) 1991 experiments. In 1990, ring F3 had a 2-m horizontal extension on the vertical vent pipes increasing the overall ring diameter to 27-m.

change enlarged the area enriched with CO<sub>2</sub>. A multi-port sampler (Hendrey et al., 1993) characterized the 3-dimensional CO<sub>2</sub> concentration gradients within the modified ring. Results indicated that the accuracy in CO<sub>2</sub> control was somewhat reduced within the modified ring. Comparing the quality of CO<sub>2</sub> concentration control in the F3 ring with that of the other three rings in 1990, it was decided that the optimum size of FACE rings for the conditions at Maricopa, was intermediate between the two diameters. Therefore, in 1991 all vertical up-rights were modified with a 1-m horizontal extension, which would provide an increased biological activity area of 314 m<sup>2</sup> with a radius of 10.0-m (Fig. 3c).

Additional scientists joined the team. It was during this period that a shift in emphasis from engineering performance to acquisition of biologically-based databases occurred. The results of these two years of CO<sub>2</sub> x water supply experiments are reported in twenty-five manuscripts submitted for publication in a special issue of Agricultural and Forest Meteorology (Dugas and Pinter, 1993).

A summary of the experimental protocols used for all FACE experiments to date, and those planned in the future is given in Table 1. A FACE wheat experiment is scheduled for

the 1992-93 season (Fig. 4). Because the need for CO<sub>2</sub>-enriched plant material continues to increase, and because the growth response of plants within the ring appears to be uniform by 2-m inside the vertical vent pipes (Mauney et al., 1993a,b), the radius of the CO<sub>2</sub> enrichment control area will be extended to 10.5-m. This corresponds to an activity area of 346.4 m<sup>2</sup>. It is our intent to conduct three addition wheat experiments during subsequent growing seasons depending on funding level. The treatments of the first two years of the FACE wheat study will repeat those of the 1990 and 1991 cotton design, i.e., CO<sub>2</sub> concentration of ambient air and air enriched to a CO<sub>2</sub> concentration of 550  $\mu\text{mol mol}^{-1}$  and WET and DRY irrigation. The experimental design for the third and fourth year of FACE wheat will focus on the CO<sub>2</sub> x nitrogen interaction by substituting the irrigation split-plot treatment with nitrogen levels.

Table 1. Summary of experimental protocol from past 1987-1991, present 1992-93, and planned future 1994-1996 FACE projects.

Year	Location	Crop	Biological Activity Area per Ring (m <sup>2</sup> )	[CO <sub>2</sub> ] ( $\mu\text{mol mol}^{-1}$ )	Water Treatment	Nitrogen
1987	Yazoo City, MS	cotton	132.7	ambient, 550 (daylight hours)	rainfed	adequate
1988	Yazoo City, MS	cotton	132.7	ambient, 550 (daylight hours)	rainfed	adequate
1989	Maricopa, AZ	cotton	132.7	ambient, 550 (daylight hours)	irrigated	adequate
1990	Maricopa, AZ	cotton	254.5	ambient, 550 (daylight hours)	irrigated 2/3 full irrigation	adequate
1991	Maricopa, AZ	cotton	314.2	ambient, 550 (daylight hours)	irrigated 2/3 full irrigation	adequate
1992-93	Maricopa, AZ	wheat	346.4	ambient, 550 (24 hr day <sup>-1</sup> )	irrigated 1/2 full irrigation	adequate
1993-94	Maricopa, AZ	wheat	346.4	ambient, 550 (24 hr day <sup>-1</sup> )	irrigated 1/2 full irrigation	adequate
1994-95	Maricopa, AZ	wheat	346.4	ambient, 550 (24 hr day <sup>-1</sup> )	irrigated	adequate no nitrogen
1995-96	Maricopa, AZ	wheat	346.4	ambient, 550 (24 hr day <sup>-1</sup> )	irrigated	adequate no nitrogen

### 24.3 Biological Measurements Collected in FACE Cotton

Numerous plant, soil, and micrometeorological parameters have been measured in the FACE cotton experiments. A summary of these biological databases derived from the five year effort is given in Table 2. Note the gradual shift in both the quantity, quality and diversity in these databases from 1987-91. A description of each of these measurements is given in more detail below. Each measurement type was assigned a specific activity area within the plots (Figs. 1c, 2b, 3b, 3c, and 4b) for the 1987-1991 growing seasons, respectively.

#### 24.3.1 CO<sub>2</sub> Fluxes and Carbon Pools - Changes in Above- and Below-ground Biomass

The growth and development of cotton plants in the FACE experiment were followed throughout the season by weekly destructive sampling (Mauney et al., 1993a,b). Measured plant parameters included stage of growth, plant height, biomass of stem, leaf, reproductive structures, roots, i.e. taproot and lateral roots, and green leaf area index. Plant maps were constructed to determine morphological development. Mineral content of aboveground biomass was determined (Huluka et al., 1993), as well as the carbon and nitrogen content of



the soil (Wood et al., 1993). At physiological maturity of the crop, representative areas within each treatment combination and replicate were hand harvested. Components of yield, i.e., mature bolls, were measured for all treatments (Mauney et al., 1993a,b). Below-ground biomass and root length density were determined at 1-2 times during the season by washing of soil cores (Prior et al., 1993).

#### 24.3.2 Leaf photosynthesis, respiration and water exchange

Individual leaf photosynthesis measurements were used to develop response functions relating assimilation rate ( $A$ ) to intercellular  $CO_2$  concentrations ( $C_i$ ) for a number of temperatures, air saturation deficits, leaf ages, and leaf growth conditions, i.e.,  $CO_2$  and soil moisture (Evans et al., 1993). In a separate effort, cotton leaf net photosynthetic measurements were obtained to characterize the full-season  $CO_2$  enrichment effects on leaf light-response functions (Idso et al., 1993). Dark respiration rates were also determined on plant material used for developing  $A-C_i$  response functions. Carbohydrate accumulation of soluble sugars and starch were also collected (Hendrix et al., 1993). On dates when intensive diurnal leaf photosynthesis measurements were taken, leaf temperatures, xylem potential, stomatal conductance and transpiration rates (Bhattacharya et al., 1993) were also collected to complete the dataset.

#### 24.3.3 Canopy Carbon and Water Exchange

From the perspective of ecosystems, it is necessary to integrate individual leaf carbon exchange rates to the canopy of a community of plants. In 1989 and 1990 a pop-on chamber system was used to make transient measurements of canopy carbon and water vapor exchange rates of the CONTROL & FACE, WET & DRY treatment combinations of one

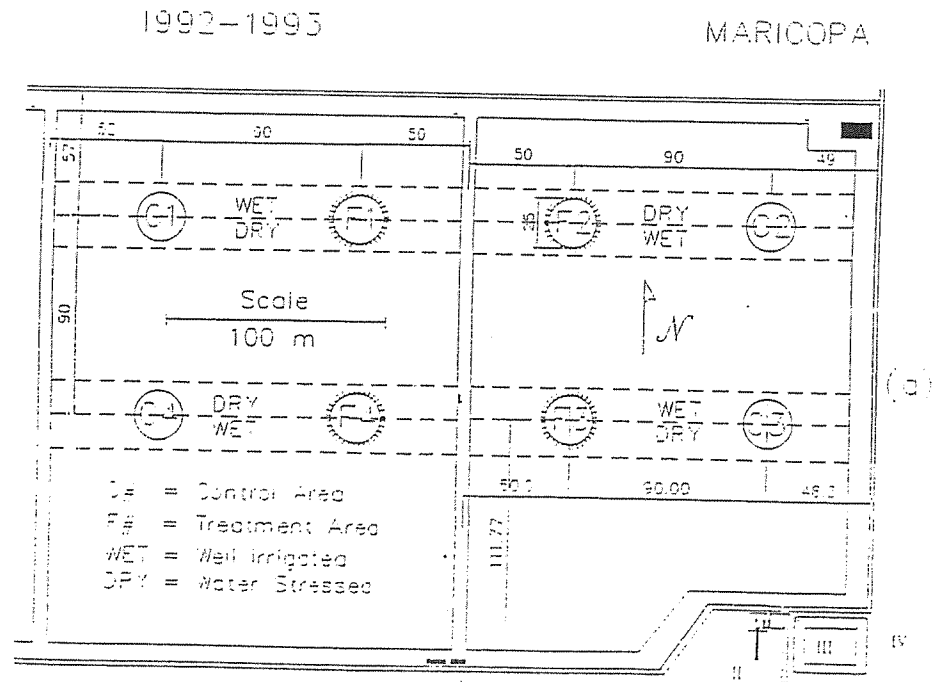


Fig. 4a: For explanations see next page

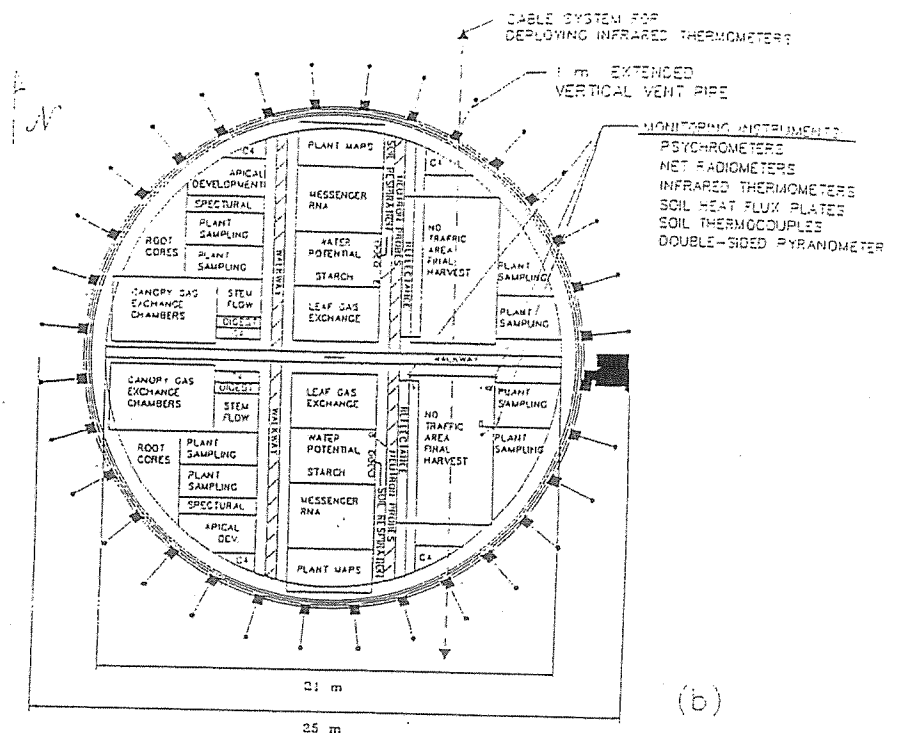


Fig. 4: (a) Field plan and overview of the plot location for the 1992-93 FACE wheat experiment in Maricopa, Arizona. Roman numerals designate the location of the instrument trailers (I), CO<sub>2</sub> storage tank and evaporator unit (II), evaporator pond (III), and irrigation canal used to supply water to evaporator pond (IV). (b) location of the assigned biological activity areas in both irrigation sub-plots of a FACE ring.

replicate, rotating to another replicate each week (Hileman et al., 1993). In 1991, two steady-state canopy gas exchange systems adapted from Garcia et al. (1990) were used to make these measurements. In addition measurements of canopy carbon exchange rates across a broad range of atmospheric CO<sub>2</sub> concentrations, ambient to 1800 mol mol<sup>-1</sup>, were obtained every 2 weeks to determine cotton canopy CO<sub>2</sub> response curves (Garcia, personnel communication).

#### 24.3.4 Changes in Soil Organic Matter from C Isotope Analysis

Because the CO<sub>2</sub> used for FACE is a by-product of petroleum refining, it is devoid of <sup>14</sup>C and has a different <sup>13</sup>C ratio than normal air. By determining the C isotope ratios of soils, plants, and the air in the FACE and CONTROL plots, the entry and incorporation of "new" C into soil organic matter and its storage and turnover was assessed (Leavitt et al., 1993).

#### 24.3.5 Soil Respiration

Soil respiration or soil CO<sub>2</sub> flux was measured with flux chambers specially fabricated for this purpose (Nakayama, 1991; Nakayama and Kimball, 1988). The measuring device consists of an open-ended plastic cylinder (200-mm diameter by 100-mm tall) installed on the soil

surface. Two successive gas samples are taken with a hypodermic syringe, the first immediately after the open-top cylinder is covered with a lid and the second after a one minute exposure period. The difference in  $\text{CO}_2$  concentration, determined with an infrared gas analyzer, between the two was used to calculate soil respiration (Nakayama et al., 1993).

#### 24.3.6 Water and Energy Fluxes and Balance

The effects of elevated  $\text{CO}_2$  on energy fluxes and evapotranspiration (ET) were

Table 2. Summary of biological-based databases collected in FACE (1987-1991)

Measurement Description	Growing Season				
	1987	1988	1989	1990	1991
Agronomic					
plant height	X	X	X	X	X
plant maps					X
no. mainstem nodes $\text{m}^{-2}$		X	X	X	X
leaf area index	X	X	X	X	X
specific leaf weight $\text{g m}^{-2}$		X	X	X	X
no. total nodes $\text{m}^{-2}$		X	X	X	X
no. squares $\text{m}^{-2}$		X	X	X	X
no. flowers $\text{m}^{-2}$	X	X	X	X	X
no. green bolls $\text{m}^{-2}$		X	X	X	X
no. abscised sites $\text{m}^{-2}$		X	X	X	X
no. lateral roots plant <sup>-1</sup>				X	X
root/shoot ratio				X	X
no. flower-tag bolls $\text{m}^{-2}$			X	X	X
no. mature bolls $\text{m}^{-2}$			X	X	X
leaf biomass ( $\text{kg ha}^{-1}$ )	• <sup>1</sup>	•	X	X	X
stem biomass ( $\text{kg ha}^{-1}$ )		•	X	X	X
root biomass ( $\text{kg ha}^{-1}$ )				X	X
green boll biomass ( $\text{kg ha}^{-1}$ )		•	X	X	X
mature boll biomass ( $\text{kg ha}^{-1}$ )	•		X	X	X
total plant biomass $\text{kg ha}^{-1}$	•	•	X	X	X
yield	X	X	X	X	X
harvest index			X	X	X
root length				X	X
root weight length <sup>-1</sup>				X	X
root dry weight density ( $\text{g m}^{-3}$ )				X	X

<sup>1</sup> Fresh weight measurement

Table 2. (cont.)

Measurement Description	Growing Season				
	1987	1988	1989	1990	1991
Canopy Level					
light use efficiency (kg MJ <sup>-1</sup> )			X	X	X
reflectance and biophysical characteristics			X	X	X
net photosynthesis ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )			X	X	X
temperature			X	X	X
apparent dark respiration ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )					X
whole plant transpiration rate ( $\mu\text{g H}_2\text{O m}^{-2} \text{ s}^{-1}$ )					X
soil respiration and CO <sub>2</sub> concentrations				X	X
plant nutrients, i.e. C, N, P, Zn, S			X	X	X
Leaf Level					
temperature			X	X	X
A-Ci curves				X	X
chlorophyll content				X	X
net photosynthesis ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )		X		X	X
xylem potential (MPa)		X		X	X
leaf transpiration rate ( $\mu\text{g H}_2\text{O m}^{-2} \text{ s}^{-1}$ )				X	X
stomatal conductance ( $\text{mol m}^{-2} \text{ s}^{-1}$ )				X	X
leaf soluble carbohydrates (g m <sup>-2</sup> ) (glucose, fructose, sucrose)	X	X			
leaf starch content (g m <sup>-2</sup> )	X	X	X	X	X
photosynthesis acclimation and limitations					X
fluorescence				X	
Leaf proteins and rubisco activity					X
Leaf spectral characteristics				X	X
pigments					X

Table 2. (cont.)

Measurement Description	Growing Season					
	Ancillary	1987	1988	1989	1990	1991
forage quality-sudangrass						X
digestibility						X
hemicellulose						X
protein						X
lipids						X
leaf anatomy						X
rhizosphere microbial populations				X	X	
mineralization						X
energy balance and ET					X	X
soil water content and ET from soil water balance					X	X
stem flow					X	X
Changes in soil C pools from plant and soil C isotopes						X
Changes in soil N pools from plant and soil N					X	X
arthropod populations				X	X	X
water use efficiency from C isotopes of C4 and C3 plants						X

determined with the residual energy balance method (Jackson et al., 1987),  $\lambda ET = R_n - G_o - H$ , where  $\lambda$  is the latent heat of vaporization.  $R_n$ , net radiation, was directly measured;  $G_o$ , the soil surface heat flux, was determined from heat flux plates and soil temperature measurements; and  $H$ , sensible heat flux, was determined from measurements of wind speed, air temperature, and infrared-thermometer-sensed canopy temperatures in two of the WET CONTROL and FACE plots at hourly intervals all season long. Additional micrometeorological instruments, including Bowen ratio apparatus, were installed outside one of the CONTROL plots to measure the "background" condition of the field as a whole (Kimball et al., 1993).

Neutron moisture sampling and time domain reflectometry (Topp et al., 1980) was used to measure soil water content at twice weekly intervals over the entire growing season in all plots. From the metered amount of irrigation applied, rainfall, estimates of soil drainage, and the changes in soil water content, weekly ET was calculated as a residual from the soil water balance, which was a second independent determination of ET (Hunsaker et al., 1993).

Sap flow gauges (Baker and van Bavel, 1987), which measure the mass flow of water within individual plant stems, were installed in one replicate of a WET FACE and WET CONTROL. These measurements were conducted during a four week period when the cotton plant had reached its peak biomass accretion (Dugas et al., 1993). At the same time microlysimeter systems (Boast and Robertson, 1982) were installed in both plots to measure

daily soil evaporation and partitioning between E and T, and together these measurements provided an independent estimate of ET (Kimball, personnel communication).

The impact of the CO<sub>2</sub> enrichment and water treatments on water use efficiency of cotton was also determined (Johnson, personnel communication). A small amount of a C4 plant, e.g., sorghum (2-m of row), was grown amongst the cotton. By measuring <sup>13</sup>C isotope ratios of the C4 sorghum and the C3 cotton, as described by Farquhar et al. (1989), cotton water use influence was ascertained.

#### 24.3.7 Remote Sensing - Reflectance and Biophysical Plant Characteristics

Variations in cotton growth rates, leaf area development, and seasonal absorbed photosynthetically active radiation caused by increases in CO<sub>2</sub> and reduction in soil moisture or nitrogen availability were monitored in the FACE experiment using radiometers that measure reflected solar and emitted thermal radiation. Ground-based, wideband radiometers were used 3 - 5 times per week throughout the season to document canopy reflectance factors in visible and near-infrared wavelength intervals comparable to those on current resource management satellite systems. Multispectral vegetation indices derived from these data were used to monitor trends in crop development and to develop functional relations with agronomic parameters of the cotton canopy (Pinter et al., 1993a), and biophysical rates associated with crop growth (Pinter et al., 1993b).

#### 24.3.8 Changes in Leaf Pigments and Spectral Characteristics

Leaf chlorophyll concentrations were monitored twice-weekly in all plots with a Minolta SPAD 502 chlorophyll meter. By properly calibrating the SPAD meter against leaf tissue analyses performed by the procedures of Lichtenthaler and Wellburn (1983), both chlorophyll a and b, as well as carotenoids and xanthophylls, were assessed (Pinter et al., 1993c).

#### 24.3.9 Canopy Temperature, Crop Water Stress, and ET

Canopy temperatures measured via infrared thermometry are especially sensitive to rates of plant transpiration and are important for assessing levels of plant stress, and seasonal water requirements (Pinter et al., 1990). They were measured in all plots about 3 times per week near midday when evaporative demand was at its maximum levels. Twice each month, radiant canopy temperatures were obtained on a diurnal basis and compared with other methods of assessing plant water status. These data were used to calibrate fixed head infrared thermometers used in diurnal estimates of energy balance in each experimental treatment and were also used for upwards scaling of single plant transpiration data to values representative of the entire canopy.

#### 24.3.10 Relationships to other Organisms - Digestibility, Decomposition and Microbial Activity

Cotton root systems exude carbon-based compounds into their surrounding soil system, thereby providing a food source for microbes. To assess the impact of full-season CO<sub>2</sub> enrichment on this plant-soil-microbe interaction, microbial populations of rhizosphere/non-rhizosphere soils (Ankumah et al., 1993), and on rhizosphere/phyllosphere were determined (Runion et al., 1993).

Some Sudangrass was planted amongst the cotton (2-m of row), and plant forage samples collected 2-3 times during the season were tested for their in-vitro digestibility. Complementary measurements of fiber, polysaccharides, lignin, protein, and phenolics were made, as well as the ultrastructure, e.g., electron microscopy, of leaf blades, cell wall thickness, organelles, etc. (Akin et al., 1993).

#### 24.3.11 Insect and Other Arthropod Populations

These populations were surveyed to determine population dynamics and abundance in all plots. Individual physiologic parameters were also studied; e.g., development rate, weight, and survival (Akey et al., 1993).

#### 24.4. Cotton Simulation Model - COTCO<sub>2</sub>

In conjunction with FACE, a new physiologically-based, mechanistic, modular-structured simulation model of cotton physiology, development, growth, and productivity has been constructed (Amthor and Kimball, 1990a,b). The overall objectives of the modeling effort were to predict cotton crop responses to increasing atmospheric CO<sub>2</sub> concentrations and potential concomitant changing climate variables.

Model development, as a component of the FACE project, has assisted in identifying the required alterations in system design to ensure that appropriate model development and validation databases are derived experimentally. Since the model must extrapolate beyond the available model development databases (Dahlman, 1985; Reynolds and Acock, 1985), explicit physiological mechanisms were used to minimize reliance on empirical relationships, which are data set dependent.

The model has been named COTCO<sub>2</sub>, for cotton response to atmospheric CO<sub>2</sub> concentration. In its present configuration, COTCO<sub>2</sub> consists of eight modules which contain sixty-eight subroutines, twelve functions and three common block include files, involving over 1000 variables and parameters in approximately 3000 lines of executable ANSI FORTRAN 77 code. Run-time for a season-long simulation on a Sun Microsystems SPARCstation-2 workstation is about thirty minutes. A description of this model is given by Wall et al. (1993).

#### 24.5 FACE Wheat

After completion of the 1991 experiment, it was felt that after measuring the response of the cotton ecosystem for 3+ years with FACE, it was time to shift to another crop. Wheat was selected because it is an important global food crop, and it grows well at Maricopa in the winter-spring. Several key personnel already had experience working with this crop and a database of organ development rates at high CO<sub>2</sub> and various temperatures and soil moisture regimes from controlled-environments was available (Wall et al., 1988). Furthermore, modeling efforts were already underway.

The objectives of FACE wheat are (1) to evaluate the effects of elevated CO<sub>2</sub> at ample and limiting levels of water on the wheat ecosystem, including its growth, yield, phenology, morphology, photosynthesis, respiration, carbohydrate status, carbon partitioning, water requirements, light use efficiency, and nutritional value to other organisms, (2) to evaluate changes in CO<sub>2</sub>, N<sub>2</sub>O, H<sub>2</sub>O vapor, and energy fluxes, in soil C and N pools, and in energy exchange parameters such as leaf conductance and spectral characteristics that are likely to feedback and affect the atmosphere and future climate change, (3) to facilitate development of wheat growth models capable of predicting the effects of increasing atmospheric CO<sub>2</sub> concentration and changing climate variables on future wheat growth, yield, energy balance, and water use, and (4) to facilitate development of remote sensing techniques capable of monitoring biophysical parameters, surface temperature, energy balance, and evapotranspiration over wide areas.

A total of Forty-seven scientists, research assistance and graduate students plan to participate (Table 3). Planned measurements include above- and below-ground biomass and yield, height, leaf area, morphology, stomatal density, anatomy, carbohydrates, phenolics, secondary plant compounds, antioxidants, digestibility, grain quality, elemental content, soil and plant carbon isotopes, evapotranspiration, water use efficiency, canopy temperature, soil water content, soil CO<sub>2</sub>, N<sub>2</sub>O and CH<sub>4</sub> emissions, nitrogen cycling, decomposition.

Table 3. List of scientists and responsibilities for measurements for FACE wheat 1992-93

Scientist	Affiliation	Measurement Responsibility
B.A. Kimball	U.S. Water Conservation Laboratory, Phx, AZ	Overall project management, energy balance and evapotranspiration (ET)
P.J. Pinter, Jr.	U.S. Water Conservation Laboratory, Phx, AZ	Aboveground biomass and numbers of individual organs, plant height, leaf area, reflectance, light interception and use efficiency, chlorophyll, canopy temperature
G.W. Wall	U.S. Water Conservation Laboratory, Phx, AZ	Morphology via plant maps, plant growth modeling
F. Wechsung	Potsdam Institute Potsdam, Germany	Morphology, leaf expansion rates, head and awn photosynthesis, wheat growth modeling
A. Trent & A. Li	University of Idaho Moscow, ID	Apical development
R.L. Garcia	U.S. Water Conservation Laboratory, Phx, AZ	Canopy gas exchange, i.e., photosynthesis, respiration, & transpiration response curves
M.S. Moran	U.S. Water Conservation Laboratory, Phx, AZ	Validation of wide-area remote-sensing ET detection techniques and models
F.S. Nakayama	U.S. Water Conservation Laboratory, Phx, AZ	Soil CO <sub>2</sub> fluxes and concentrations. Also N <sub>2</sub> O and CH <sub>4</sub>
A.R. Mosier	USDA-ARS, Soil, Plant Nutrient Research Ft. Collins, CO	Soil N <sub>2</sub> O and CH <sub>4</sub> fluxes
D.J. Hunsaker	U.S. Water Conservation Laboratory, Phx, AZ	Soil water content and ET
R. Rauschkolb H. Cho	University of Arizona MAC, Maricopa, AZ	Farming operations and soil, plant nutrients, i.e., C, N, P, Zn, S
G. Wechsung	Humbolt University Berlin, Germany	root biomass and root length density, soil and plant nutrient dynamics
A. Frumau & H. Vugts	Free University of Amsterdam Netherlands	Micrometeorology and Evapotranspiration
S.W. Leavitt	University of Arizona and Tucson, AZ	Changes in soil C pools from plant and soil C isotopes



Table 3. (cont.)

Scientist	Affiliation	Measurement Responsibility
S.P. Long & C. Osborn	University of Essex Colchester, England	Photosynthesis acclimation and limitations, fluorescence
G. Nie	Brookhaven National Laboratory Long Island, NY	Leaf photosynthetic proteins
A. Guintoli & F. Miglietta	Consiglio Nazionale Delle Ricerche Firenze, Italy	Fluorescence, grain development rates
L. Jahnke	University of NH Durham, NH	Antioxidants
D. Clark	Arizona State University Tempe, AZ	Secondary compounds
G.R. Hendrey & K.F. Lewin & J. Nagy	Brookhaven National Laboratory Long Island, NY	Consultation about the operation of the FACE system
J. Ham & R. Senack	Evapotranspiration Laboratory Manhattan, KS	Transpiration from stem flow gauges
W. Hunt	Natural Resource Ecology Laboratory, Ft. Collins, CO	Decomposition of plant residues, N, lignin
C.F. Morris	Western Wheat Quality Laboratory, Pullman, WA	Wheat grain quality
J.S. Amthor	Woods Hole Research Center, Woods Hole, MA	Wheat growth modeling
R.F. Grant	University of Alberta Alberta, Canada	Wheat growth modeling
T. Sinclair	University of Florida Gainesville, FL	Wheat growth modeling Leaf N content
D.L. Hendrix	Western Cotton Research Laboratory, Phx, AZ	Carbohydrate storage pools in individual organs
D.H. Akey	Western Cotton Research Laboratory, Phx, AZ	Arthropod populations
D.E. Akin	Russel Agricultural Research Center, Athens, GA	Digestability, hemicellulose, protein, lipids, anatomy

Table 3. (cont.)

Scientist	Affiliation	Measurement Responsibility
M. Estiarte & J. Peñuelas	Institut de Recercai Barcelona, Spain	Phenolics, stomatal density
H.B. Johnson	Grassland Soil and Water Research Laboratory Temple, TX	Water use efficiency from C isotopes of C4 and C3 plants
S. Malone	Grassland Soil and Water Research Laboratory Temple, TX	Anatomy
D.L. Suarez	U.S. Salinity Laboratory Riverside, CA	Soil CO <sub>2</sub> modeling
E.A. Paul	Michigan State University East Lansing, MI	Consultation about soil C pool changes and isotopic analyses, microbial digestion
L. H. Allen	University of Florida Gainesville, FL	Consultation about operation of FACE system
C. V. Vu	University of Florida Gainesville, FL	Rubisco activity
T. Kartschall & S. Grossman	Potsdam Institute Potsdam, Germany	Wheat growth modeling

photosynthesis, respiration, photosynthetic proteins, chlorophyll, fluorescence, reflectance and light use efficiency, leaf spectral characteristics and pigments, and insect populations. Four or more wheat modelers will also participate.

#### 24.5.1 Experimental Protocol - (1992-93, 1993-94)

A similar protocol as that used in the 1991 cotton experimental will be implemented for the first two years of the FACE wheat project. As illustrated in Fig. 4, there will be 4 replicates, consisting of 4 FACE rings and 4 CONTROL rings at ambient CO<sub>2</sub>, each split into DRY and WET halves. Note the change in location for all rings in the FACE wheat plot layout (Fig. 4), when compared to that of the cotton (Figs. 2-3). After three years of cotton research on the same plot area, we altered the location of the rings so that we could monitor the sequestering of CO<sub>2</sub> in the soil for the wheat study, while monitoring the decay of the sequestered CO<sub>2</sub> in the cotton study independently. The CONTROLS will have plenum and vertical vent pipes similar to those of the FACE plots, but there will be no blowers. Water will be supplied through a subsurface drip irrigation system. The WET irrigation amount will be based on replacement of evapotranspiration from the prior week, with corrections for any rainfall. Because precipitation quantities are greater during the winter months and because wheat requires less water than cotton, a reduction in irrigation water (adjusted for rainfall)

will be restricted in the DRY half of each FACE plot to 1/2 that of the well-watered WET amount in the other half. This irrigation level will ensure that water stress treatment will be obtained. The variety selected is Yecora Rojo, a semi-dwarf hard red spring wheat developed by the CIMMYT Research Center in Mexico. It is widely grown in Arizona and is the variety whose organ development rates are available (Wall et al., 1988). Wheat will be planted the first week in December, 1992 in 0.24-m row spacings with plants spaced 15- to 20-mm apart giving a plant population of approximately 140 plants m<sup>-2</sup>.

#### 24.5.2 Experimental Protocol - (1994-95, 1995-96)

Upon completion of the two years of CO<sub>2</sub> by water, study, we plan to conduct two additional years of research introducing a nitrogen treatment. Following the final harvest in May 1994, a large irrigation will be applied to leach as much nitrogen as possible below the root zone. A crop will be sown and removed from the field while still green, as done by Kimball and Mauney (1992). The soil, e.g., Trix clay loam, has an organic matter content of about 0.5%, so this site should be fairly good for producing limiting levels of soil nitrogen. The CO<sub>2</sub> treatments will be the same, and the nitrogen treatments will be ample (N+) and none-added (N-). There will be subplots within the larger plots where small amounts of <sup>15</sup>N-labeled fertilizer will be added, and then nitrogen transformations and N<sub>2</sub>O emissions will be monitored.

#### 24.5.3 Databases added to protocol since FACE cotton experiment

At present, it is our intent to maintain and expand the quantity and diversity of biological measurements in the FACE wheat compared to those measured in the cotton. Slight modifications in some measurements, particularly those which pertain to morphology and growth and development, will be tailored for wheat. However, a similar experimental protocol is planned. Some additional measurements have recently been added to the plan as noted below. Other types of measurements can be added in subsequent years as additional personnel become involved in the project.

#### 24.5.4 Wheat morphology, phenology and biomass partitioning

Apex development will be monitored via dissections to determine initiation rates of leaf, spikelet and floret primordia. Individual plants will be tagged with different colored wire to determine culm distribution patterns, i.e., MS, T0, T1, T2, T10, etc. Plant maps will be constructed to quantify the culm distribution patterns. Leaf appearance and elongation rates will be quantified on tagged plants by culm type. Detailed biomass partitioning amongst plant parts, i.e. crown, stem segments, leaf sheaths, leaves, chaff and grain across culm types will be determined every 5-7 days. Detailed data on individual kernel developmental rates will be taken to quantify the allocation of carbon and nitrogen into the developing grain on a per-culm basis. These results, along with those derived from the belowground root biomass, will enable quantification of the individual plant organ partitioning coefficients. The overall intent of this exercise will be to quantify the physiologically-based rate functions required to build and/or validate a wheat model sensitive to atmospheric CO<sub>2</sub> concentrations.

#### 24.5.5 Leaf Photosynthesis, Photorespiration

Additional measurements using 1% vs. 21% O<sub>2</sub> will be collected to assess P<sub>i</sub> limitations (Harley and Sharkey, 1991). An integrating sphere leaf chamber and the steady-state gas exchange system will be used to make maximum quantum yield measurements. In addition to the gas exchange measurements, leaves will be analyzed for soluble and total proteins, and enzyme assays will be conducted to evaluate rubisco activity, activation state and concentration of active centers. Leaf fluorescence measurements will also be collected on selected days.

#### 24.5.6 Above- and Below-ground Nutrient Dynamics

Aboveground biomass samples will be partitioned by height into crown, stem segments, leaf sheaths, leaves, chaff and grain. A belowground root sample will be taken with a portable root core device. Cores will be partitioned by depth and soil removed with a hydropneumatic root washer. Root length and area will be determined with a video camera imaging system, and final dry weight will be ascertained. Nutrient content, i.e., N, C, P, Ca, Mg, Na, K, Zn, Cu, Mg, and Fe, will be determined for all biomass samples at six stages of growth, i.e., 2-leaf, tillering, stem elongation, anthesis, grain filling, and at physiological maturity. Similar nutrient content will be determined on soil samples except that N-total will be partitioned into organic fraction,  $\text{NO}_3^-$ , and  $\text{NH}_4^+$ . Soil, pH, redox potential and volumetric water content for each core by depth will also be determined.

#### 24.5.7 Soil $\text{CO}_2$ Distribution

Soil  $\text{CO}_2$  flux will be determined indirectly from the  $\text{CO}_2$  distribution profile in the soil (Suarez and Simunek, 1992). The distribution of  $\text{CO}_2$  in the soil profile will be determined by sampling soil air for  $\text{CO}_2$  at various depths in the soil (Nakayama and Kimball, 1988). The air sample will be taken using narrow-bore tubing permanently inserted into the soil.

#### 24.5.8 Changes in Leaf Pigments and Spectral Characteristics

High-resolution, field-portable spectroradiometers will be used to examine fine details of canopy and single leaf reflectance spectra. Concurrent samples of leaf material will be taken to the laboratory for analysis of actual pigment content. Derivative spectra and shifts in the "Red edge" will be examined to correlate changes in leaf pigments and nutritional status as described by Demetriades-Shah and Steven (1988).

#### 24.5.9 Extension to Large-Area ET and Plant Growth evaluation

A method for field-scale mapping of ET utilizing remotely sensed spectral data and on-site measurements of meteorological conditions was developed by Jackson (1985). This technique has been successfully applied to mature agricultural fields using ground-, aircraft- and space-based sensors (Moran et al., 1990) and will be further tested with data from the FACE wheat experiments.

A method for incorporating remotely sensed spectral and thermal data into simulation models of crop growth and ET has been described for wheat (Maas et al., 1992; Moran et al., 1992). Though this approach shows promise for large-area estimations of ET rates and biomass production, it needs verification on a smaller scale and incorporation of the  $\text{CO}_2$  effect. The FACE experiment will provide important validation data sets for wheat.

#### 24.5.10 Relationships to Other Organisms - Digestibility/Decomposition

Digestibility will be determined as was done for cotton. In addition, the effects of FACE on decomposition will be determined. At the end of each growing season, samples of the plant residue, mainly stems and senesced leaves will be taken to the laboratory along with 150 g samples of surface soil. The plant material will be incubated with the soil, and rates of decomposition will be determined. Ancillary measurements of the N and lignin contents of the plant material will also be made (Hunt, personnel communication).

#### 24.5.11 $\text{N}_2\text{O}$ Fluxes and Soil Nitrogen Cycling

The FACE experiments will provide a unique opportunity to examine N cycling and  $\text{N}_2\text{O}$  emissions under field conditions. We plan to apply small amounts of  $^{15}\text{N}$  labeled fertilizer manually to 2-m by 2-m subplots within the larger  $\text{CO}_2$  plots. Plant samples will be collected both from the subplots at the end of the growing season, and biomass, total N, and  $^{15}\text{N}$  will

be determined. Soil core samples will also be taken and total N,  $\text{NO}_3^-$ , and  $\text{NH}_4^+$  will be determined. Nitrogen isotope ratios will be determined in soil and plant digests and extracts using a modified Rittenberg method (Hauck, 1982). These measurements will allow calculation of fertilizer N recovered in plants and soils.

$\text{N}_2\text{O}$  emissions will be measured in all subplots and at other positions outside the subplots using the same technique as that for the soil  $\text{CO}_2$  emissions described above. Sampling will be done from all cylinders near midday several times during the season and from selected cylinders over a 24 hr day on a few intensive diurnal measurement days.

#### 24.5.12 Messenger RNA

A large sample of leaf material will be collected periodically and archived. Storage of leaf material via liquid  $\text{N}_2$  will enable post-hoc enzyme activity and/or protein blots/N-terminal analysis of plant material.

#### 24.6 Wheat Growth Model

Development of a capability to predict the response of the wheat ecosystem to increasing  $\text{CO}_2$  and changing climate is an important component of the FACE wheat project. The measurements described in the previous section will provide data with which to validate wheat growth models under conditions as representative of future fields as it is possible to create them today. These data will be compiled, edited, and published in a standard IBSNAT (1986) format, as was done previously with the cotton data (Kimball et al., 1992). Thus, they will be available to anyone wishing to test his/her wheat growth model.

Considerable effort has already been expended on impact studies of future wheat production (Smith and Turpak, 1989). However, the models used had a simple multiplication factor for the effects of  $\text{CO}_2$ , and they had never been validated for their  $\text{CO}_2$  response aspects against field data. Their results must be regarded with skepticism, lest they later prove to have been misleading. The GCTE Operational Plan (Steffen et al., 1992) lists as Task 3.1.2 the need to develop robust, reliable models capable of predicting the effects of changed atmospheric composition and climate on phenology, photosynthesis, respiration, carbohydrate status, carbon partitioning among the various organs, water balance, nutrient content, and plant competition. The FACE wheat program will cooperate in the development and validation of at least four such models for wheat. A similar model to that of COTCO2 (Wall et al., 1993) called WHEAT is nearing completion by Amthor (unpublished), and tests of it will be made during the course of the FACE wheat experiments. In a separate effort Grant (1992a,b) and Grant et al. (1992) have developed an ECOSYS model which can account for the  $\text{CO}_2$ -climate effects on the above-listed processes, and which should be adaptable to wheat. Tests of this model also are planned.

#### 24.7 Conclusions

The FACE technology has proven an effective research option to investigate the influence of full-season  $\text{CO}_2$  enrichment of terrestrial vegetation. The project has grown significantly since the early pilot studies in 1987 to a full-blown effort involving scientist from numerous disciplines, agencies and foreign countries. The economy of scale for FACE has been proven (Kimball, 1993). The combining of all experimental efforts on a single project fosters mutual information exchange and interpretations of data amongst the participants which has a synergistic effect, one that remains unequalled by isolated individual efforts.

With continued progress by the IGBP-GCTE in organizing research on a global scale, and with proposals being made for several FACE experiments on a variety of ecosystems, it will be desirable to try to coordinate the research with these other groups. It is our intention to cooperate as much as possible. We welcome other researchers to join our program.

**Acknowledgements.** Numerous individuals have been involved in the conceptual development, actual planning, implementation and execution of the FACE research program in the past five years. The contributions from scientific and technical support personnel from the United States Department of Agriculture-Agricultural Research Service, Brookhaven National Laboratory, Department of Energy-Office of Health and Environmental Research, and several Universities is greatly appreciated. In particular, thanks is extended to Dr. Jack R. Mauney for his historical account on the biological databases collected during the early FACE cotton experiments. Special thanks are extended to Keith F. Lewin, Robert L. La Morte and Matthew L. Reaves for their assistance in preparation of the figures.

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