AGRICULTRUAL PRODUCTIVITY AND WATER USE: EFFECTS OF GLOBAL CHANGE

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PROJECT SUMMARY

We propose to conduct global change research over the next three years with the following objectives: (1) Determine the long-term effects of elevated CO_2 on the physiology, growth, wood production, fruit yield, fruit nutritional quality, and water use efficiency of sour orange trees, as well as its effects on soil structure and carbon. This is a continuation of an ongoing open-top chamber experiment started in 1987, which is the longest such continuous CO_2-enrichment experiment ever conducted. (2) Assess likely impacts of global change on the productivity of agricultural crops via synthesis and integration of existing large accumulated experimental data base plus additional crop growth modeling. (3) Determine effects of elevated CO_2 on the physiology, growth, yield, N_2-fixation, persistence, and soil carbon sequestration of alfalfa using the free-air CO_2 enrichment (FACE) approach. This experiment is the logical follow-on to prior such FACE experiments on cotton, wheat, and sorghum conducted at Maricopa, AZ. (4) Determine effects of elevated CO_2, water supply, and grazing pressure on productivity, shrub-grass competition, carbon sequestration, and water relations of the piñon-juniper rangeland ecosystem, also using the FACE approach. This new project, called Carbon Exchange and Sequestration in Arid Regions (CESAR), would utilize CO_2 from a huge geologic source within this ecosystem, and it would involve a large consortium of universities, ARS, private industry, and others. Achieving Objectives 3 and 4 is contingent upon obtaining outside funding.

OBJECTIVES

1. Determine the long-term effects of elevated CO_2 on the physiology, growth, wood production, fruit yield, fruit nutritional quality, and water use efficiency of sour orange trees, as well as its effects on soil structure and carbon sequestration beneath the trees.

2. Assess likely impacts of potential global change on productivity of agricultural crops via synthesis and integration of large accumulated experimental data bases.

   [Note: Objectives 3 and 4 are contingency plans dependent upon obtaining outside funding.]

3. Determine effects of elevated CO_2 on the physiology, growth, yield, N_2-fixation, persistence, and soil carbon sequestration of alfalfa.

4. Determine effects of elevated CO_2, water supply and grazing pressure on the productivity, shrub-grass competition, carbon sequestration, and water relations of the piñon-juniper rangeland ecosystem.

NEED FOR RESEARCH

Description of the Problem to be Solved

1. Sour orange: Whether enough carbon can be sequestered in the boles of tress and in the soil beneath them to significantly slow the rate of rise of atmospheric CO_2 concentration is an important question facing global change research, as are the implications of the ongoing CO_2 rise for human nutrition. *Sour orange* represents such long-lived woody species, which is being studied to determine (1) whether an initial CO_2-induced enhancement in wood and fruit production will be maintained over a tree's life span, (2) whether quality of wood and fruit will change, and (3) whether increases will occur in soil carbon storage beneath the trees.

2. Synthesis and Integration: Policymakers often have difficulty perceiving principles from the vast array of facts before them, so a common refrain is that the products of scientific research are "data rich and knowledge poor." Currently, about 2 papers are being published every 3 days about the effects of CO_2 and other environmental variables on agricultural crops, from which more definitive knowledge about global change effects on agriculture needs to be synthesized. Furthermore, available assessments may be suspect. The Inter-governmental Panel for Climate Change is now writing its third major assessment of likely impacts of global change on agriculture (as well as many other facets of world society), but most of the predictions are based on simple plant growth models which ignore some important plant processes (IPCC, 2001).

   Four simple models (Tubiello et al., 1999; Jamieson et al., 2001) were able to simulate the responses of wheat to elevated CO_2 interacting with soil water and soil nitrogen supplies as observed in our free-air CO_2 enrichment (FACE) wheat experiments. Nevertheless, such simple models as tabulated by the IPCC (2001) cannot address some important aspects of plant growth responses to elevated CO_2. For example, they "grow" the crops at air temperature rather than at the crop's own temperature; yet, we have shown that elevated CO_2 causes wheat canopies to warm 0.6 to 1.2°C above air temperature due to the direct effects of the elevated CO_2 on the plants' stomatal apertures (Kimball et al., 1992, 1995, 1999). Such warming would be in addition to any global warming of air temperature, and it could cause similar consequences, such as changes in yield and major shifts in optimal production regions of crops. A second effect that is not adequately addressed by simple daily-time-step models is that plants make their photosynthate during daytime, yet they continue to translocate material and grow at night. Therefore, daily temperature patterns may be very important in determining plant responses to elevated CO_2. One prediction of general circulation models is that night temperatures are likely to warm more in the future than daytime temperatures (Collatz et al., 2000; Easterling et al., 1997; Hansen et al., 1995). Therefore, another generation of assessments needs to be done -- with detailed process-oriented models capable of simulating all known effects of elevated CO_2, as well as of other interacting environmental variables, on crop physiology, growth, yield, carbon sequestration, and water relations.

3. Alfalfa: Alfalfa is a perennial deep-rooted legume crop that has the potential to respond to elevated CO_2 with deep sequestration of soil carbon, even at low soil nitrogen and thereby slow the rate of rise of the atmospheric CO_2 concentration. It is an important forage crop in the U.S. (24 million acres; 4th in acreage behind corn, wheat, and soybeans; USDA, 2000) that grows well in Arizona. Specific scientific reasons to focus on alfalfa are: (1) being deep-rooted, alfalfa can sequester carbon at deeper depths below the plow layer where it may be able to be stored for much longer periods, (2) being perennial, alfalfa grows the year around, so the interaction between elevated CO_2 and temperature can be studied, and (3) being a legume, the effects of elevated CO_2 on nitrogen fixation can be examined, as can the importance of nitrogen for C sequestration.

4. Piñon-juniper: Piñon-juniper is an expansive ecosystem whose character may be changed by global change. This vast mid-elevation ecosystem serves as rangeland for substantial cattle production and watershed catchment for much of the Western U.S. It contains more than a dozen National Parks and Monuments, is home to numerous Native American groups, has a history of varied natural resource management (fire suppression, logging, grazing), and is under great development pressure from surrounding regions (Wilkinson 1998). Little research has addressed biosphere-atmosphere interactions of this region, yet paleoecological studies and climate change models indicate this region may be highly sensitive to global change (Cole,1985; Nielson, 1995; Grissino-Mayer et al., 1997). Land use (fire suppression, grazing) has had widespread impacts on this region (Fleischner, 1994; Brown and McDonald, 1995; Moore et al., 1999), yet we do not understand the implications of these activities for C exchange and sequestration, nor the nature of their interaction with atmospheric and climatic change. Insect outbreaks associated with climate change appear to be increasing (Walker et al., 1998). Their impacts on ecosystem C fluxes and sequestration can be significant but are little understood in arid regions. Of the region's major vegetation types, the piñon–juniper woodland is the third most extensive in the continental U.S. Therefore, there are several reasons to determine the effects of elevated CO_2 and other interacting environmental variables on this important ecosystem.

Relevance to ARS National Program Action Plan

The project is relevant to all components. While the primary emphasis is on determining and assessing the impact of global change on *agricultural ecosystems* and to develop strategies for adaptation, this research inherently involves studying all aspects of *carbon cycling* from photosynthetic carbon assimilation to soil carbon sequestration, the latter of which can mitigate the rate of global change. Production of *trace gases*, such as N_2O, will also be addressed. In addition, the influences on surface energy balance and evapotranspiration will be assessed, thereby contributing to the *water cycle* component.

Potential Benefits

The main benefit will be an enhanced ability to prepare for potential global change. Knowing the probable impacts of global change on crop production and water use in each region will give researchers and growers the incentive to develop strategies for coping with problems and maximizing benefits, as well as for sequestering carbon to mitigate the rate of global change. The growth models developed as part of this research should prove to be useful tools for developing such strategies.

Anticipated Products

Scientific publications will be produced that describe, as well as synthesize and integrate, the effects of elevated CO_2 and interacting environmental variables on plant physiology, growth, yield, light use efficiency, carbon sequestration, and water use of crops and rangeland for various regions and global change scenarios. Process-based plant growth models that have been more perfected and validated than currently exist will also be products of this research.

Customers

Other agencies, such as the Department of Energy and Environmental Protection Agency, need the information to set policy regarding carbon storage credits, energy sources (i.e., coal versus nuclear), CO_2 emissions (whether to tax or not), and land use (reforestation). Agricultural policymakers will also use the information to formulate resource conservation plans for the next Farm Bill, especially if there is a "green payments" program that encourages carbon sequestration. And, of course, farmers eventually will use the information.

SCIENTIFIC BACKGROUND

1. <u>Sour orange trees</u>:

A search of the CRIS system for “global change” or “CO_2" or “carbon dioxide” revealed that 198 projects are coded for work on these topics within ARS and that another 547 are also outside of ARS. However, only about 40 pertain to assessing the impact of global change, especially of elevated CO_2, on agricultural ecosystems. CO_2 enrichment research is being conducted at the ARS Gainsville, Beltsville, Auburn, Temple, Raleigh, and Ft. Collins locations. Some of the non-ARS projects were free-air CO_2 enrichment (FACE) projects on desert in Nevada and on trees in Wisconsin and North Carolina, and the latter will be discussed shortly. However, our long-term study on sour orange trees appears to be unique.

Beyond the CRIS system, there have been many reports of the growth responses of trees to atmospheric CO_2 enrichment; however, the vast majority of them have been of relatively short duration. In a recent review of the literature, for example, Idso (1999) evaluated the results of 180 such experiments, finding that only two of them extended beyond four years duration. In addition, all of the short-term studies (three years or less) dealt with trees growing in different types of rooting media in containers located in growth chambers or greenhouses, as opposed to the undisturbed soil of the natural environment. Because of these protocol deficiencies, these latter studies are clearly incapable of answering important questions related to carbon sequestration in trees and soils.

Some newer studies are beginning to address these deficiencies. The free-air CO_2 enrichment (FACE) experiment at Duke University, for example, as well as a similar study at Oak Ridge National

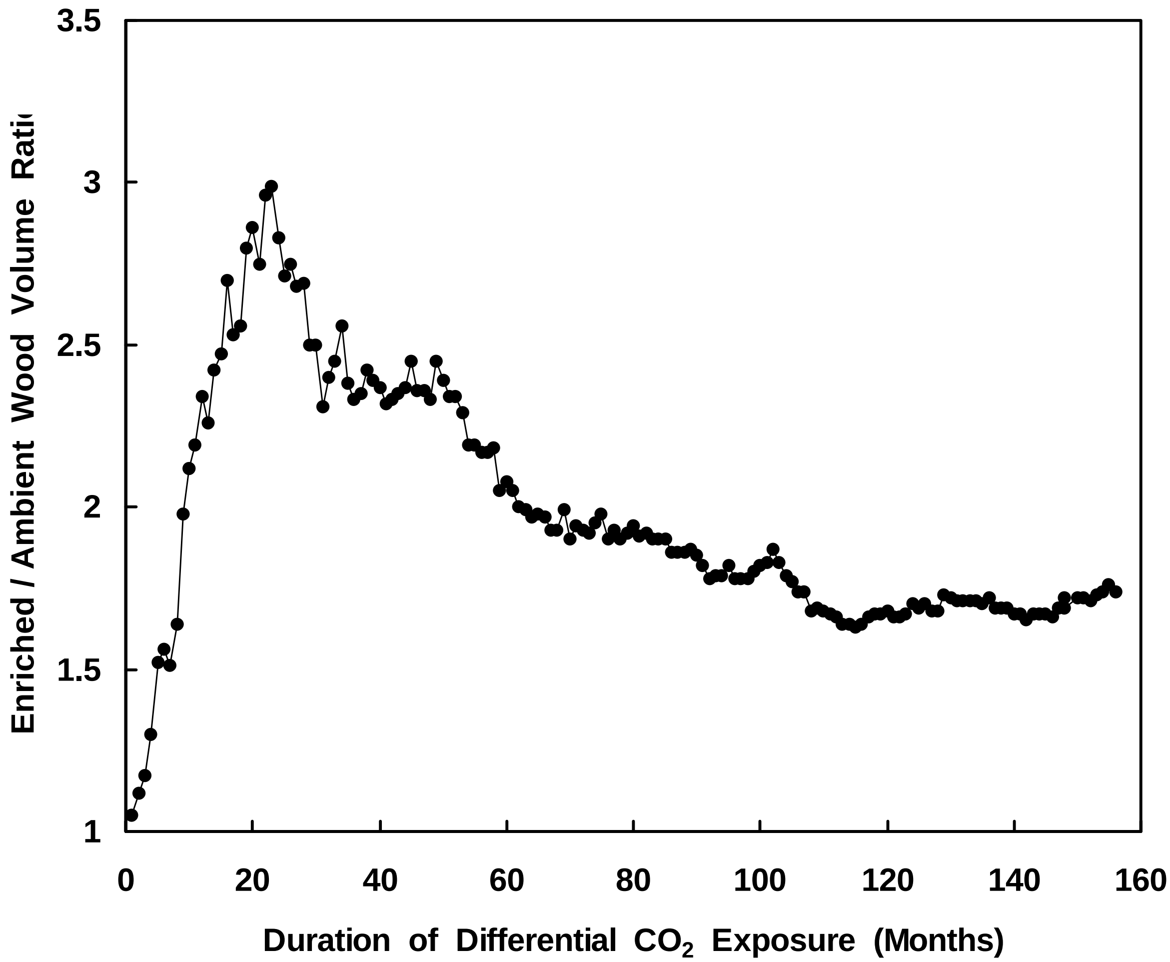

Figure 1. Ratio of volume of sour orange tree wood in CO_2-enriched chambers (700 :mol mol^{-1}) to that in ambient chambers (400 :mol mol^{-1}) versus duration of exposure to elevated CO_2.

Laboratory, are being conducted on trees growing in their natural habitat. However, in both of these experiments the trees were already at least a decade old before the CO_2 treatments were established. FACE experiments on aspen and on poplar have begun in Wisconsin and Italy, respectively, on plantation saplings, but it will be several years before results are available. Open-top chamber experiments have also been initiated on scrub oak in Florida and on longleaf pine in Alabama. However, these studies are generally only projected to last about ten years. Although much important information will unquestionably be obtained from these experiments, what is learned may not be representative of the long-term equilibrium responses of the trees.

To illustrate this latter point, we present a 13-year history of the relative above-ground (trunk + branch) wood volume of our sour orange trees (Figure 1). These data were obtained from a relationship between trunk circumference and trunk and branch volume that we determined specifically for our trees at the ends of the second and third years of the experiment (Idso and Kimball, 1992). As can be seen from the results, the CO_2-enriched trees experienced a large initial CO_2-induced growth enhancement that increased their wood volume relative to that of the ambient-treatment trees by a factor of 3.0 at the 23-month point of the experiment. Thereafter, this ratio declined, rapidly at first – as has been observed in other studies (Idso, 1999) – but then more slowly. At the end of nine full years, however, the enriched/ambient wood volume ratio leveled off; and for the past 50 months, it has remained essentially constant at a mean value of 1.69 (which has also been the ratio for fruit yield).

Clearly, an experiment of only ten years' duration could well miss the important fact that the relative growth advantage enjoyed by CO_2-enriched trees early in their history may not be totally lost over the long haul, as some have supposed, but may continue indefinitely at a very significant, albeit reduced, level. In addition, it is unclear what different results might have been obtained if our sour orange trees had not been very small (30 cm tall) and very young (on the order of six months old) when the experiment was begun. This point, too, is extremely important; for the effects of atmospheric CO_2 enrichment very early in a plant's life cycle may be of great importance to how it responds to continued CO_2 enrichment later in life (Van Der Kooij et al., 1996; Jitla et al., 1997; Miller et al., 1997; Farage et al., 1998). Hence, experiments begun at the ten-year point of a tree's existence may also fail to reveal its true equilibrium response.

2. Synthesis and Integration:

That elevated CO_2 affects plant growth has been known for more than two centuries. Starting at about the beginning of the last century, specific studies to exploit the greater growth at higher CO_2, and thereby obtain higher agricultural yields, were conducted. Although impractical for open-field agriculture, CO_2 enrichment became a standard recommended horticultural practice in greenhouses during the 1960's wherever the greenhouses were closed and unventilated. In the late 1970s there was increasing concern about the rapid increase in atmospheric levels of CO_2 and what that might do to open-field agriculture and to natural ecosystems. This concern motivated Kimball (1983a) to review the prior work in this area, and he assembled some 430 of the early observations. He analyzed these data and concluded, i.e., synthesized, that a doubling of atmospheric CO_2 concentration would increase yields about 30%, on average, if global warming was minimal. Several similar synthesis papers have been written since, which include more recent literature and/or which examine particular classes of plants and/or particular plant processes or responses (Kimball, 1983b, 1986, 1993; Cure, 1985; Cure and Acock, 1986; Kimball and Idso, 1983; Poorter, 1993; Idso and Idso, 1994; Ceulemans and Mousseau, 1994; Morison, 1995; Wullschleger et al., 1997; Cotrufo et al., 1998; Curtis and Wang, 1998; Wand et al., 1999; Norby et al., 1999; Nakagawa and Horie, 2000; Reddy and Hodges, 2000).

Since the 1960s when computers first became widely available, plant scientists have developed models to simulate plant growth and yield. Because experiments cannot be conducted on every soil type and in every local climate, these models are an extremely valuable tool for encoding knowledge gained in relatively few experiments conducted under particular conditions and then providing the means to predict what will happen in other locations. They are especially useful for forecasting what may happen over wide regions under various scenarios of global change. Therefore, several such integrative studies have been done to predict the likely effects of global change on the agricultural productivity of various regions of the U.S. and of the world, as tabulated by the recent third assessment of the IPCC (2001). However, as discussed under the "Need for Research," we are concerned that the relatively simple models that have been used in such integrative studies are not able to simulate some plant responses to elevated CO_2, which may be important in determining overall crop responses.

In the past, we have developed models for guayule growth (Kimball, 1981), for energy relations of greenhouse crops (Kimball, 1986b), and for cotton growth (Wall et al., 1994; COTCO2 Model). In all three cases, after initial progress, work stopped in order to devote more time to experiments on effects of elevated CO_2 on plant growth. With the onset of the FACE wheat experiments in 1992-3, we became active in the GCTE (Global Change Terrestrial Ecosystems, a division of the IGBP or International Geosphere-Biosphere Programme) Wheat Network, a world-wide group of wheat modelers and experimentalists who are developing the capability to predict likely effects of global change on future wheat productivity. We contributed our data from two FACE x water and two FACE x N experiments to the effort. These data were desired by the group because they were needed for validating CO_2 response aspects, because they were of high quality and obtained at frequent intervals, and because there were many ancillary data. To date, at least ten modeling papers have utilized our FACE wheat data for validation (Grant et al., 1995a,b, 1999, 2001; Grossman et al., 1995, 1999; Kartschall et al., 1995; Barnes et al., 1997; Tubiello et al., 1999: Jamieson et al., 2001). For the first 5 months of 2000, we supported a Ph.D. graduate student, Mr. Talbot Brooks, to work with Dr. Robert Grant, author of the *ecosys model*, at the University of Alberta, in developing a C4 photosynthesis sub-model suitable for simulating the effects of elevated CO_2 and other environmental variables on sorghum photosynthesis. Thus, we have been at the periphery of plant growth modeling for some time.

In the aforementioned CRIS search, projects on modeling effects of global change appeared from the ARS Ft. Collins and Beltsville locations. The RZWQM root zone water quality model from Ft. Collins is a promising farm management tool, but questionable for the detailed physiological questions addressed herein. At Beltsville, the GLYCIM soybean model and the CPM cotton model appear to have the necessary physiological process detail, but they were written largely by Basil Acock who has recently retired, and we understand the group is being reorganized, so the opportunities for collaboration appear to be in limbo.

3. Alfalfa FACE Project:

Starting in 1983, researchers from the U.S. Water Conservation Laboratory (USWCL) and the adjacent Western Cotton Research Laboratory (WCRL) studied the effects of increased CO_2 at ample and limiting levels of water and nitrogen on cotton using open-top chambers (e.g., Kimball et. al., 1992; Kimball and Mauney, 1993). Recognizing limitations of the open-top chamber approach, the USWCL and WCRL scientists participated with other researchers, especially some from Brookhaven National Laboratory (BNL), in the design and implementation of a free-air CO_2 enrichment (FACE) project to conduct open-field CO_2 enrichment experiments. The first successful biological experiment was conducted in 1989 (Hendrey, 1993), followed by more cotton experiments in 1990 and 1991 with an additional water stress treatment. The results of these experiments are reported in 21 papers in a special issue of *Agricultural and Forest Meteorology* (Dugas and Pinter, 1994). In 1992 the FACE effort shifted from cotton to wheat, and two FACE experiments incorporating ample and limiting supplies of water were conducted from 1992-1994 and two more with ample and limiting supplies of soil nitrogen from 1995-1997. About 100 scientists from 43 different research organizations in eight countries participated in the cotton and wheat experiments (Wall and Kimball, 1993; Pinter et al., 1996). About 54 papers have resulted from the wheat experiments (see Curriculum Vitae for Kimball, Wall, and Pinter for lists), and several more are in preparation. Next the focus shifted to sorghum, a C4 crop, and two FACE experiments at ample and limiting water were conducted in1998 and 1999. Three papers (Rillig et al., 2001; Ottman et al., 2001; Cousins et al., 2001) have been accepted for publication from the sorghum experiments, and several more are in preparation or submitted.

A few experiments have been done to determine the response of alfalfa to elevated CO_2, but their results have not been very consistent. Daley et al. (1988) grew alfalfa in open-top chambers at CO_2 concentrations of ambient +0, +75, +150, and +300 :mol mol^{-1}, and they found that photosynthesis of young leaves was stimulated +40% by the +150 treatment. However, significant enhancement of yield, i.e. above-ground biomass, only occurred in 3 of 9 harvests, suggesting that much of the enhanced photosynthate was going to the large root system. In a similar study, Bunce (1993, 1995) grew alfalfa in CO_2-enriched open-top chambers, which had about as many weeds as alfalfa plants. The alfalfa biomass was altered +302, -31, and +47% and the combined alfalfa plus weed biomass was altered +56, -9, and +38% by enrichment to 700 :mol mol-1 over each of three years of the experiment, respectively. In the Swiss FACE experiment, in which the main species are ryegrass and white clover, Lücher et al. (2000) grew alfalfa in sub-plots within the FACE rings. The alfalfa had been inoculated with effective or ineffective nodulating strains of *Rhizobium*. They observed increases in growth of about +49% for the alfalfa with the effective strain of *Rhizobium* and elevated CO_2 at 600 :mol mol-1. On the other hand, the alfalfa with the ineffective strain averaged only about a 12% growth increase when ample N fertilizer was applied, and surprisingly decreased about 25% in growth with elevated CO_2 and low levels of N. We should also note that white clover (*Trifolium repens*), which is also a legume, increased above-ground growth by about 25% averaged over several years due to the elevated CO_2 (Hebeisen et al., 1997), but similar to Bunce (1993, 1995), they also found large year-to-year variation in the CO_2 response. Sgherri et al. (1998, 2000) recently described another CO_2-enriched open-top chamber experiment on alfalfa that was also subjected to a short (5 day) drought. They examined several biochemical compounds related to photosynthesis and oxidative stress, but apparently have not yet reported on any agronomic aspects. We conclude from these works that there can be substantial variability in the response of alfalfa to elevated CO_2, and it is likely that, besides the effectiveness of the symbiotic *Rhizobium*, the partitioning between above- and below-ground organs may be very important. Above-ground agricultural yield may be enhanced at the expense of the below-ground root system with carbon-sequestration potential or vice versa.

For the reasons presented under the "Expected Significance" section, alfalfa is a crop whose response to CO_2 deserves more study especially in view of its potential for carbon sequestration and N_2-fixation. Following our demonstration that the FACE approach is the method of choice for conducting such research, about 28 FACE projects are now in operation or planned for various ecosystems around the world (http://cdiac.esd.ornl.gov/programs/FACE/whereisface.html). However, except for the small ancillary experiment in the Swiss FACE Project (Lücher et al., 2000), none has yet studied alfalfa; therefore, we are proposing to study it as the next logical continuation of the Maricopa FACE Project. However, it is contingent upon obtaining additional outside funding, and a proposal to obtain such funding is pending with NASA.

4. Piñon-juniper Rangeland CESAR-FACE Project:

As discussed in the "Need for Research," the piñon-juniper (PJ) ecosystem covers extensive mid-elevation areas in the Western U.S., and, because of its importance, additional research should be done to ascertain its response to elevated CO_2 and other interacting environmental variables. Free-air CO_2 enrichment (FACE) is the logical approach. However, unlike prior agricultural crop experiments at Maricopa AZ, the PJ ecosystem will have to be studied the year around; and because it is more heterogenous with larger vegetation, we will have to use larger diameter FACE rings. Therefore, the annual CO_2 requirement for conducting the research will be substantially larger than at Maricopa. If the price for CO_2 were the same as at Maricopa, the expense could be extremely prohibitive. Fortunately, however, an enormous geologic pool of CO_2 has been discovered within the PJ ecosystem near Springerville AZ, which is east-northeast of Phoenix near the New Mexico border, and the companies developing the CO_2 well field have offered CO_2 for a FACE project at a small fraction of the price paid in the prior Maricopa experiments. Moreover, the gas is quite pure with no toxins, and it is at favorable pressures for easy incorporation into a plastic pipe FACE distribution system. The presence of this CO_2 source greatly increases the feasibility for conducting such a piñon-juniper rangeland FACE project.

Led by Dr. George Koch from Northern Arizona University, Flagstaff AZ, a consortium of institutions and private companies has proposed to create a Science and Technology Center near Springerville AZ, for the study of Carbon Exchange and Sequestration in Arid Regions (CESAR). Besides utilizing the cheap CO_2 for FACE experiments, the Center would also feature flux towers to measure the present-day carbon, water, and energy fluxes, and it would also have a strong training and education component. Consortium members would include Northern Arizona University, Arizona State University, The University of Arizona, Ridgeway Industries (the company with the CO_2 development rights), Tucson Electric Power (an electric power company whose holdings within the well field include excess buildings and land they are willing to donate to the project), as well as ARS scientists from the U.S. Water Conservation Laboratory and several rangeland research groups. Additional scientists from other institutions around the world are likely to become involved as well.

The CESAR Project is not yet fully organized, but outside funding is being sought. If it becomes a reality, we USWCL scientists would expect to contribute our expertise about operation of FACE experiments. We likely would also participate in the measurement of water and energy relations and in the development of techniques for remote sensing of the CO_2 and other treatment effects.

If the CESAR Project gets underway on rangeland, then at some point in the future it likely would become advantageous to move the agricultural crop FACE operations from Maricopa to Springerville. The much lower CO_2 cost would enable agricultural crops to be studied at much higher CO_2 concentrations than have heretofore been possible.

APPROACH AND RESEARCH PROCEDURES

Objective 1 - Sour Orange Trees

Experimental Design

To date, this experiment is the longest of its type ever conducted, and therefore, it has become extremely valuable. It began in July of 1987 by planting eight sour orange tree (*Citrus aurantium*, L.) seedlings directly into the ground at Phoenix AZ and enclosing pairs in four clear-plastic-wall open-top chambers. The trees have been maintained under optimum conditions of water and nutrient supply; and since mid-November 1987, half have been continuously exposed to ambient air of approximately 400 ppm CO_2 and the other half to air enriched with an additional 300 ppm CO_2 to a concentration of approximately 700 ppm. Details of the study are given in Idso and Kimball (1997), where we present results obtained over the first eight years of the experiment.

We are now in the 14th year of the study, and we estimate we will need to monitor the growth of the trees for about five more years in order to convincingly demonstrate that, after a large initial growth stimulation followed by a slow decline, the growth response to elevated CO_2 of the trees levels out at a constant value (Figure 1), i.e., we need to show that this value is maintained for a sufficiently long time that it we are confident it will be maintained over the remainder of the trees' life span.

While obtaining these important long-term growth data, as described by Idso and Kimball (1993, 1997), we also continue to observe yearly fruit production (numbers and fresh and dry weights) and fruit vitamin C and folic acid concentrations (via yearly determinations made by collaborators - Drs. Kevin Goodner and Wilbur Widmer - who are expert in these techniques). We also make near-weekly determinations of new branch lengths and fresh and dry weights, as described by Idso et al. (2000), new leaf numbers, areas and fresh and dry weights, as well as leaf fall-rates and leaf starch and chlorophyll concentrations, as described by Idso et al. (1993, 1996). In conjunction with other collaborators, we have additionally embarked upon some exploratory programs to determine what we can learn about CO_2 effects on tree water use efficiency (via carbon isotope analyses of cores removed from the trees' trunks, in which Dr. Steve Leavitt is expert) and wood density (via x-ray densitometry analyses of the same cores, in which Dr. James Burns is expert). Likewise, we have launched an exploratory study into what we can learn about the effects of atmospheric CO_2 enrichment on the production of the glycoprotein glomalin, which is produced by fungi that live in symbiotic association with the orange tree roots, as well as the impact of CO_2-induced variations in this substance on soil aggregation, via techniques in which our collaborator, Dr. Matthias Rillig, is expert (Rillig et al., 1999).

Eventually, at the end of the experiment, we plan to conduct a detailed above- and below-ground biomass inventory of the leaves, branches of various size classes, trunks, and roots of various size classes and depths. Concentrations of C and N in the tree organs will be determined as well as those of the soil beneath the trees, radiating outward from the trees in concentric circles and downward to the bottom of the root zone. We will also enlist the help of our collaborators in studying the glomalin distribution in the soil at that time, as well as the distributions of other organic compounds that might be present; and we will investigate mycorrhizal characteristics and components of the soil food-webs we encounter beneath the trees.

For the sour orange tree experiment, Idso is responsible for the biological measurements, and Kimball is responsible for maintaining the CO_2 treatments.

Contingencies

We have already had much experience in all aspects of the studies we are currently conducting on the sour orange trees, and we feel confident we shall be able to successfully achieve the several goals.

Collaborations

Achievement of our primary objective – determining the long-term effect of atmospheric CO_2 enrichment on the growth and fruit production – can be done without outside collaboration. All of our collaborations deal with ancillary goals, such as medicinal attributes (Idso et al., 2000b) and vitamin C (Idso et al., 2001). Others in the data analysis stage include bulk wood density and water use efficiency from carbon isotopic analyses, as well as glomalin production from arbuscular mycorrhizal fungi.

Necessary (w/i ARS): Dr. Kevin Goodner, Citrus & Subtropical Products Lab., Winter Haven FL
Necessary (external to ARS): Drs. Steven Leavitt and Jim Burns, University of Arizona; Dr. Matthias Rillig, University of Montana;

Objective 2 - Synthesis and Integration

Experimental Design

One approach will be to assemble and analyze data reported in the literature from experiments around the world on the effects of elevated CO_2 and other interacting variables on plants, similar to past efforts by Kimball and by Idso (e.g., Kimball 1983a,b, 1985, 1986a, 1993; Kimball and Idso, 1983; Enoch and Kimball, 1986; Idso et al., 1997, 1988a,b; Idso, 1988, 1989, 1990, 1991a,b, 1992, 1993, 1995, 1997, 1999, 2000a,b; Rosenberg et al., 1990; Kimball et al., 1990, 1993a,b, 1997; Idso and Kimball, 1993; Idso and Idso, 1994). Generally, we plan to extract response values from the literature, organize them into logical subgroups, and then calculate means and confidence intervals (usually with necessary log transformations of ratio-type data, e.g. Kimball, 1983a)

An initial project will be to extract published data from free-air CO_2 enrichment (FACE) experiments conducted over the last decade. Several topics will be examined, including the effects of elevated CO_2 on: photosynthesis, stomatal conductance, canopy temperature, water use, plant water potential, leaf area index, shoot and root biomass accumulation, agricultural yield, radiation use efficiency, specific leaf area, tissue nitrogen concentration, nitrogen yield, tissue carbohydrate and other carbon-based compound concentrations, phenology, soil microbiology, soil respiration, trace gas emission/consumption, and soil carbon sequestration. Data will also be aggregated from species within functional groups (e.g., grape and cotton are both woody perennials and therefore might be expected to have similar responses, and if they do, then it is likely other woody perennials would behave similarly). Differences in responses of the various crops and functional groups will also be examined with regard to other interacting variables such as water supply, soil nutrient levels, temperature, etc. Comparisons will also be made between responses observed in the FACE experiments and those reported in prior reviews of chamber-based experiments.

A second synthesis and integration project will be a review of global warming and atmospheric CO_2 enrichment effects on carbon sequestration in soils and vegetation. This subject currently has worldwide attention. The recent (sixth) Conference of Parties (COP) of the Framework Convention on Climate Change (FCCC) meeting in The Hague deadlocked on this issue. Until the diverse array of scientific findings related to this topic can be harmonized within a robust conceptual framework in which all parties can have confidence, progress in developing rational domestic and international energy policies will be severely limited. In reviewing this subject, we will deal with both agricultural lands and natural ecosystems, such as grasslands and forests. We will look at both biomass production and decomposition, evaluating how these processes are affected by increasing temperature and atmospheric CO_2 concentration, singly and in combination; and we will determine how potential global change-induced alterations in these processes might increase or decrease terrestrial carbon storage in agricultural and natural settings. We anticipate this literature review will also tell us something about the current 'missing carbon' sink.

Many of the simple plant growth models use the concept of “radiation use efficiency” to calculate carbon fixed per unit of photosynthetically active radiation absorbed by the green vegetation. To simulate growth under elevated CO_2 , the modelers simply use a larger value for this efficiency, but each one appears to be getting his/her values from particular experiments. Therefore, we plan to conduct a systematic review of the literature on the topic and then to synthesize a more general relationship between radiation use efficiency and atmospheric CO_2 concentration.

Another approach will be to utilize plant growth models, particularly *ecosys*, to assess the likely impacts of global change on the productivity and water requirements of agricultural crops, starting with wheat and sorghum. In nonspecies-specific *ecosys*, all simulation are performed at the plant/soil biochemistry scale, and integrated to provide organ, organism, soil layer, and landscape scale output. Specifically, *ecosys* uses components of the Farquhar model for determination of C3 and C4 photosynthesis, numerical solutions for soil chemistry (including N and C cyling) and energy balances, and explicit C allocation routines for growth and development, as well as sequestration. The *ecosys* model has been well validated for wheat (Grant et al., 1995a,b, 1999b, 2001b), and we expect it soon will be also for sorghum. It has also been validated with respect to several non-agricultural species, such as aspen, black spruce, and moss (Grant et al., 1999a; 2001a). Thus, using various scenarios of rising CO_2 and changing climate and scaling methodologies employed by the IPCC (2001), we propose to obtain more robust estimates of the impacts of global change on agricultural crops than obtained previously with simple models, such as cited in the third assessment by the IPCC (2001). The results will also be compared to those obtained previously with the simple models.

We also propose to conduct such impact studies for cotton with a sophisticated hourly-time-step model, but more work will have to be done with regard to model selection and development. No requisite species characteristic files have been written for *ecosys* nor has any validation work been done with it for cotton. Similarly, Wall et al. (1994) developed an initial working version of COTCO2 specifically to simulate the effects of elevated CO_2 and other climate variables on cotton, but it too has not been validated. It is also our understanding that a “cotton production model” (CPM) has recently been completed by the ARS Remote Sensing and Modeling Laboratory, Beltsville MD (V.R. Reddy, personal communication), which also has an hourly time step. However, its author has retired and it too has not been validated. Therefore, it is anticipated more model development work will have to be done for at least one of the cotton models before assessments can be made. However, such will not be undertaken until the wheat and sorghum works are completed, and by that time the situation with cotton may have changed.

Kimball, Idso, Wall, and Pinter are all responsible for synthesis work, and Kimball and Wall will do the modeling work.

Contingencies

If funding is obtained for Objectives 3 and/or 4, the Synthesis and Integration work will lower in priority but not be abandoned. This strategy is justified because we are in a unique position to pursue Objectives 3 and 4. We have teams of interested multidisciplinary collaborators capable of collecting near comprehensive sets of data, and we have the expertise to do the free-air CO2 enrichment. Thus, we should try to achieve these objectives before team members become too engaged in other projects and disperse.

Collaborations

Necessary (outside ARS): Dr. Robert Grant, Univ. of Alberta; Mr. Talbot Brooks, Ariz. State Univ.

Objective 3 - Afalfa FACE Project (Contingent on obtaining outside funding)

Experimental Design

We propose to conduct a FACE experiment on alfalfa (*Medicago sativa* L.) at ample and limiting supplies of water for at least 3 years using a similar experimental design as that described previously with cotton, wheat, and sorghum (Figure 2; Wall and Kimball, 1993; Hunsaker et al., 1996; Kimball et al., 1999; Ottman et al., 2001).

A general objective is to determine the interacting effects of elevated CO_2 (FACE), soil water supply, and temperature on an alfalfa (Medicago sativa L.) ecosystem. Specific objectives and hypotheses are as follows:

1. Determine effects of elevated CO_2, water supply, and temperature on biomass (net primary productivity) and leaf area production, forage yield, and plant phenological development.

   Hypothesis 1a. There will be a significant growth response (~30%) at 200 :mol/mol of CO_2 above ambient under well-watered (Wet) conditions.

   Hypothesis 1b. There will be a somewhat larger growth response to 200 :mol/mol of CO_2 above ambient under the water-stress (Dry) treatment.

   Hypothesis 1c. There will be a larger relative response to elevated CO_2 under hot summer conditions than under cool winter temperatures.

   Hypothesis 1d. Phenology will not be significantly affected by elevated CO_2.

   Hypothesis 1e. Elevated CO_2 will have a positive effect on stand persistence.

2. Determine effects on sequestration of carbon in soil organic matter, soil CO_2 concentration, and soil respiration.

   Hypothesis 2a. There will be a significant increase in soil organic carbon due to elevated CO_2, especially at deeper depths.

Hypothesis 2b. There will be significant increases in the emissions of soil CO_2 in proportion to the biomass responses.

3. Determine effects on stomatal conductance, canopy temperature, energy fluxes, evapotranspiration, and soil water contents.

Hypothesis 3a. At times of minimal water stress, in the FACE plots there will be reduced stomatal conductances.

Hypothesis 3b. Elevated CO_2 will increase (make less negative) plant water potentials, more so under the water-stress than the well-watered treatment.

Hypothesis 3c. At times of minimal water stress, in the FACE plots there will be higher canopy temperatures (due to reduced stomatal conductances; accept 3a),

Hypothesis 3d. And higher sensible heat fluxes with reduced evapotranspiration.

Hypothesis 3e. And net radiation will be slightly reduced due to slightly more up-going long-wave radiation from warmer canopy temperature.

Hypothesis 3f. For a few days following irrigations, soil water contents in the rooted zone will be higher in the FACE plots for both Wet and Dry treatments.

Hypothesis 3g. Because of water conservation while soil water is not limiting, the soil water contents in the Wet plots at the end of each cutting cycle and growing season will be wetter under FACE,

Hypothesis 3h. And because of greater root ramification in the FACE-Dry plots, the soil water contents in the Dry plots will be drier under FACE.

4. Determine effects on photosynthetic biochemistry and net leaf and canopy photosynthesis.

Hypothesis 4a. Carbohydrates will accumulate in the FACE-grown alfalfa leaves and C3 pathway photosynthetic processes will be somewhat inhibited, particularly regeneration of RuBP,

Hypothesis 4b. But nitrogen will be non-limiting in legume alfalfa, so there will be a substantial increase in photosynthesis at elevated CO_2.

5. Determine effects on nitrogen fixation.

Hypothesis There will be significant effects on the amounts of N_2 fixed that parallel the effects on biomass accumulation, as listed for Hypothesis 1.

6. Determine impacts on soil C and N mineralization and total microbial activity.

Hypothesis Rates of mineralization and total microbial activity will be significantly increased by elevated CO_2.

7. Develop techniques for remote detection of net primary productivity of the alfalfa ecosystem subjected to varying levels of CO_2, water supply, and seasonal temperature.

Hypothesis 7a. A functional relationship exists between the fraction of absorbed photosynthetically active radiation (fAPAR) captured by the plants for potential use in photosynthesis and multispectral vegetation indices (e.g. normalized difference vegetation index, NDVI) that can be used to predict potential carbon accumulation in the alfalfa ecosystem.

Hypothesis 7b. The relationship developed in 6a will not be significantly affected directly by elevated CO_2, thereby implying that relationships between remotely detected signals and plant biophysical parameters will not change with time as the atmospheric CO_2 concentration increases in the future.

8. Determine impacts on herbivorous insects and consequent plant damage.

Hypothesis There will be greater plant damage due to insect herbivory under elevated CO_2 while at the same time rates of insect development will be slowed.

Equally important objectives include assembling a database of the above measurements and developing and validating plant growth models (Holt et al., 1975; Denison and Loomis, 1989; Grant et al., 1995a,b) capable of predicting the effects of the increasing atmospheric CO_2 concentration and any concomitant climate change on the alfalfa ecosystem.

CO_2 treatment: A FACE apparatus will be used to enrich 25-m diameter circular plots by 200 µmol mol^{-1} CO_2 above ambient during the daylit period year-round (cutoff threshold will be an air temperature # 5 $^{\circ}$C) (Figure 2). Enrichment will occur from 50% emergence, through stand establishment, and for each successive cutting over the 3-year study. To account for any effect of the blowers, the Control rings at ambient CO_2 will have air flow like the FACE rings (Pinter et al., 2000). However, because we do not plan to operate at night, blower effects should be non-significant. We plan to stop enrichment at night because (1) studies on the effects of elevated CO_2 on dark

respiration have been inconclusive, (2) the blower effect on the microclimate at night (Pinter et al., 2000) introduces unnatural temperature and humidity effects which somewhat compromise direct use of the data for model validation, and (3) the savings in CO_2 costs can be better spent on additional measurement activities.

Irrigation treatment: We plan to impose our water supply treatments using a flood irrigation system, as done previously in our sorghum experiments (Ottman et al., 2001: Conley et al., 2001; Wall et al., 2001), by using a strip-split-plot design similar to that in Figure 2 with either side of each ring receiving a Wet or Dry treatment. Ideally, irrigations for the Wet (amply water supply) treatment would be initiated after 30% of the available water in the root zone is depleted, and they would be irrigated with an amount calculated to replace 100% of the potential evapotranspiration since the last irrigation (Fox et al., 1992). Practically, however, the irrigations and cuttings have to be coordinated in order to be able to operate machinery in the field without damaging the soil. Therefore, the Wet treatment will receive two or three irrigations per cutting, while the Dry treatment will receive one irrigation per cutting. The irrigations for the Wet treatment will be applied after the hay has been removed from the field and again at about half the interval of time to the next cutting. The irrigation for the Dry treatment will be applied after the hay has been removed from the field. In a study conducted in the San Joaquin Valley (Frate et al., 1988), irrigating once per cutting at the beginning of the growth cycle resulted in 87% of the yield of twice per cutting. Alfalfa is most susceptible to yield loss from water stress at the beginning of the regrowth cycle (Doorenbos and Pruitt, 1977; Brown and Tanner, 1983).

Adjustments will be made for any rainfall; but because the seasonal consumptive water use is typically over 2030 mm (Erie et al, 1982), while seasonal rainfall averages only about 150 mm, this adjustment is minor; and excellent control of the water supply is anticipated in this semi-arid desert region, as evidenced by the wide range in volumetric water content obtained in the prior FACE x water wheat and sorghum experiments (Hunsaker et al., 1996; Conley et al., 2001).

Crop culture: The alfalfa will be grown similar to commercial crops in the area using recommended agronomic practices. The cultivar CUF 101 was selected because it is a well-adapted to the low elevation deserts of Arizona and is the most widely grown cultivar in this area. It will be sown in October at a rate of 25 kg seed ha^{-1}. The seeds will be inoculated with an effective strain of *Rhizobium* bacteria. The soil will be sampled before sowing and analyzed for phosphorus concentration as a guide for pre-plant phosphorus fertilization. The soil will also be sampled in the fall of each year and analyzed for phosphorus as a guide for annual phosphorus fertilizer application in the winter. Phosphorus is the only plant nutrient that we expect to apply as fertilizer. Because alfalfa is a legume, we do not plan to fertilize with N, and indeed the effects of elevated CO_2 on N_2-fixation will be an important aspect of this experiment. Weeds will be controlled as needed following recommendations.

Biomass production and other agronomic measurements: Forage yield will be determined by cutting the main plots eight times per year at early flowering. More detailed measures of alfalfa growth will be recorded from sub-plots at each of the eight harvests and on weekly intervals for at least three cutting cycles each year (spring, summer, and fall). These more detailed measures of plant growth include leaf biomass, stem biomass, plant height, and green leaf area index. Besides the main effects of elevated CO_2 on alfalfa growth at ample and limited water supply, after the 3 years of study over a wide range of summer to winter temperature extremes, the interactive effects between CO_2 and temperature will be examined, as will the effects of solar radiation. Because the annual pattern of solar radiation change leads that of temperature by a month or more, we anticipate being able to separate the effects of these two important environmental variables.

Soil carbon sequestration: Sequestration of carbon in the soil may be significantly increased by elevated CO_2, especially at deeper depths. We have gained experience examining soil organic carbon (SOC) change through repeated application of isotopic tracer techniques in FACE cotton (Leavitt et al., 1994), wheat CO_2xH2O (Leavitt et al., 1996), wheat CO_2xN (Leavitt et al., 2001), and sorghum (mss. in preparation). This method depends on the plant having or acquiring a carbon isotope composition that is sufficiently different from soil organic carbon that new carbon from the experiment entering the soil will alter SOC isotopic composition. The larger the difference in isotopic composition between SOC and inputs, the more sensitive the tool. In this study, we will dominantly employ a commercial CO_2 gas from geologic deposits which is most economical in our area. It has a *^{13}C= -5‰ for enriching FACE plots. By itself, it will result in FACE alfalfa plants with *^{13}C= -26‰, providing a tracer about 4.5 to 5‰ ^{13}C-depleted relative to SOC measured as -21 to -21.5‰ at the end of the previous sorghum experiments. In Control plots, the tracer will be a little stronger as the plants will have *^{13}C= -27‰, about 5.5 to 6‰ ^{13}C-depleted relative to the original SOC. Other studies have found this isotopic separation was sufficient to estimate carbon inputs. However, a stronger label would increase the power of this isotopic method to detect differences. Therefore, we plan to strengthen the isotopic tracer signal in the FACE plots by blending the main geologic CO_2 with at least 15% petroleum-derived CO_2 (*^{13}C= -40‰), which is somewhat more expensive. This will produce alfalfa plants of at least -27‰ if not even more ^{13}C-depleted. Extensive soil core samples will be taken before and after the experiment. Then, with estimates of new carbon from both FACE and Control plots, the difference will indicate net effect of CO_2 enrichment. Besides determination of new C from isotopes, we also will measure total C using standard chemical analyses, but this measure is less sensitive because of the large amount of C already present in soil. In addition, collection and *^{13}C analysis of soil CO_2 at 2 depths will provide results for additional comparison between FACE and Control for belowground processes.

Photosynthesis and plant water relations: Leaf photosynthesis, conductance, and transpiration rates will be surveyed with a portable closed-exchange (transient) leaf gas (CO_2, H_2O) exchange system with a 250 cm^3 transparent cuvette (LI-COR, Inc., Model LI-6200, Lincoln, Nebraska, U.S.A.). Additional measurements of gas exchange rates will be made at solar noon (maximum photosynthetic rates) from the beginning of a soil dehydration cycle to just prior to rehydration (immediately following an irrigation until just prior to the subsequent one). We will monitor the leaf relative water content gravimetrically, and, with thermocouple psychrometers, we will measure total leaf water potential and its osmotic and turgor components.

Carbohydrate levels in the leaves of alfalfa vary with time during cutting cycles, and they are predicted by at least 3 alfalfa models (Holt et al., 1975; Denison and Looms, 1989; Grant et al., 1995a,b). We observed them to increase dramatically in leaves of wheat grown in elevated CO_2, particularly fructans (Nie et al., 1995b), and such accumulation has been shown to inhibit photosynthesis (Azcon-Bieto, 1983). Therefore, we will sample alfalfa leaves and stems, as well as roots whenever possible, when photosynthetic measurements are made. The samples will be flash frozen in liquid nitrogen and stored in a -80^{o}C freezer. The samples will be freeze-dried, ground to pass a 20-mesh screen, and assayed for soluble sugars (sucrose, glucose fructose) and low and high molecular weight fructans and starch (Hendrix, 1992, 1993; Hendrix and Peelen, 1987). Key elements in the biochemical regulation of sucrose synthesis include bisphosphate (F2, 6bP) and sucrose phosphate synthase (SPS) (Stitt, 1991). For leaves stored at -80^{o}C we will monitor levels for sucrose, FbP, F2-6bP, SPS, and cytosolic FbPase in conjunction with measurements of photosynthesis. Because carbohydrate accumulation in leaves will change with CO_2 and water-stress, these studies may also provide insight into how carbohydrates may serve as feed-back inhibitors of photosynthetic gene expression in the field, as proposed from laboratory studies (Krapp, 1991,1993; Sheen, 1990, 1992).

Biochemical assessment of the dark reactions of the photosynthetic apparatus will be performed concurrently with the survey of leaf photosynthesis and A/Ci curves. Leaves will be sampled, quick-frozen in liquid nitrogen, and stored at -80^{o}C. These frozen leaf samples will be analyzed for soluble and total proteins, and enzyme assays will be conducted to evaluate Rubisco activities, activation states, and concentrations of active centers. Proteins will be extracted from an equal area of the frozen leaf samples. The extracted proteins will then be size-fractionated by polyacrylamide gel electrophoresis and either transferred to nitrocellulose membrane or stained with coomassie blue. The amount of protein in the individual lanes will be quantified by densitometric scanning of the stained protein bands. Rubisco, LHCII and the *a* and *b* subunits of the chloroplast ATPase are very abundant proteins and can be quantified easily with this procedure. We have antisera to Rubisco, PEPCase, and the b-subunit of the ATPase. We will use these to probe the proteins immobilized on the nitrocellulose filters, which will provide accurate identification and quantification of the proteins. Results from the western blots will help confirm those from the densitometric scans.

Biochemical assessment of light reactions of the photosynthetic apparatus, the quantum yield of PSII and any changes in photochemical and non-photochemical quenching will be monitored with a portable fluorometer (Model PAM-2000, Heinz Walz GmbH, Germany). Interpretation of fluorescence measurements will be used to determine how elevated CO_2 affects apparent electron transport and quantum yield in water-stressed compared with well-watered leaves. As was done in FACE wheat, thylakoid membranes will be isolated from the frozen leaf samples, with a modification of the protocol described by Nie et al. (1995a,b), to determine how additional thylakoid membrane proteins, particularly those associated with electron transfer complexes (PSI, PSII, cyt b6/f), are effected by elevated CO_2 and water stress. Thylakoid membrane proteins will be size fractionated by SDS-PAGE and either transferred to nitrocellulose membrane or stained with coomassie blue. Proteins will again be quantified by western blotting using antigens to key polypeptides of each complex. We have antisera to PSI and PSII reaction center proteins as well as cyt b6/f. We will use these to probe the proteins immobilized on the nitrocellulose filters, which will provide accurate identification and quantitation of the proteins.

We propose to seek a molecular explanation for any observed *down-regulation* of photosynthetic proteins. We will examine the expression of genes encoding the proteins assayed above by the steady-state level of mRNA using northern blots. The leaves from each treatment will be wrapped in aluminum foil and immediately frozen in liquid nitrogen and stored in a -80 ^{o}C freezer. Total cellular RNA will be isolated from the frozen leaf samples using buffers containing guanidine hydrochloride followed by precipitation with lithium chloride (Hird et al., 1991). Equal quantities of glyoxal denatured RNA will be size fractionated by agarose gel electrophoresis and either stained or transferred to nylon membrane. The immobilized mRNA will then be probed with radiolabeled DNA fragments specific for a number of chloroplast and nuclear genes. In particular, we will look at steady-state levels of mRNA from *psaA/B*, *psbA*, *psbD*, *atpB*, *rbcL*, representative of chloroplast genes and nuclear from genes encoding LHCII, RbcS, FbPase, and SbPase.

From the perspective of the ecosystem, it is necessary to integrate individual leaf carbon exchange rates to the whole-plant, canopy and community levels. Therefore, eight steady-state whole-canopy gas exchange systems (Garcia et al. 1990; Brooks et al., 2001) will be used to monitor CO_2 and H_2O vapor exchange rates for all treatment combinations for two replications. All eight chambers will be run simultaneously and controlled from a central control trailer located on site. In addition, at bi-weekly intervals we intend to measure canopy carbon exchange rates across a broad range of atmospheric CO_2 concentrations (ambient to 1800 **:** mol mol^{-1}) and to monitor any changes in canopy architecture due to CO_2 or water level with a portable canopy analysis system (Model LI-6000, Li-COR, Lincoln, NE).

Energy and soil water balances and evapotranspiration: Canopy temperatures will be measured using carefully calibrated infrared thermometers that are switched between FACE and Control plots weekly (Kimball et al., 1994, 1995, 1999). Net radiation, soil heat flux, and sensible heat flux will be measured, and latent heat flux (i.e. evapotranspiration) will be determined using a residual energy balance approach (Kimball et al., 1994, 1995, 1999). Soil water contents will be measured using neutron scattering equipment and time domain reflectometry, similar to the methods used previously by Hunsaker et al. (1994, 1996, 2000) and Conley et al. (2001). Utilizing irrigation and rainfall amounts along with the soil water content data, a second estimate of evapotranspiration will be calculated as a residual in the soil water balance.

N_2 fixation: The fixing of inorganic atmospheric N_2 to organic forms of N is an important aspect of the alfalfa ecosystem. We will determine the overall effects of elevated CO_2 on nitrogen yield from measurements of biomass production, as described previously, and of the nitrogen concentrations of the tissues. In addition, we will determine the effects of elevated CO_2 on the symbiotic N_2 fixation in 2m x 2m sub-plots within the main plots. In half of each of these sub-plots, non-N-fixing plants will grown, but the entire sub-plot area will be fertilized with a small amount of fertilizer that has been highly enriched with ^{15}N. Following Lüscher et al.(2000), the percentage of N derived from symbiosis will be calculated from {%N = [1-(^{15}N atom excess $_{\text{fixing crop}}$)(^{15}N atom percentage excess $_{\text{reference crop}}$)]

* 100} for both Control and CO_2 enriched plants (McAuliffe et al., 1958; Witty, 1983; Boller and Heichel, 1983). The reference non-N_2-fixing plants will be a near-isogenic non-fixing line of alfalfa germplasm (< 2% or less N_2-fixing activity; Barnes et al., 1990) and/or other non-fixing plants such as *Taraxacum officinale* L. (a species with deep tap root) or *Lolium perenne* L. (a species with shallow fine roots; Lüscher et al., 2000). A non-fixing alfalfa line would be preferred for the reference crop because then there would be more homogeneity in the crop canopy and few differences in rooting patterns and non-symbiotic N uptake. In addition, the number of nodules formed per plant will be determined by counting the number of nodules formed under both FACE and ambient CO_2 conditions. The effectiveness of the formed nodules and N_2 (C_2H_2) fixation activity of the nodules will be determined by the acetylene reduction technique (Havelka et al., 1982).

Soil N_2O and CO_2 emissions and soil CO_2 concentrations: We also propose to measure CO_2 and N_2O emissions throughout the growing season. Soil CO_2 will be collected at depths of 15, 30 and 50 cm in situ by means of permanently installed standpipes open at those depths. CO_2 from samples taken every 3 weeks will be cryogenically separated and isotopically analyzed. For surface soil efflux, PVC collars (ca. 20 cm diameter) will be partially pushed into the soil, and CO_2 flux will be measured using a LI-COR gas analyzer after placement of a cover over the open top of the collars. CO_2 samples will also be drawn into evacuated flasks, and they too will be cryogenically separated and analyzed isotopically. These collections and analyses should provide information about relative rates at which CO_2 is escaping from the soils of the FACE and Control plots, and the isotopic composition should reveal the relative contributions from different sources, especially in FACE plots where plant respired CO_2 should be very ^{13}C depleted relative to the SOC originally present in the soil.

Nitrous oxide emissions will be measured at about monthly intervals throughout the season within 1 m x 1 m soil subplots. Two subplots will be located in each FACE and Control ring for a total of 16 subplots. Measurements will also be made on a diurnal basis on selected days. Nitrous oxide flux will be measured using a non-steady-state (closed) chamber method (Matthias et al., 1980; Livingston and Hutchinson, 1995). We will analyze N_2O in air samples using a gas chromatograph equipped with an electron capture detector. Each chamber is made of opaque corrugated plastic and is temporarily deployed for 30 minutes on a 0.5 by 0.5 m base permanently inserted in the center of each soil subplot. Plastic syringes (50 mL) are used to sample air within each chamber at the time of deployment and 30 minutes later. Air samples are then immediately transferred from syringe to evacuated 10 mL Wheaton bottles for subsequent N_2O analysis in the laboratory. Gas samples from known standards are injected into evacuated Wheaton bottles in the field in order to assess potential losses of N_2O from the bottles during transport to Tucson. N_2O emission rate (g m^{-2} day^{-1}) is computed from the measured rate of increase of N_2O concentration in the chamber and the known height of the chamber. Chamber height is adjusted during the season in order to accommodate increased plant canopy height.

In addition to sampling N_2O emissions, denitrification rate will be evaluated using the acetylene inhibition method (Ryden et al., 1987; Terry et al., 1986) in undisturbed soil cores collected from each subplot. Simultaneously, microbial respiration will be evaluated as the amount of CO_2 evolved from soil in 1-L incubation jars using a CO_2 trap-titrimetric method (van Kessel et al., 1993). After incubation and sampling, the soil cores (ca. 15 cm long by 5 cm diameter) will be dried to measure the water content, bulk density, and inorganic nitrogen (Keeney and Nelson, 1982). The N_2O flux and denitrification rate measurements should give important information about the relative loss of fertilizer N due to elevated CO_2 under water stress and well-watered conditions.

Remote sensing measurements: Measurements of solar reflectance and thermal emittance will be used to characterize dynamic agronomic and biophysical parameters of the alfalfa canopy on a continuous basis throughout the 3 year duration of the experiment. Data will be acquired several times a week at the whole canopy scale using ground based, wide band sensor systems having visible and near-infrared capabilities. Observations will be made at a time corresponding to a standardized solar zenith angle of 57° to minimize bi-directional reflectance factor complications that caused by widely varying illumination angles at different times of the year. Commonly used multispectral vegetation indices (VIs), such as the normalized difference vegetation index (NDVI), will be used to develop predictive relationships with percent cover, plant height, green leaf area index, and biomass of the alfalfa crop. These non-invasive, repeated measures of plant growth are expected to have less variation associated with them compared to traditional biomass samples. They will thus be of considerable value in differentiating treatment effects on regrowth of alfalfa after each harvest event. Multispectral VIs will also be used to estimate the fraction of absorbed photosynthetically active radiation (fAPAR) captured by the canopy for potential use in photosynthesis (Pinter, 1993; Pinter et al 1994). This is an important biophysical parameter required for determining the effect of elevated CO_2 on radiation use efficiency (RUE, the amount of biomass produced per unit of absorbed PAR). Temporal derivatives of VIs will also be examined for their utility in detecting alfalfa growth rates and in further quantifying differences between experimental CO_2 and irrigation treatments.

Fundamental questions regarding the direct effects of elevated CO_2 on spectral properties of alfalfa will be addressed using field portable spectroradiometers capable of measuring hyperspectral features (wavelength regions 3 to 10nm in width) in the visible, near-infrared, and short wave infrared portions of the spectrum. Canopy measurements will be taken at a standardized solar zenith angle of 57°. Single leaf data will be obtained using an external integrating sphere. Depending upon rates of plant growth and size of individual leaflets, these data will be acquired as often as weekly intervals during the height of the growing season. Data analysis will investigate treatment effects on narrow spectral features, spectral derivatives, overall spectral shape, and red edge position using traditional regression techniques.

The temporal course of plant water status between irrigations will be characterized non-destructively using several thermal indices that have been developed by USWCL scientists. Infrared thermometers will be used to obtain canopy surface temperatures from all replicates and treatment combinations about 1 hour after solar noon when plants are exposed to maximum atmospheric evaporative demand. The data will be used to compute the crop water stress index (CWSI) and estimate the ratio of actual to potential canopy evapotranspiration (Jackson et al., 1981; Idso et al., 1981). The water deficit index (WDI) which utilizes the thermal infrared plus an estimate of plant cover derived from VIs is expected to provide an accurate estimate of plant water status early in the regrowth period when plants are small and canopy cover is incomplete (Moran et al., 1994). These remote indices of plant water status will be compared with less frequent measures of physiological water stress to provide a functional rationale for their use. Spatial variability within the FACE experimental field will be assessed periodically from a light aircraft or helicopter equipped with a thermal scanning radiometer.

Herbivorous insects: Elevated-CO_2-grown plants can affect the growth of herbivorous insects, as we have shown previously on cotton (Butler et al., 1985; Akey et al., 1988, 1989), and Awmack and Harrington (2000) have shown for alfalfa aphids on faba bean. However, alfalfa is especially noteworthy for attracting herbivorous insects, which attack during various times of the year and have different modes of feeding. Normally little active control is done, and at the time of cutting, the insects' food supply is removed and there is an abrupt change in microclimate (Pinter et al. 1975), which more or less resets the clock for the next cycle. We anticipate that this situation will provide opportunities for study of the effects of the elevated CO_2 and water stress treatments on these insects. Much of the work will be serendipitous, i.e., taking advantage of naturally occurring infestations. However, the initial infestations may be unevenly distributed, which would make interpretation of the data difficult. Therefore, we plan to augment the natural populations with infestations using insects in cages in some sub-plot areas. In particular, we will study the response of Lygus bugs, a serious pest complex on several crops in the U.S. and the world, and a foliage-feeding moth or leaf miner.

Plant growth modeling: We plan to utilize the data from the FACE alfalfa experiment to validate several aspects of *ecosys*, a mechanistic plant growth model with regard to several plant processes, namely the effects of CO_2, water supply, and seasonal weather on: biomass and leaf area production; sequestration of soil carbon; N_2-fixation, leaf photosynthesis, stomatal conductance, and plant water status; and canopy temperature, energy fluxes, and soil water content. Thus, the results from our specific experiments conducted at one place for a short time can be applied to future global change scenarios useful for exploring the policy implications of C sequestration and agricultural productivity in wider regions. At the same time, we expect that ecosys will provide a theoretical framework, which likely will help interpret the experimental data.

The names of the scientists on the FACE Alfalfa Project Team and their areas of responsibility for measurements are listed in the following table.

Investigator	**Measurement responsibiltiy**
Bruce A. Kimball	Overall management and energy and water balance measurements
Paul J. Pinter, Jr.	Remote sensing measurements
Gerard W. Wall	Plant water relations and photosynthesis measurements
Michael Ottman	Above-ground biomass production and other agronomic measurements
Steven W. Leavitt	Soil carbon sequestration and soil atmosphere measurements
Allan D. Matthias	Soil respiration and N_2O emissions measurements
Andrew N. Webber	Biochemical compounds involved with photosynthesis

George W. Koch	Mechanistic modeling of stomatal response to elevated CO_2
Dean Martens	N_2-fixation and other soil microbiological measurements
Bruce Hungate	Nitrogen cycling as related to elevated CO_2 and hydrology
Stephen Prior, Brett Runion & Allen Torbert	Carbon and nitrogen mineralization. And possibly root biomass.
David H. Akey and Jacquelyn L. Blackmer	Responses of herbivorous insects
Robert F. Grant & Talbot J. Brooks	Plant growth and soil process modeling

Contingencies

Conduction of the alfalfa FACE project is contingent upon obtaining additional outside funding. If funding cannot be obtained, we will do more of the Objective 2 synthesis and integration.

Collaborations

Necessary (within ARS); – Dr. Dean Martens, Soil Microbiologist, Tucson, AZ; Drs. David Akey and Jacquelyn Blackmer, Entomologists, Phoenix, AZ.; Drs. Stephen Prior and Allen Torbert, Auburn, AL and Allen Torbert, Temple, TX.

Necessary (external to ARS); – Dr. Michael Ottman, Department of Plant Sciences, University of Arizona; Dr. Steven Leavitt, Tree Ring Laboratory, University of Arizona; Dr. Robert Grant, University of Alberta; Mr. Talbot Brooks, Department of Geography, Arizona State University; Dr. Andrew Webber, Department of Plant Biology, Arizona State University; Drs. George Koch and Bruce Hungate, Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ.

Objective 4 - Piñon-juniper Rangeland FACE Project (Contingent on obtaining outside funding)

Experimental Design

Final details of the experimental design have not been decided, but a design under consideration is to build nine large (24-m diameter) FACE rings [using general methodology described in Hendrey (1993) and Miglietta et al. (1997)], with three rings at current ambient CO_2 concentration, three rings at 550 :mol mol^{-1}, and three rings at 750 :mol mol^{-1}. Because of the critical role of water in regulating ecosystem processes in the arid southwest, each ring (ambient, 550, and 750) will be split into two halves, one receiving 50% more than naturally occurring precipitation. Sub-plots will be clipped periodically at different frequencies to simulate varying grazing pressure.

Choosing an experimental design with adequate statistical power to discern ecologically important differences in the face of the heterogeneity of the piñon-juniper ecosystem is a concern. Rather than utilize a grid lay-out like we have in our Maricopa FACE field, it is likely that matched trio areas would be identified for each replicate block that wouldn't be a specified distance apart. The areas would be matched according to similar gross vegetation structure, slope, aspect, and soil properties. Probably a full growing season's worth of measurements will be done to establish a baseline condition for each plot before the CO_2 and other treatments are imposed.

The design with three CO_2 concentration levels, in contrast to most other elevated CO_2 experiments (and all FACE experiments), will allow us to test whether the effects of elevated CO_2 on C exchange and sequestration are linear over this range of CO_2 concentrations, spanning most of the projected increases in atmospheric CO_2 concentrations through the next century (IPCC, 2001). Some of the hypotheses to be tested and approaches used in these experiments include:

Hypothesis 1: Elevated CO_2 will increase rates of CO_2 uptake by ecosystems from the atmosphere, but (1) these increases will be larger in woody species (dominated by the more responsive "C3" photosynthetic pathway) than grasses (dominated by the less-responsive "C4" photosynthetic pathway) , and (2) partitioning of the extra C taken up in elevated CO_2 among ecosystem compartments varying in turnover time will strongly influence the way rates of C exchange translate to long-term C sequestration in wood and soils.

We will measure rates of net ecosystem C exchange in ambient and elevated CO_2 concentrations using open gas exchange systems (Garcia et al., 1990; Brooks et al., 2001). The geological source of CO_2 is 'dead', containing undetectable amounts of ^{14}C (this CO_2 differs only slightly from atmospheric CO_2 in stable C isotope [^{13}C] composition). We will use this depletion in ^{14}C to trace carbon flow to pools of varying turnover times, including soil fractions and wood. Collaborators and PIs include experts in radiocarbon tracing (Leavitt) and soil C analysis, including fungal pools. Measurements of natural abundance of ^{14}C will be made on the accelerator mass spectrometer in the Laboratory of Isotope Geochemistry at the University of Arizona. Because our design includes two levels of elevated CO_2, we will be able to quantitatively assess the effects of increased CO_2 (550 to 750 :mol mol^{-1}) on carbon partitioning and the implications for long-term C sequestration. Additionally, we will use ^{13}C labeling techniques to track rates of C uptake and incorporation into soil by different plant growth forms (see below).

Hypothesis 2: Elevated CO_2 will favor shrub encroachment into grasslands, and the increase in C uptake and sequestration caused by invading deep-rooted shrubs will be greater than the direct effect of elevated CO_2 on rates of C exchange and sequestration in the grassland ecosystems.

Sampling for biomass production of the grasses will be done, whereas the wood volume increase of the shrubs will be made from dimensional measurements and allometric relationships. Numbers of plants of each species will be tabulated. In addition, the dominant woodland (piñon, juniper, rabbitbrush) and grassland species differ in stable isotope composition from each other due to their different photosynthetic metabolisms, and the differences are reflected in the soils developed under these different plant groups. This provides a powerful C isotope tracer which we will use to document the incorporation into grassland soil (*^{13}C = - 15 ‰) of C from the deeply rooted shrubs (*^{13}C = -23 to -27 ‰), and how this process is affected by elevated atmospheric CO_2.

Hypothesis 3: Elevated CO_2 will cause partial stomatal closure during times when there is sufficient moisture, which will decrease transpiration per unit of leaf area of both C3 shrubs and C4 grasses. Consequently, canopy temperatures of both shrubs and grasses will increase. Water use efficiency will increase under elevated CO_2 for both. However, there will be a CO_2 growth response of the C4 grasses only drier conditions, whereas the C3 shrubs will respond under moist conditions as well. An ancillary hypothesis is that the elevated CO_2 will enable the grasses to withstand greater grazing pressure only under the drier conditions.

Soil water content will be monitored by TDR or neutron scattering, and water use determined from changes using a soil water balance (e.g. Hunsaker, et al., 1996). Net radiation, canopy temperatures, air temperatures, and soil heat fluxes will be measured components of the energy balance, while water use will be determined as the residual (e.g. Kimball et al., 1999). Plant water potential will be determined occasionally using sampled leaves in pressure bombs and psychrometers (e.g. Wall et al., 2001).

Not all responsibilities have been defined for the Piñon-Juniper FACE Project Team, and several more members will be added in the future. However, it is likely the following scientists will have areas of responsibility as listed in the following table.

Investigator	**Measurement responsibility**
George W. Koch	Overall management and mechanistic modeling of stomatal response to elevated CO_2
Bruce A. Kimball	Initial establishment of FACE apparatus and energy and water balance measurements
Paul J. Pinter, Jr.	Remote sensing measurements
Gerard W. Wall	Plant water relations and photosynthesis measurements
Jack Morgan	Biomass productivity and photosynthesis
Steven W. Leavitt	Soil carbon sequestration and soil atmosphere measurements
Bruce Hungate	Nitrogen cycling as related to elevated CO_2 and hydrology
Robert F. Grant & Talbot J. Brooks	Plant growth and soil process modeling

Contingencies

Contingent upon obtaining outside funding. If such funding is not obtained, we will focus more on Objective 2 synthesis and integration.

Collaborations

Necessary (within ARS) – Dr. Jack Morgan, Ft. Collins, CO

Necessary (external to ARS) – Drs. George Koch (Leader of CESAR Project) and Bruce Hungate, Ecologists, Northern Arizona University, Flagstaff, AZ; Dr. Steven Leavitt, Tree Ring Laboratory, University; Dr. Robert Grant, Plant Growth Modeler, University of Alberta; Mr. Talbot Brooks, Modeler, Arizona State University.

PHYSICAL AND HUMAN RESOURCES

1. Sour orange trees: The physical and human resources available and necessary to accomplish our research objectives over the next three years are basically the same as they have been since the beginning of this very-long-term project. We have the needed equipment, and about 1.0 FTE of technician time will be directed to the project.

2. Synthesis and integration: We have the requisite PC computers and access to higher speed Unix computers at Arizona State University (ASU). We have a small library at the USWCL and access to the library at ASU. We also utilize the resources of the National Agricultural Library. Two FTE of technician time will be directed to the project. However, as mentioned at the outset, this activity will have lower priority than FACE experiments on alfalfa and/or piñon-juniper if they get funded. In the latter case, the two FTE of technician time would be mostly directed there.

3. Alfalfa FACE Project: The USWCL has the requisite FACE apparatus, as used in the 1998 and 1999 FACE sorghum expeiments, for maintaining controlled elevated CO_2 concentrations over open-field plots, although a CO_2 storage tank would need to be leased or purchased. The University of Maricopa, Maricopa Agricultural Center, has the land and necessary farming equipment. They also can supply irrigation water, and a Specific Cooperative Agreement or other arrangement would be made with them to conduct the experiment. The USWCL also has the necessary balances and drying ovens for biomass measurements; the data loggers and micro-meteorological instruments for energy balance measurements; the pressure bombs, leaf psychrometers, and other sensors for determining plant water status; and the infrared thermometers, black-body calibrator, hyperspectral radiometer, and other sensors for remote sensing aspects. Two FTE of permanent technician time would be directed to the project, but this will not be enough. Outside funding is being sought for more temporary technical assistance and also for an engineer to be responsible for installation and maintenance of the FACE and irrigation treatments. Our collaborative partners also require more technicians (or graduate students).

4. Piñon-juniper FACE Project: This will be a new project, so considerable equipment will have to be purchased and installed. Fortunately, Tucson Electric Power has a generating station with excess buildings and land within the CO_2-well field whose use they have offered to the project. Power, water, and communication connections can also be made at their facility, so it is likely that no installation of long cable or pipe lines will be needed. Nevertheless, in spite of the TEP infrastructure, FACE apparatus will have to be be built and installed.

If the Alfalfa FACE Project gets funded and is in operation at Maricopa, many USWCL instruments and the technicians listed above will not be available for the Piñon-Juniper FACE Project at Springerville. Therefore, additional instruments and technical help will have to be obtained. On the other hand, if the Alfalfa FACE Project does not get funded, the USWCL resources can be directed to Springerville.

MILESTONES AND EXPECTED OUTCOMES

	Research Objective or Area of Study			
Date	**1. Sour Orange Trees (Idso, Kimball)**	**2. Synthesis & Integration (Kimball, Idso, Wall, Pinter)**	**3. FACE Alfalfa Expt. (Kimball, Wall, Pinter)**	**4. FACE Piñon-Juniper Rangeland Expt. (Kimball, Wall, Pinter)**
Jan. 2001	Harvest fruit & tabulate monthly growth increments for 2000.	Review of free-air CO_2 enrichment (FACE) effects on agricultural crops completed.	Proposal submitted to NASA for funding.	Rough plan and pre-proposal completed.
Jan. 2002	Ditto for 2001 + folic acid production + soil fungal growth & glomalin & soil structure.	Above FACE review published, and review of carbon sequestration completed. Paper on elevated CO_2 effects on canopy temperature and crop production areas written.	If selected for NASA funding, will install FACE apparatus and plant alfalfa in fall 2001. If not, then timetable will pushed forward accordingly until funding finally achieved.	Second stage planning completed, and proposal prepared and submitted to funding agency.
Jan. 2003	Ditto for 2002 + wood density & strength + water use efficiency.	First regional study with *ecosys* on wheat completed. Paper written on relationship between radiatio use efficiency and CO_2 concentration.	Tabulation and review of first year's growth, remote sensing, energy and water balance, photosynthesis and water relations, and other data.	If selected for funding, initiate experiment in spring of 2002. If not, timetable will be delayed accordingly until funding finally achieved.
Jan. 2004	Ditto for 2003 + differing sunlit & shaded growth & fruit & antioxidant + history of leaf starch & sugar production + leaf senescence & fall history.	First regional study with *ecosys* on sorghum completed. Cotton model selected and regional study intiated.	Tabulation and review of second year's growth, remote sensing, energy and water balance, photosynthesis and water relations, and other data.	Tabulate and review of initial season's baseline measurements at ambient CO_2.
Mar. 2004	End of time for this proposed project plan.			
Jan. 2005	Ditto for 2004. This marks the minimum length of time to be sure that the CO_2-enriched trees have achieved a constant relative growth advantage over the ambient-treatment trees (Figure 1) that can reasonably be expected to continue throughout the remaining life of the trees.		Tabulation and review of third year's growth, remote sensing, energy and water balance, photosynthesis and water relations, and other data. Papers written on these topics.	FACE treatment starts spring of 2004. Tabulation and review of first season's growth, remote sensing, energy and water balance, photosynthesis and water relations, and other data.

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