E&PD Management Unit

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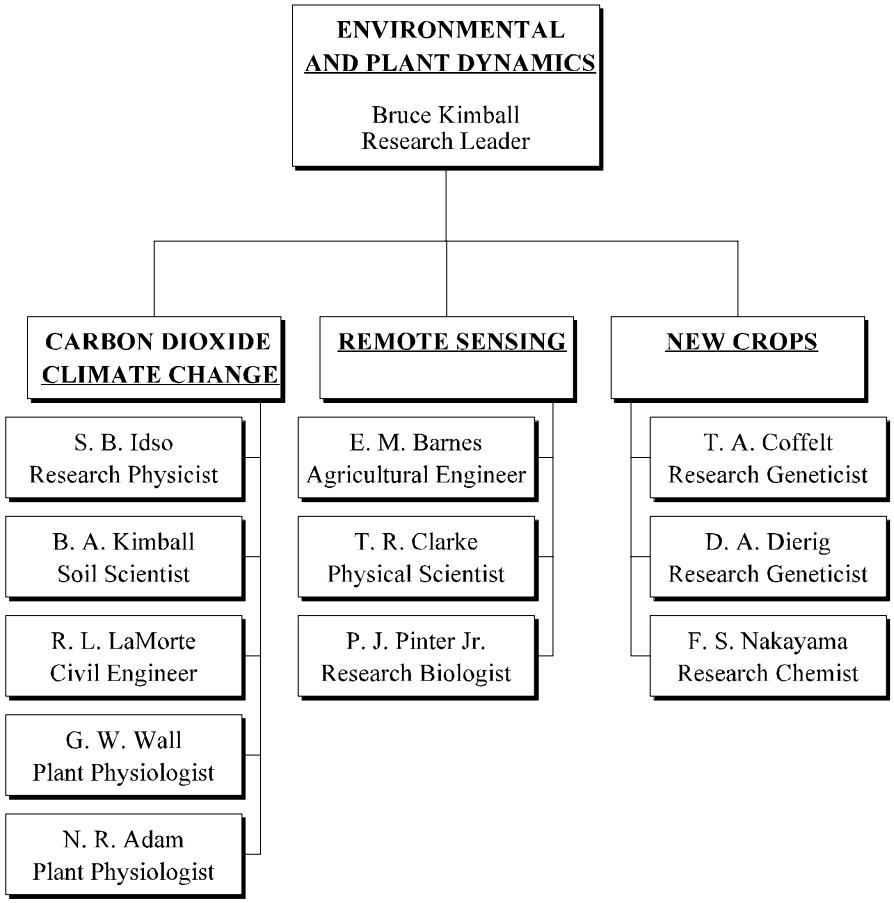


Mission

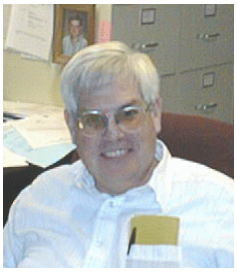
The Environmental and Plant Dynamics Research Group seeks to develop optimum resource management strategies for meeting national agricultural product requirements within the context of possible changes in the global environment. There are three main research thrusts: The first is predicting the effects of the increasing atmospheric CO_2 concentration and climate change on the yield and water use of crops in the future. The second thrust seeks to develop remote sensing approaches for observing plant conditions and biophysical processes which are amenable to large scale resource monitoring using aircraft- and satellite-based sensor systems. The third research thrust is to develop new industrial crops with unique high value products and lower water requirements for commercial production within the context of changing environments.

E&PD RESEARCH STAFF

NEAL R. ADAM, B.S., M.S., Ph.D., Plant Physiologist
Research regarding physiological, biochemical and molecular responses of wheat to CO2 enrichment in FACE crop canopy experiment. Establish protocol for enzyme activity assays, SDS-PAGE and other biochemical procedures on leaf samples. Design and implement data collection and processing tools.

EDWARD M. BARNES, B.S., M.S., Ph.D., Agricultural Engineer
Remote sensing applications for farm management; consideration of approaches that integrate remotely-sensed measurements with crop growth models and decision support systems.

THOMAS R. CLARKE, B.A., Physical Scientist
Remote sensing for farm management, thermal and optical radiometry, and instrument calibration.

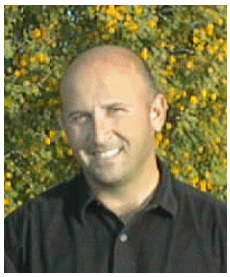


TERRY A. COFFELT, B.S., M.S., Ph.D., Research Geneticist-Plants
Breeding, genetics, and germplasm evaluation of new crops--guayule, lesquerella, and vernonia; development of acceptable production practices.

DAVID A. DIERIG, B.S., M.S., Ph.D., Research Geneticist-Plants
Breeding, genetics, germplasm collection and evaluation of new industrial crops with unique, high-value products, including lesquerella, vernonia, and guayule.

SHERWOOD B. IDSO, B.S., M.S., Ph.D., Research Physicist
Effects of atmospheric CO_2 enrichment on biospheric and climatic processes.

BRUCE A. KIMBALL, B.S., M.S., Ph.D., Research Leader for E&PD and Supervisory Soil Scientist
Effects of increasing atmospheric CO_2 and changing climate variables on crop growth and water use; free-air CO_2 enrichment (FACE), and CO_2 open-top chambers and greenhouses; micrometeorology and energy balance; plant growth modeling.



ROBERT L. LaMORTE, B.S.E., Civil Engineer
Instrumentation, operation and data collection for the control of atmospheric CO2 in global change experiments on agricultural crops.

FRANCIS S. NAKAYAMA, B.S., M.S., Ph.D., Research Chemist
New crops such as guayule (for latex rubber and resin), lesquerella (hydroxy fatty acid) and vernonia (epoxy fatty acid); including extraction and analytical techniques and by-product uses for the various components; Editor-in-Chief of Industrial Crops and Products, an International Journal.

PAUL J. PINTER, JR., B.S., M.S., Ph.D., Research Biologist
Applications of remote sensing technology to management of agricultural resources and research in plant sciences; effects of elevated CO_2 on biophysical properties of plants.

GERARD W. WALL, B.S., M.S., Ph.D., Plant Physiologist
Derivation of experimental databases to quantify growth, development, and physiological response of agronomic crops to full-season CO_2 enrichment; development of deterministic and stochastic digital simulation models of the soil-plant-atmosphere continuum in response to a CO_2 enriched environment.

PLANT GROWTH AND WATER USE AS AFFECTED BY ELEVATED CO_2 AND OTHER ENVIRONMENTAL VARIABLES

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PLANT GROWTH AND WATER USE AS AFFECTED BY ELEVATED CO_2 AND OTHER ENVIRONMENTAL VARIABLES

MISSION

To predict the effects of elevated CO_2 and climate change on the photosynthesis, growth, yield, and water use of crops under optimal and limiting levels of water and fertility.

THE FREE-AIR CO_2 ENRICHMENT (FACE) PROJECT: PROGRESS AND PLANS

B.A. Kimball, Supervisory Soil Scientist; P.J. Pinter, Jr., Research Biologist;
G.W. Wall and N.R. Adam, Plant Physiologists; R.L. LaMorte, Civil Engineer;
M.M. Conley, Physical Science Technician; J. M. Triggs and T.J. Brooks, Research Technician

PROBLEM: The CO_2 concentration of the atmosphere is increasing and is expected to double sometime during this century. Climate modelers have predicted that the increase in CO_2 will cause the earth to warm and precipitation patterns to be altered. This project seeks to determine the effects of such an increase in CO_2 and any concomitant climate change on the future productivity, physiology, and water use of crops.

APPROACH: Numerous CO_2 enrichment studies in greenhouses and growth chambers have suggested that growth of most plants should increase about 30% on the average with a projected doubling of the atmospheric CO_2 concentration. However, the applicability of such work to the growth of plants outdoors under less ideal conditions has been seriously questioned. The only approach that can produce an environment today as representative as possible of future fields is the free-air CO_2 enrichment (FACE) approach. Therefore, the FACE Project was initiated; and three experiments were conducted on cotton from 1989-1991(Hendrey, 1993; Dugas and Pinter, 1994). Then, from December 1992 through May 1994, two FACE experiments were conducted on wheat at ample and limiting levels of water supply, with about 50 scientists from 25 different research organizations in eight countries participating. Another two FACE wheat experiments were conducted on wheat from December 1995 through May 1997 at ample and limiting supplies of soil nitrogen. Over 50 papers have been published (e.g., Kimball et al., 1995, 1999; Pinter et al., 1996) or are in press from these wheat experiments, and more are being prepared.

Much of the CO_2 enrichment research that has been conducted in the past has been with C3 plants and relatively little with C4 crops such as corn, sugarcane, or sorghum. The neglect of C4s was because their photosynthetic process was known to respond relatively less to elevated CO_2. However, their stomata do partially close in elevated CO_2, thereby suggesting the possibility of some water conservation. Therefore, with grants (one to USWCL and one to The University of Arizona) from the NASA/NSF/DOE/USDA/EPA (TECO III) Program, we conducted two more FACE experiments on sorghum during the summer-fall growing seasons of 1998 and 1999. Our hypothesis was that there would be only a small enhancement of growth due to the FACE treatment when the plants have ample water; but under water-stressed conditions, there would be a substantial growth enhancement resulting from the water conservation due to the partial stomatal closure.

Similar to the previous FACE experiments, measurements with the sorghum included leaf area, plant height, above-ground biomass, morphological development, canopy temperature, reflectance, chlorophyll, light-use efficiency, energy balance, evapotranspiration, soil and plant elemental analyses, soil water content, photosynthesis, stomatal conductance, video observations of roots from minirhizotron tubes, soil CO_2 and N_2O fluxes, and changes in soil C storage from soil and plant C isotopes. Some soil cores for roots also have been obtained. As before, all of the data will be assembled in a standard format for validation of plant growth models.

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FINDINGS: Grain samples from the final FACE wheat harvests were subjected to a battery of nutritional and bread-making quality tests, as described in more detail by Kimball et al. (2001). The water stress treatment improved quality slightly with grain protein concentrations increasing relatively about 2% and bread loaf volumes about 3%. In contrast, a low soil nitrogen supply decreased quality drastically with protein decreasing about 36% and loaf volume about 26%. At ample water, elevated CO_2 from FACE decreased quality somewhat with protein decreasing 5% (relatively) in the irrigation experiments; but in the nitrogen experiments at ample N (which had a higher level of nitrogen than in the irrigation experiments), there was no effect of CO_2 on grain quality. Loaf volume was similarly decreased 2% by elevated CO_2 in the irrigation experiments and not affected at high nitrogen in the nitrogen experiments. Elevated CO_2 tended to make the deleterious effects of low nitrogen worse, with for example, protein decreasing 33% at ambient CO_2 and 39% under FACE. Loaf volume similarly decreased 22% at ambient and 29% under FACE.

As reported in more detail by Ottman et al. (2001), stover yield from the FACE sorghum experiments responded slightly to CO_2; and over the two seasons and over the Wet (ample) and Dry (water stress) irrigation treatments, it averaged 848 g m^{-2} for the control and 928 g m^{-2} for FACE (+9 %). In the Dry plots, grain yield increased due to elevated CO_2 from 472 to 553 g m^{-2} (+17%) in 1998 and 106 to 142 g m^{-2} (+34%) in 1999. In the Wet plots, however, grain yield was not influenced by elevated CO_2 in 1998, but decreased due to elevated CO_2 from 476 to 424 g m^{-2} in 1999 (-12%). Sorghum phenological development was not affected in a consistent manner by elevated CO_2. Elevated CO_2 had the general effect of slowing growth in the Dry plots and accelerating growth in the Wet plots during the vegetative stages, but causing the reverse after anthesis during grain fill. Leaf senescence was accelerated in the Wet plots and may have been partially responsible for the lack of grain yield response to CO_2 with ample water. In water-stressed sorghum, stomatal closure due to elevated CO_2 had a negative effect on growth early and only later in the growth cycle were the positive benefits of soil water conservation realized.

INTERPRETATION: The FACE wheat grain quality data suggest that future elevated CO_2 concentrations will exacerbate the deleterious effects of low soil nitrogen on grain quality; but with ample fertilizer nitrogen, the effects will be minor.

The FACE sorghum data suggest that under conditions with ample water, the higher future atmospheric CO_2 concentrations may cause a slight decrease in sorghum yield due to an accelerated grain-filling period. On the other hand, under water-stress conditions, which are typical of much of the rain-fed areas where sorghum is grown in the U.S. and in Africa and other developing countries, the future higher levels of CO_2 are likely to increase productivity by 15% or more.

FUTURE PLANS: Analyses and reporting of the results from the FACE wheat and especially from the sorghum experiments will continue. Consensus from the participating investigators is that a FACE alfalfa experiment should be conducted next. Specific reasons to focus attention on alfalfa as an experimental crop are as follows: (1) Being deep-rooted, alfalfa could potentially sequester carbon at greater depths below the plow layer where the carbon may be stored for much longer periods than is possible with many other plants. (2) Because alfalfa is a perennial crop that grows the year around with about 8 cuttings per year in our climate, growth observations can be obtained over a very wide range of temperatures and, therefore, the interaction between elevated CO_2 and temperature can be studied. (3) Because alfalfa is a legume, the effects of elevated CO_2 on nitrogen

fixation can be examined as can the importance of nitrogen for C sequestration. (4) Alfalfa is an important crop in the U.S. (4th in acreage behind wheat, corn, and soybeans), and it grows well in Arizona. Unfortunately, funding is not currently available to conduct such an experiment; a proposal has been submitted NASA to obtain such funding.

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FREE-AIR CARBON DIOXIDE ENRICHMENT (FACE): EFFECTS ON SORGHUM EVAPOTRANSPIRATION IN WELL-WATERED AND WATER-STRESSED IRRIGATION TREATMENTS

M. M. Conley, Physical Science Technician; B. A. Kimball, Supervisory Soil Scientist;
P. J. Pinter, Jr., Research Biologist; D. J. Hunsaker, Agricultural Engineer;
G. W. Wall and N. R. Adam, Plant Physiologists; and R. L. LaMorte, Civil Engineer

PROBLEM: The 1996 Intergovernmental Panel on Climate Change (IPCC) projects that, if 1994 CO_2 emission levels are sustained, the global atmospheric CO_2 concentration will reach 500 μmol mol^{-1} by the year 2100. Such an increase in CO_2 concentration is likely to decrease plant evapotranspiration (ET) and increase plant water-use efficiency (WUE).

APPROACH: Eight 490 m^2 rings were subjected to two levels of CO_2 (F = FACE, C = Control) and two levels of soil water supply (W = Wet, D = Dry) as described in detail by Ottman et. al. (2001).

Soil water content measurements: Volumetric soil moisture content was determined with the neutron probe (Hydroprobe Model 503 DR, Campbell Pacific Co., Martinez CA). The calibration equation used was: $\theta = 0.015 + 0.156 * (\text{count} / \text{standard count})$ where θ = volumetric soil water content (θ: m^3 H_2O / m^3 soil). Measurements were taken at 0.3 m intervals to either 1.8 m or 3.0 m depths during the 1998 and 1999 seasons, respectively.

Active root depth was determined by estimating the expected water extraction front from 0-1.76 m (Robertson et al., 1993). ET was calculated only for the zone of soil containing active roots. ET was calculated during drying periods by using a soil water balance equation (Jensen et al., 1990).

FINDINGS: Temporal changes in soil water content: During 1998, volumetric soil water content measurements (Fig. 1a) showed two distinct dry-down periods for the Dry plots - the first from day of year (DOY) 228-254 and the second from DOY 275-330. We observed three dry-down periods in the Dry treatment during 1999: DOY 198-218, DOY 228-258, and DOY 268-290. During 1998, periods of plant stress in Dry treatments, as inferred from a 30% drop in soil moisture below field capacity, occurred during DOY 247-253 and DOY 290-325, whereas they occurred from DOY 202-218, DOY 244-258, and after DOY 280 until maturity during 1999 (Fig. 1b).

Evapotranspiration: During the first year (1998), major differences in cumulative ET between Wet and Dry treatments were apparent by DOY 273 (Fig. 2a). Aside from a divergence during DOY 260-290, when plants were undergoing reproductive growth, FD and CD treatments showed similar patterns in cumulative ET. However, significant differences between CO_2 treatments were evident in Wet plots beginning on DOY 260. FW plots evapotranspired 60 mm (±117 mm) or 11% less than CW over the entire growing season. Seasonal ET in the Dry treatment revealed FD evapotranspired 1 mm (±22 mm) or 0% less than CD. During 1999, differences in cumulative ET between Wet and Dry treatments began at DOY 220, becoming more pronounced after DOY 240 (Figure 2b). ET differences between CO_2 levels were evident within the Wet irrigation treatment from DOY 230 through the end of the season when FW had consumed 58 mm (±34 mm) or 9% less water than CW plants. Seasonal ET in the Dry treatment revealed FD evapotranspired 25 mm (±14 mm) or 6% less than CD.

Figure 1: Mean volumetric soil water contents (from 0.0 to 1.8 m depth) in the Control-Dry (CD), FACE-Dry (FD), Control-Wet (CW), and FACE-Wet (FW) plots for the 1998 (1) and 1999 (b) sorghum growing seasons. Bars denote standard errors. Each mean is the average of four replicates. A indicates when only Wet plots were irrigated. B indicates when both Wet and Dry plots were irrigated. NS, *, **, *** = Not significant at P > 0.25 and significant at P < 0.25, 0.15, 0.05, respectively.

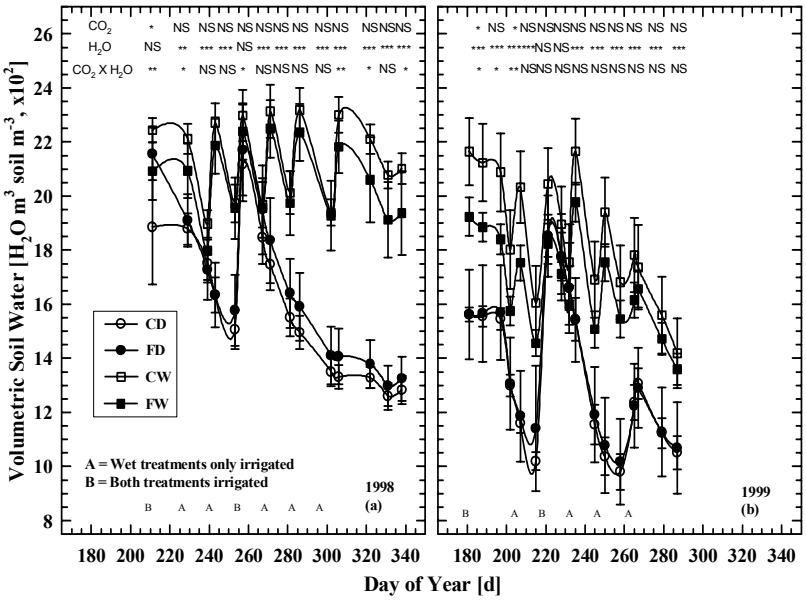
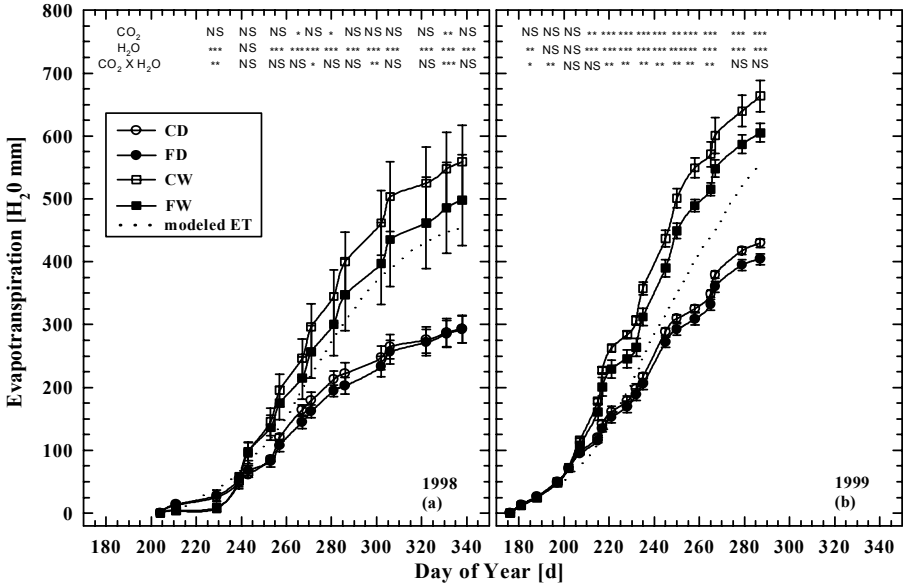


Figure 2: Mean cumulative evapotranspiration (ET) for the Control-Dry (CD), FACE-Dry (FD), Control-Wet (CW), and FACE0-Wet (FW) plots in 1998 (a) and 1999 (b) sorghum growing seasons. Bars denote standard errors. Each mean is the average of four replications. The dotted line represents modeled ET from the Arizona Meteorological Network AZMET (Brown, 1987) adjusted for sorghum. NS, *, **, *** = Not significant at P > 0.25 and significant at P < 0.25, 0.15, 0.05, respectively.



Water-use Efficiency: Grain yield and total above-ground biomass were measured in 1998 and 1999 by Ottman *et al.* (2001). Water-use efficiency based on grain yield (WUE-G) was calculated as the ratio of grain yield per square meter, per mm of ET (Table 1). Water-use efficiency based on total biomass (WUE-B) was calculated as the ratio of biomass per square meter, per mm of ET (Table 2). Based on the two year average, the WUE-G for FD was 0.18 (±0.21) $g/m^2/mm$ or 19% greater than CD, and FW was 0.08 (±0.1) $g/m^2/mm$ or 9% greater than CW; WUE-B for FD was 0.5 (±0.49) $g/m^2/mm$ or 17% greater than CD, and FW was 0.42 (±0.33) $g/m^2/mm$ or 16% greater than FW. This suggests an increasing WUE due to CO_2 enrichment with increasing water stress. Additionally, CO_2 enrichment caused a larger relative increase in grain yield than in total biomass. Such yield increases in grain and total biomass are likely without additional use of water resources at higher than ambient CO_2 concentrations.

Table 1: Sorghum grain yield (Ottman et al. 2001), ET, WUE-G, and the percent difference in WUE-G based on yield and due to FACE enrichment.

	1998			FACE	1999			FACE
	Yield (g/m^2)	ET (mm)	WUE-G ($g/m^2/mm$)	WUE-G % diff.	Yield (g/m^2)	ET (mm)	WUE-G ($g/m^2/mm$)	WUE-G % diff.
FACE-Dry	553 (±30)	292 (±21)	1.93 (±0.24)	15% (±24%)	142 (±33)	404 (±9)	.36 (±.09)	45% (±26%)
Control-Dry	472 (±58)	293 (±22)	1.68 (±0.32)		106 (±18)	429 (±6)	.25 (±.04)	
FACE-Wet	677 (±22)	498 (±72)	1.43 (±0.24)	15% (±21%)	424 (±21)	605 (±15)	.70 (±.05)	-3% (±6%)
Control-Wet	670 (±11)	559 (±58)	1.24 (±0.15)		475 (±13)	664 (±25)	.72 (±.05)	

Table 2: Sorghum biomass (Ottman et al. 2001), ET, WUE-B, and the percent difference in WUE-B based on biomass and due to FACE enrichment.

	1998			FACE	1999			FACE
	Yield (g/m^2)	ET (mm)	WUE-B ($g/m^2/mm$)	WUE-B % diff.	Yield (g/m^2)	ET (mm)	WUE-B ($g/m^2/mm$)	WUE-B % diff.
FACE-Dry	1332 (±80)	292 (±21)	4.64 (±0.61)	12% (±17%)	970 (±69)	404 (±9)	2.41 (±0.22)	26% (±15%)
Control-Dry	1176 (±73)	293 (±22)	4.13 (±0.55)		822 (±58)	429 (±6)	1.91 (±0.16)	
FACE-Wet	1658 (±43)	498 (±72)	3.51 (±0.57)	22% (±23%)	1551 (±54)	605 (±15)	2.56 (±0.15)	8% (±3%)
Control-Wet	1554 (±5)	559 (±58)	2.87 (±0.30)		1566 (±17)	664 (±25)	2.37 (±0.11)	

Increased plant water stress due to increased water demand coupled with decreased total applied water can explain the large decrease in yield, biomass, and WUE in 1999 relative to 1998.

Elevated CO_2 increased WUE-G by 0.13 (±0.16) $g/m^2/mm$ or 14% and WUE-B by 0.46 (±0.41) $g/m^2/mm$ or 16%. CO_2 enrichment caused partial stomatal closure, reduced stomatal conductance, and decreased transpiration per unit of leaf area in both Wet and Dry plots (Wall et al., 2001). Sorghum did not exhibit an increased leaf area in Wet plots (Ottman et al., 2001) so there was conservation of water (Figure 2a,b). In the Dry plots, CO_2-enriched plants had reduced stomatal conductance (Wall et al., 2001), which conserved water and enabled them to grow further into a drying cycle. Cumulative evapotranspiration of FD and CD plants were similar (Figure 2a,b). FD plants grew more (Ottman et al., 2001) and had a greater WUE than CD plants. Therefore, we accept the hypothesis that elevated CO_2 will cause sorghum to decrease ET under wet conditions and to increase WUE under both wet and dry conditions.

INTERPERTATION: Our data show that future water requirements for irrigated sorghum should decrease slightly, provided global warming is minimal. Under rain-fed conditions, where sorghum is more likely to experience water stress, elevated CO_2 will likely cause a productivity increase in total biomass and specifically grain yield. Moreover, under ample-water and, especially, water-limited conditions, increases in CO_2 are likely to cause WUE to increase substantially.

FUTURE PLANS: We plan to run ANOVA analysis of theta by depth using SAS. We will make ET and WUE measurements on alfalfa (Kimball et al, this volume).

COOPERATORS: See Kimball et al. (this volume).

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ENERGY BALANCE AND EVAPOTRANSPIRATION OF SORGHUM: EFFECTS OF FREE-AIR CO_2 ENRICHMENT (FACE) AND SOIL WATER SUPPLY

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PROBLEM: The CO_2 concentration of the atmosphere is increasing and is expected to double sometime during this century. Climate modelers have predicted that the increase in CO_2 will cause the earth to warm and precipitation patterns to be altered. Such increases in CO_2 and possible climate change could affect the hydrologic cycle and future water resources. One component of the hydrologic cycle that could be affected is evapotranspiration (ET), which could be altered because of the direct effects of CO_2 on stomatal conductance and on plant growth. Therefore, one important objective of the Free-Air CO_2 Enrichment (FACE) Project (Kimball et al. this volume) is to evaluate the effects of elevated CO_2 on the ET of sorghum and other crops.

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

Briefly, the FACE apparatus consists of the following: Four toroidal plenum rings of 25 m diameter constructed from 12" irrigation pipe were placed in a sorghum field at Maricopa, Arizona, shortly after planting. The rings had 2.5-m-high vertical pipes with individual valves spaced every 2 m around the periphery. Air enriched with CO_2 was blown into the rings, and it exited through holes at various elevations in the vertical pipes. Wind direction and speed were measured adjacent to each FACE ring, and CO_2 concentration was measured at the center of each. A computer control system used wind direction information to turn on only those vertical pipes upwind of the plots so that the CO_2-enriched air flowed across the plots no matter which way the wind blew. The system used the wind speed and CO_2 concentration information to adjust the CO_2 flow rates to maintain desired CO_2 concentrations at the centers of the rings. The FACE CO_2 concentration was elevated by 200 ppm CO_2 above ambient (about 360 ppm in daytime) 24 hr/day all season long. Four matching Control rings with blowers to provide air flow but no added CO_2 were also installed in the field. Some additional measurements were made in mid-field areas between the FACE and Control plots where neither CO_2 nor air flow were altered.

In addition to the CO_2 treatments, varying soil water supply was also a factor. Using a split-plot design, the main circular CO_2 plots were divided into semicircular halves, with each half receiving an ample irrigation regime (Wet) or else receiving a water-stress treatment (Dry). Using flood irrigation, the Wet plots were irrigated on roughly a two-week schedule, whereas the Dry plots were irrigated only twice (shortly after planting and at mid-season).

The determination of the effects of elevated CO_2 on $\boldsymbol{ET}$ by traditional chambers is fraught with uncertainty because the chamber walls that constrain the CO_2 also affect the wind flow and the exchange of water vapor. Therefore, as done previously in the FACE cotton and wheat experiments (Kimball et al., 1994, 1995, 1999), a residual energy balance approach was adopted whereby $\boldsymbol{ET}$ was calculated as the difference between net radiation, $\boldsymbol{R}_n$, soil surface heat flux, $\boldsymbol{G}_0$, and sensible heat flux, $\boldsymbol{H}$:

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

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

The instruments were calibrated carefully before the start of the experiments and again between the experiments in early 1999. During 2000, final calibrations were performed, including some comparisons among instruments from various manufacturers to use as reference standards. Analysis of the data from the standards comparisons is completed, and now analysis of the field data themselves is progressing.

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

The sorghum growth response to elevated CO_2 amounted to about +9% averaged over two seasons and over the Wet and Dry irrigation treatments (Ottman et al., 2001; Kimball et al., this volume); so, based on the growth and energy balance comparisons from the past cotton and wheat experiments, we hypothesize that there was a slight reduction in sorghum **ET**. However, another basis for this hypothesis is that such a slight reduction was deduced from an analysis of the soil water balance by Conley et al. (this volume). They found that **ET** was reduced about 11% in Wet plots due to the FACE treatment, as averaged over the two growing seasons; whereas in the Dry plots, it was increased an average 3%.

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

FUTURE PLANS: We plan to complete the analysis of the micrometeorological data from the two FACE sorghum experiments and write the corresponding manuscript.

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

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RELATIONSHIPS OF A-CI PARAMETERS AND ENZYME ACTIVITIES IN SORGHUM EXPOSED TO FREE-AIR CO_2 ENRICHMENT (FACE)

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BACKGROUND: With the expected doubling of atmospheric levels of CO_2 sometime in the 21st century, it is important to understand how this change will affect our way of life and, more generally, how it will affect plant life. The Free Atmospheric CO_2 Enrichment (FACE) facility at The University of Arizona Maricopa Agricultural Center is helping to determine how plants respond and acclimate to long-term exposure to elevated levels of CO_2 in the field.

Plants acclimate to changes in CO_2 concentration through changes in the amounts and activities of enzymes required to reestablish a balance within the photosynthetic apparatus. An earlier report (Adam *et al.*, 1997) presented data from a gas-exchange technique in which photosynthesis (A) was measured at a range of intracellular CO_2 concentrations (Ci), which provided information on changes within the photosynthetic apparatus of spring wheat, a so-called C_3 plant. Adam *et al.* (1998) showed that in spring wheat, the slope of the A-Ci relationship at those low values of Ci could be used to assess the changes in the activity and content of Rubisco due to growth in increased levels of CO_2. The work with spring wheat also indicated that, in order to fully assess the response of crop plants to elevated CO_2, various growth stages and canopy, a profile must be measured.

Similar experiments were conducted in 1998 and 1999 on sorghum, a warm-season crop with a different carbon-trapping mechanism, therefore, called a C_4 plant. The carbon-trapping enzyme of sorghum is PEPCase which, unlike Rubisco, fixes only carbon. The product of the reaction catalyzed by PEPCase is then shuttled to Rubisco and into the Calvin cycle. The relationships between the A-Ci curves and metabolic processes of C_4 plants are not fully understood. One objective of these experiments was to investigate the effect of growth in elevated CO_2 on these C_4 pathway enzymes in sorghum, as well as on the A-Ci relationships.

APPROACH: *Sorghum bicolor* (L.) Moench (cv. Dekalb 54) was planted in an open field at The University of Arizona Maricopa Agricultural Research Center, located 50 km south of Phoenix, Arizona (33.1 °N, 112.0 °W). Sorghum was planted on July 13 and 14, 1998, and again on June 14 and 15, 1999 (Kimball et al., 1999). Fifty percent emergence occurred July 30, 1998, and July 1, 1999. Following sowing, FACE apparatus was erected on site to enrich the CO_2 concentration of the ambient air (ca. 370 μmol mol^{-1} during daytime) by 200 μmol mol^{-1} above ambient. Water was applied as a split plot factor using flood irrigation such that "Wet" plots received ample water while "Dry" plots received only two irrigations and were severely stressed. All plots received 278.7 kg ha^{-1} N.

For the first sampling date (the 4th and 5th leaf stages in 1998 and the 2nd through 5th leaf stages in 1999), gas exchange analyses were conducted on the uppermost fully-expanded leaf (referred to as the top leaf) and on the top minus one leaf. Thereafter, measurements were made on the top leaf and the top minus two leaf. Photosynthesis (A) rates were measured over a range of intercellular CO_2 (Ci) levels, generating an A-Ci curve. At the end of each curve, the leaf was frozen as quickly as possible with a liquid nitrogen-cooled clamp and stored in liquid nitrogen. Activity of Rubisco,

PEPCase and PpdK were assayed from leaves collected from both years. Parameters estimated from the A-Ci curves were the initial slope, the bend (or inflection) of the curve, and the asymptote.

FINDINGS: The A-Ci curves in the early growth stages (i.e., 2nd and 3rd leaf stages) were found to resemble a C_3-type curve. However, as the plants reached the 4th and 5th leaf stage, the curves were more similar to a typical C_4 curve (Fig. 1a), in that the initial slope of the curve is steeper. We also found that the curves became more C_3-like as water stress became more severe (Fig. 1b) and became more C_4-like after irrigation (Fig. 1c). Step-wise regression showed that the enzyme activities measured explained 50% or less of the variation seen in the parameters of the A-Ci curves (Table 1) and that the initial activity of Rubisco was the enzyme parameter most closely related to all three portions of the curve.

INTERPRETATION: The initial activity of Rubisco was not expected to be related to all three portions of the curve. In addition, PEPCase was expected to be related to the initial slope of the curve. It is likely that factors other than enzyme activity are more important in determining the shape of the A-Ci curve and that the initial activity of Rubisco is responding to the factors that determine the shape of the curve. Although leaf samples from some of the measurement dates remain to be assayed, it appears that factors other than enzyme activity or concentration (for example, water status of the plant) will be more important in determining the response of sorghum plants to future climatic changes.

FUTURE PLANS: Biochemical assays will be conducted on both the remaining samples to determine whether these relationships change. In addition, further analysis of the effects of growth in CO_2 enrichment and water stress on these relationships will be conducted.

COOPERATORS: See Kimball et al., this volume. However, we especially wish to acknowledge the collaborative efforts of Andrew Webber of Arizona State University for helpful advice and the use of his laboratory. We also thank Jonathan Triggs and Jose Olivieri for technical assistance.

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Figure 1. Response of Photosynthesis (A) to Changes in Intercellular CO_2 concentration for the uppermost, fully-expanded leaf of sorghum in (a) pre-water stress conditions, (b) pre-irrigation and (c) post-irrigation conditions.

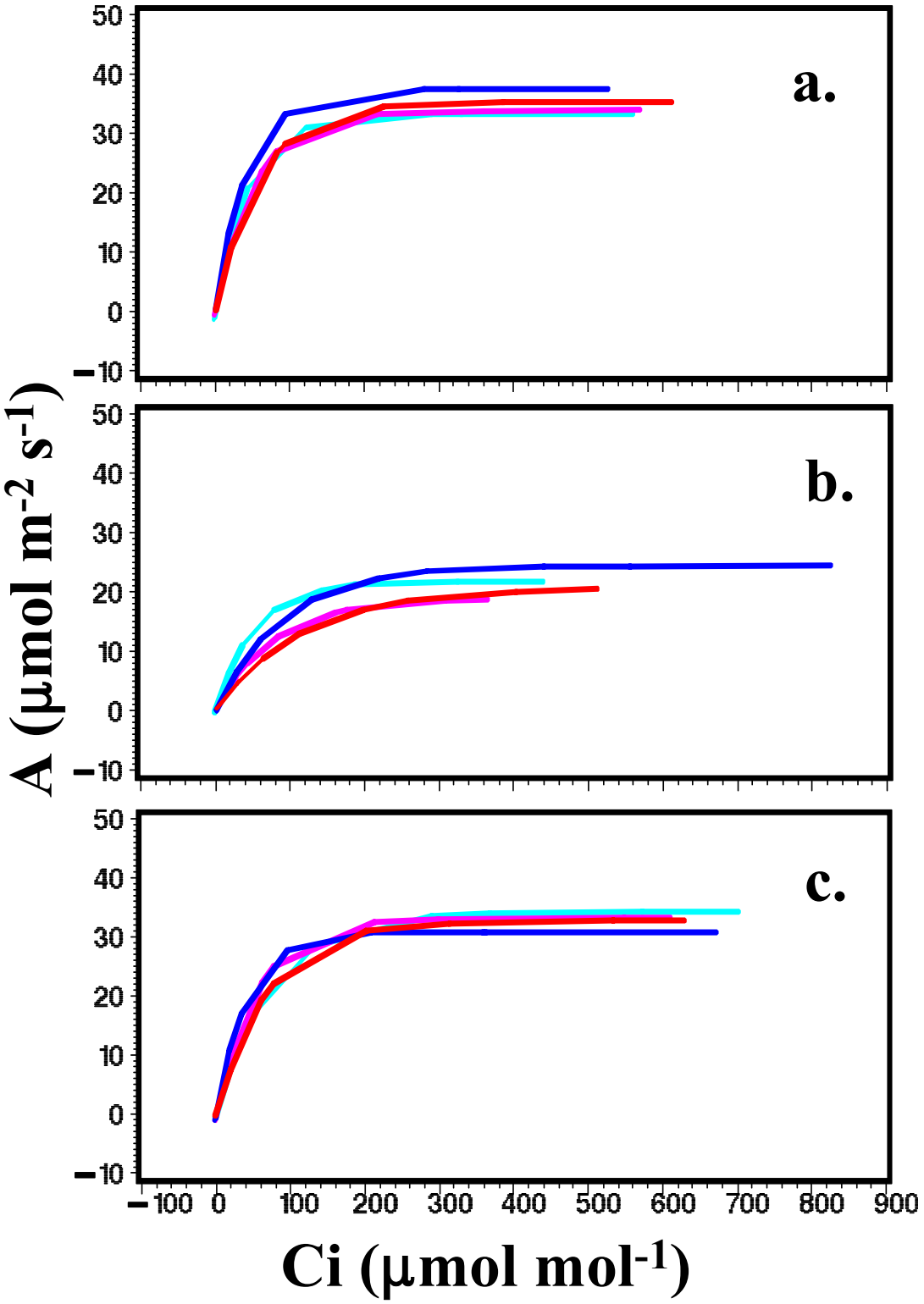

Table 1. Results of step-wise regression analysis of A-Ci curve parameters (Initial slope, Bend, and asymptote) with enzyme activities (Rubisco initial activity, Rubisco full activity, PEPCase optimal activity, PEPCase physiological activity, and PpdK activity).

Parameter	Variable	Model R-Square	Pr > F
Initial Slope	Rubisco Initial Activity	0.5137	< 0.0001
	Rubisco Full Activity	0.5453	0.077
'Bend' of Curve	Rubisco Initial Activity	0.3656	< 0.0001
	PpdK Activity	0.4337	0.0216
	Rubisco Full Activity	0.4779	0.0544
Asymptote	Rubisco Initial Activity	0.3182	< 0.0001
	PpdK Activity	0.4153	0.0075

Effects of Elevated CO_2 and Water and Nitrogen Stress on Phenology of Spring Wheat

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PROBLEM: Terrestrial ecosystems will probably experience a significant increase in atmospheric CO_2 during this century. The potential effect of this change on important food and fiber crops has been the subject of extensive research by U.S. Water Conservation Laboratory (USWCL) scientists for more than a decade. In our research, we have been using Free-air Carbon dioxide Enrichment (FACE) to expose plants to supra-ambient levels of CO_2 because FACE minimizes the microenvironmental and climatic artifacts that are often associated with studies using open-top chambers or greenhouses. Various performance measurements have shown that the FACE facility provides very good temporal and spatial control of CO_2 concentrations and is a cost-effective means for large scale fumigation experiments.

Our strategies for CO_2 exposure and the sophistication of ambient CO_2 control plots have evolved over the years as FACE has been applied to different crops. Modifications have also been made as experimental objectives have changed and as more was learned about system behavior under different environmental conditions (Pinter *et al.*, 2000). When the focus of our FACE project shifted from cotton to spring wheat, a decision was made to enrich the plots on a 24h day^{-1} basis. Because of the extra costs involved, the control plots used during the first two years experimentation in wheat (1992-93 and 1993-94) had dummy plastic manifolds and standpipes but were not equipped with blowers. However, with the new 24h day^{-1} protocol, we began to suspect that the blowers used to introduce CO_2-rich air into the elevated CO_2 arrays was causing some slight additional microturbulence in the boundary layer above the canopy, especially during calm nights. Although subtle and difficult to measure with the mechanical cup anemometers in our micrometeorological instrumentation, the additional disturbance resulted in a slight increase in air and radiant canopy temperatures in the blower-equipped plots at night. We also suspected and later confirmed that the slightly higher temperatures were associated with differences in plant phenology and end-of-season senescence rates.

Wheat FACE experiments were carried out for another two years, through the 1995-96 and 1996-97 growing seasons. For these experiments, however, blowers were installed in the experimental control plots (hereafter called blower plots), and we did not observe differences between FACE and blower plots in nighttime air temperatures, canopy temperatures, plant phenology, or senescence. The objectives of this report are to clarify that CO_2 did not have a direct effect on wheat developmental rates and to discuss the overall stimulatory effect of CO_2 on final grain harvest from all four years of the experiment.

APPROACH: FACE field experiments were conducted at The University of Arizona Maricopa Agricultural Center (MAC). Spring wheat (*Triticum aestivum*, L. cv Yecora Rojo) was sown in mid-December, emerged on or about January 1, and was harvested near the end of May in each year. Irrigation was accomplished via subsurface drip tubing. A split, strip plot design incorporated two levels of CO_2 as the main treatment effect during all four years with levels consisting of ambient Control (~360 μmol mol^{-1}) and elevated FACE (a nominal 550 μmol mol^{-1})

concentrations. The secondary treatment variable during the first two years (CO_2 by water experiments, 1992-93 and 1993-94) included two levels of irrigation: Wet (100% of consumptive requirements) or Dry (50% of Wet). The secondary treatment variable in the final two years (CO_2 by N experiments, 1995-96 and 1996-97) was applied nitrogen: High (~388 kg ha^{-1} yr^{-1}) and Low (~77 kg ha^{-1} yr^{-1}). More complete details on experimental design and treatment conditions may be found in Kimball *et al.* (1999), Hunsaker *et al.* (2000), and Pinter *et al.* (2000).

Plants were sampled at 7-10 day intervals using similar techniques all four years. Main stems were assigned growth stages according to the Zadoks scale of plant development. Dates when plants reached the midpoint of eight principal growth stages (tillering, stem elongation, booting, heading, anthesis, milk development, soft dough, and ripening) were computed via linear interpolation and then averaged for all replicates within a treatment combination. We computed the difference in chronological time required to reach a specific growth stage between plots with and without blowers (i.e., control minus FACE for 1992-93 and 1993-94) and also between plots having blowers but exposed to either ambient or elevated CO_2 levels (i.e., blower minus FACE for 1995-96 and 1996-97). Paired "t" tests were used to determine the statistical significance of these differences in phenology.

FINDINGS: Qualitative observations of plants growing in the field revealed relatively large differences in phenology (at heading, anthesis, and maturity) between control and FACE plots in the CO_2 by water experiments that were not evident when comparing blower and FACE plots in CO_2 by N experiments. Zadoks growth stage data from the periodic plant samples confirmed these observations. Development in plots with blowers was accelerated by 2 to 5 days compared to plots without blowers. The CO_2 treatment by itself appeared to have minimal effect on developmental rates. Paired "t" test comparisons showed the average differences in phenology between elevated CO_2 FACE plots and the ambient CO_2 control (without blowers) during 1992-93 and 1993-94 were relatively large and highly significant in both the Wet and Dry irrigation treatments (Table 1). The chronological differences in developmental rates between FACE and blower treatments during 1995-96 and 1996-97 were less than 0.5 days and only under deficit nitrogen conditions were the differences between CO_2 treatments statistically significant.

Table 1. Mean differences in the time required to reach each of 8 primary growth stages (tillering → ripening) between different Blower and CO_2 configurations used during the FACE Wheat experiments at Maricopa, AZ.

	Is Blower Present ?			**mean time difference days ± 1 SE**	**value from paired *"t"* test**
Comparison[†]	**ambient CO_2 treatment**	**elevated CO_2 treatment**	***n***[‡]		
CO_2 by Water Experiment (1992-93 & 1993-94)					
CW minus FW	No	Yes	64	3.2 ± 0.33	9.80 ***
CD minus FD	No	Yes	64	2.3 ± 0.23	10.10 ***
CO_2 by Nitrogen Experiment (1995-96 & 1996-97)					
BH minus FH	Yes	Yes	57	0.4 ± 0.31	1.25 NS
BL minus FL	Yes	Yes	57	0.4 ± 0.18	2.20 *

*, *** Significant at the 0.05 or 0.001 probability levels, respectively.
[†] Treatment abbreviations: C, Control; B, Blower; F, FACE; W, Wet irrigation; D, Dry irrigation; H, High nitrogen; L, Low nitrogen.[‡] Refers to the total number of paired observations. Only 3 replicates were available for *t* test comparisons during most of the 1996-97 experiment.

INTERPRETATION: One of the unique findings of the 1992-93 and 1993-94 FACE experiments was the apparent accelerating effect of elevated CO_2 on plant development and rates of canopy senescence (as reported by Pinter et al., 1996; and photograph in Kimball et al., 1995). A reexamination of those data in light of the slight differences in temperature we now know to exist between the treatments and the new phenology results from the CO_2 by N experiments have led us to conclude that the blower effect was sufficient to explain most of the apparent developmental acceleration. The subtle, blower-related rise in nighttime temperatures had cumulative effects on long-term developmental processes of the wheat plant. We now believe that elevated CO_2 *per se* had very little effect on the rates of plant development in well-watered and amply-fertilized spring wheat.

What effect might the blowers have had on CO_2 enhancement of final grain yields? We had originally reported only a 10% increase in yield for wheat exposed to CO_2 at 550 μmol mol^{-1} and supplied with adequate water and nutrients, suggesting that plants in the Control treatment had additional opportunity to "catch up" with the sink-limited FACE plants (Pinter et al., 1996; and Kimball et al., 1995). We now believe that this 10% figure was probably an <u>underestimate</u> of the true CO_2 effect that might have been observed during the first 2 seasons had the controls been properly equipped with blowers and the grain filling duration of both CO_2 treatments been similar. In fact, during the 1995-96 and 1996-97 experiments, grain yields for adequately fertilized and well-watered wheat showed a 15% increase associated with a nominal +200 μmol mol^{-1} CO_2 elevation. This translates into a CO_2 enhancement (β) factor of ~28% for a doubling of atmospheric CO_2 concentrations, an increase which compares favorably with the 33% average for agricultural crops reported by Kimball (1983).

There is little argument that the FACE technique approaches natural conditions more closely than open-top chambers or other means of exposing plants to elevated CO_2. Important advantages include an unmodified light environment, unrestricted rooting volume, and large experimental areas. Despite our results showing that the blowers cause a slight modification of crop microclimate, the advantages of FACE over open-top chambers and greenhouses still outweigh disadvantages by a considerable margin. However, these findings emphasize the importance of equipping control plots in FACE facilities with blowers and point out a disadvantage to nighttime CO_2 enrichment in our region.

FUTURE PLANS: A manuscript dealing with interactive effects of elevated CO_2 and water or nitrogen stress on wheat plant growth and final yields is in preparation.

COOPERATORS: S. Leavitt, A. Matthias, T. Thompson, and S. White, The University of Arizona, Tempe AZ; B. Roth, P. Murphree, J. Chernicky, and R. Rauschkolb (deceased), Maricopa Agricultural Center, Maricopa AZ; K. Lewin, J. Nagy, and G. Hendrey, Brookhaven National Laboratory, Uptown NY; and F. and G. Wechsung, S. Grossman-Clarke, and T. Kartschall, Potsdam Institute for Climate Research, Potsdam, Germany. We also thank R. Rokey, S. Gerszewski, R. Seay and D. Pabian for technical assistance in the field and R. Altamarano, M. Baker, C. O'Brien, H. Steinman, and K. West for processing the plant samples.

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LONG-TERM CO_2 ENRICHMENT OF SOUR ORANGE TREES: EFFECTS ON PRODUCTIVITY AND CARBON SEQUESTRATION

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PROBLEM: Many people believe the ongoing rise in the air's CO_2 content is the greatest problem ever to be faced by humanity based on the assumption that it could lead to catastrophic global warming via intensification of the planet's natural greenhouse effect. However, elevated concentrations of atmospheric CO_2 also provide many benefits, some of which tend to ameliorate the global warming problem. Earth's trees, for example, account for approximately two-thirds of the planet's photosynthesis, by which means they remove prodigious amounts of CO_2 from the air and sequester its carbon in their tissues and the soil beneath them, thereby slowing its rate of rise in the atmosphere. Consequently, we seek to determine the direct effects of atmospheric CO_2 enrichment on all aspects of the growth and development of trees concentrating on the long-term aspects of the phenomenon; for until someone conducts an experiment measured in *decades*, we will never know the ultimate impact of the ongoing rise in the air's CO_2 content on the planet's most powerful contemporary carbon sink.

APPROACH: In July 1987, eight 30-cm-tall sour orange tree (*Citrus aurantium* L.) seedlings were planted directly into the ground at Phoenix, Arizona. Four identically-vented, open-top, clear-plastic-wall chambers were then constructed around the young trees which were grouped in pairs. CO_2 enrichment – to 300 ppmv (parts per million by volume) above ambient – was begun in November 1987 in two of these chambers and, other than for brief maintenance and construction periods, has continued unabated since that time. Except for this differential CO_2 enrichment of the chamber air, all of the trees have been treated identically, being irrigated and fertilized as deemed appropriate for normal growth (Idso and Kimball, 1997).

As in all prior years, we continue to measure the circumferences of the trunks of the trees at the midpoint of each month; and from these data, we calculate monthly values of total trunk plus branch volume on the basis of relationships developed specifically for our trees (Idso and Kimball, 1992). Then, from wood density (dry mass per fresh volume) measurements we have made over the past several years, we calculate monthly values of the total dry weight of the trunk and branch tissue of each tree. Results for December, January, February and March – the winter period of minimal trunk expansion – are then averaged to give a mean value for the year, from which the preceding year's mean value is subtracted to yield the current year's production of trunk and branch biomass.

We likewise continue our yearly fruit measurements, counting the number of fruit to reach maturity on each tree, weighing the fruit, and calculating the total dry weight of fruit produced in each of the CO_2 treatments from previous determinations of fruit percent dry weight. Also from previously derived relationships (Idso and Kimball, 1992), we evaluate the number of new leaves produced each year from our trunk circumference measurements; and from bimonthly assessments of leaf dry weight similar to those of Idso et al. (1993), we calculate the total dry weight of leaves produced each year. These results, added to the trunk and branch dry weights and fruit dry weights, then give us the total aboveground dry weight production per year for all of the trees in the two CO_2 treatments.

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When viewed in their entirety, the results continue to be encouraging. They indicate that the trees of both CO_2 treatments may be close to achieving a stage of maturity characterized by a near-steady-state of yearly aboveground biomass production (Fig. 1). For the last five years of the experiment, for example, the values of total aboveground biomass in the CO_2-enriched trees were 107, 90, 95, 116, and 89 kg/tree; while those for the ambient-treatment trees were 62, 51, 57, 61, and 55 kg/tree, producing a five-year-mean CO_2-enriched/ambient-treatment ratio of 1.74.

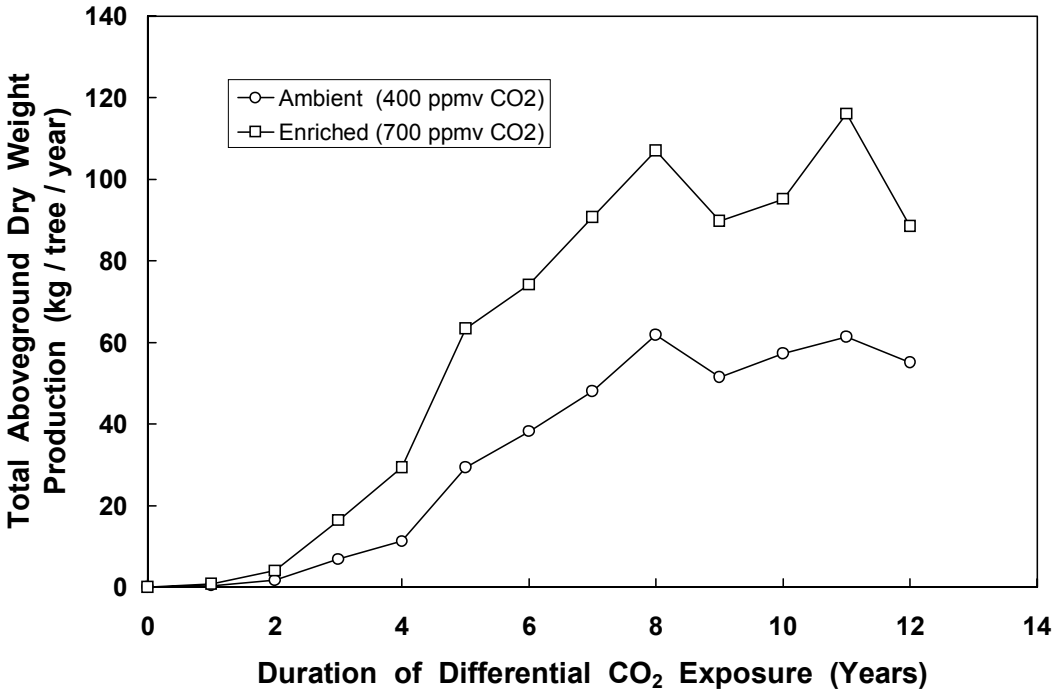

Figure 1. Yearly total aboveground biomass production in the ambient and CO_2-enriched sour orange trees as a function of time since the start of the experiment.

The fruit production component of the total aboveground productivity has been a little more erratic; nevertheless, it too appears to be approaching an asymptotic upper limit (Fig. 2). For the last five years, for example, harvested fruit biomass has been 47, 38, 38, 58, and 36 kg/tree in the CO_2-enriched trees; while in the ambient-treatment trees it has been 25, 13, 23, 31, and 22 kg/tree, producing a five-year-mean CO_2-enriched/ambient-treatment fruit production ratio of 1.87.

We have also discovered that in the spring of each year the CO_2-enriched trees experience an enormous growth enhancement. This initial stimulation begins immediately upon bud-burst; and three to four weeks later, the new branches of the CO_2-enriched trees may be four times more massive than those of the ambient-treatment trees. Furthermore, because there are more branches on the CO_2-enriched trees, they may have as much as six times more total new-branch biomass than the ambient-treatment trees. Shortly thereafter, however, a decline sets in and the CO_2-enriched/ambient-treatment new-branch biomass ratio of the trees ultimately levels out at a

value commensurate with the long-term total aboveground productivity ratio of the CO_2-enriched and ambient-treatment trees; i.e., at a value of approximately 1.74.

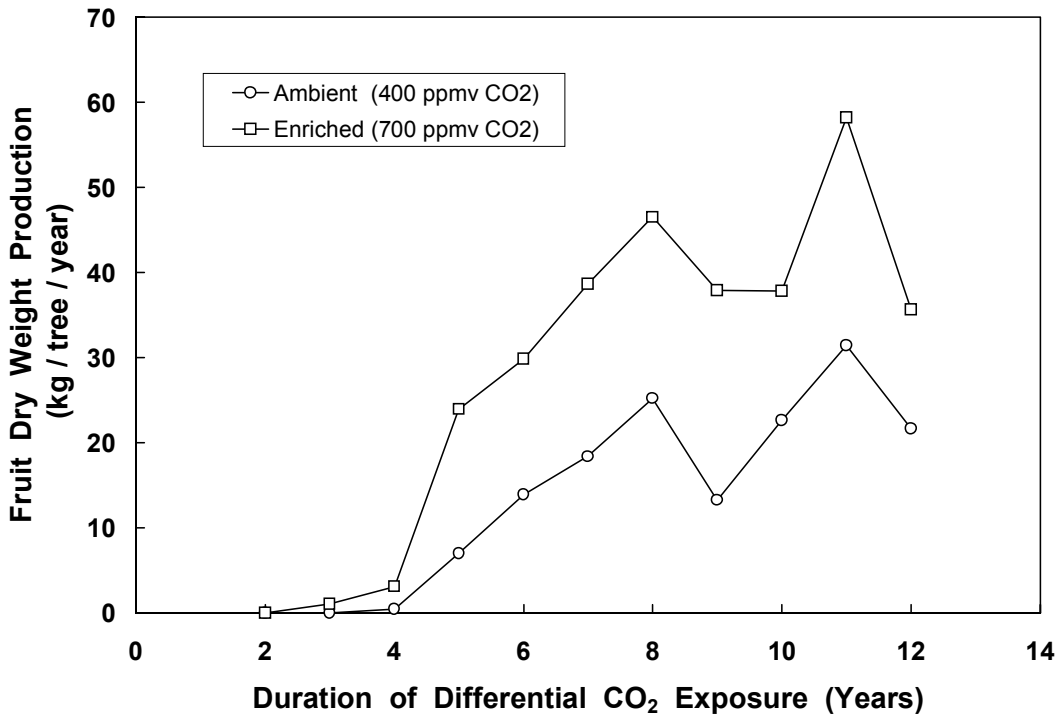

Figure 2. Yearly fruit dry weight production in the ambient and CO_2-enriched sour orange trees as a function of time since the start of the experiment.

INTERPRETATION: What is the ultimate fate of the CO_2 the people of the world yearly emit to the atmosphere? Will the trees of the planet be sufficiently stimulated by the ongoing rise in the air's CO_2 content to remove enough of it from the atmosphere to prevent a significant CO_2-induced warming of the globe? The results of our ongoing study provide important insight into these questions and may help our government craft appropriate policies to meet this global environmental challenge. In the meantime, our findings continue to demonstrate that carbon dioxide is an effective aerial fertilizer, significantly increasing the size, growth rate, and fruit production of sour orange trees exposed to more of this aerial fertilizer than is normally in the air. It is also possible that this phenomenon may be partially responsible for the progressively earlier occurrence of the spring "green up" of the Northern Hemisphere's vegetation, which has been observed over the past few decades in satellite studies of surface reflectance and in the increasingly earlier occurrence of the spring draw-down of the air's CO_2 content that is evident in studies of the atmosphere's seasonal CO_2 cycle (Idso et al., 2000).

FUTURE PLANS: We plan to continue the sour orange tree experiment as long as it takes to determine if the trees truly achieve steady-state yearly growth rates and if the CO_2-enriched trees are maintaining a growth advantage over the ambient-treatment trees that can reasonably be

expected to continue indefinitely. We are also continuing our investigation of the ultra-enhanced spring branch growth phenomenon that we have observed in the CO_2-enriched trees, having just completed three full years of pertinent measurements. In addition, we have initiated several new research thrusts related to the central problem of carbon sequestration. In cooperation with a soil microbiologist, we are investigating the role of atmospheric CO_2 enrichment in stimulating the growth of soil fungi that grow in symbiotic association with the sour orange tree roots and produce a glycoprotein called glomalin, which has been proven to enhance soil aggregation and the stability of soil aggregates. And in cooperation with several scientists who are expert in various types of tree-ring analyses, we are studying cores of the sour orange tree trunks for CO_2-induced differences in cell size, wood density and strength properties. We are also collecting some new data sets that should shed even more light on the effects of atmospheric CO_2 enrichment on the trees' physiology, including weekly assessments of leaf fall and leaf concentrations of chlorophyll, starch and various sugars. Finally, we are entering upon our tenth year of fruit vitamin C measurements and our fourth year of fruit folic acid measurements.

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ELEVATED ATMOSPHERIC CO_2 ALLEVIATES WATER-STRESS-INDUCED MID-AFTERNOON DEPRESSION IN WHEAT CARBON GAIN

G.W. Wall, Plant Physiologist; B.A. Kimball, Supervisory Soil Scientist; P. J. Pinter, Jr., Research Biologist; R.L. LaMorte, Civil Engineer; and S.B. Idso, Research Physicist

PROBLEM: Atmospheric CO_2 concentration is on the rise, which can affect stomatal conductance and water absorption processes in wheat (*Triticum aestivum* L.). Elevated CO_2 also affects internal plant water potential, and as is well known, it also affects photosynthesis. The degree to which each of these processes is affected will determine the overall productivity of wheat in the future. An imperative exists, therefore, to determine the effect that a future high CO_2 -world will have on dawn to dusk trends in stomatal conductance (g_s), total (Ψ_W), osmotic (Ψ_π), and turgor (Ψ_P) leaf potentials and net assimilation rate (A) of wheat grown under elevated CO_2 and adequate and limited soil-water content.

APPROACH: A 2-year field study on a hard red spring wheat (cv. Yecora Rojo) crop was conducted in an open field at The University of Arizona Maricopa Agricultural Center located 50 km south of Phoenix, Arizona. Seeds were sown into flat beds at 0.25-m row spacings on December 15, 1992, (130 plants m^{-2}) and December 7-8, 1993 (180 plants m^{-2}). The crops were harvested on May 25-27, 1993, and on June 1, 1994. Following sowing, a free-air CO_2 enrichment (FACE) apparatus was erected on site to enrich the CO_2 concentration of ambient air (~350 µmol mol^{-1}) to 550 µmol mol^{-1} treatment level (main plots) for 24 h per day from 50% emergence until physiological maturity. A subsurface drip-tape irrigation system provided two soil-water content (I) treatments; 50% (Dry) and 100% (Wet) replacement of potential evapotranspiration (split-plot). Treatments combinations, therefore, consisted of Control-Dry (CD), FACE-Dry (FD), Control-Wet (CW) and FACE-Wet (FW). There were four replications of each treatment combination.

A portable closed gas exchange system (0.25 L transparent cuvette) was used to make in situ measurements of g_s and A on uppermost fully expanded sunlit leaves from dawn to dusk. Leaves were excised approximately 5 mm away from the leaf collar to measure Ψ_W with a pressure chamber. Measurements of Ψ_W and Ψ_π were also made at midday with leaf thermocouple psychrometers using standard psychrometric techniques, whereas turgor pressure (Ψ_P) was derived ($\Psi_P=\Psi_W-\Psi_\pi$).

FINDINGS: FACE reduced g_s by 30% at mid-morning (2.5 h prior to solar noon), 34% at midday (solar noon), and 34% at mid-afternoon (2.5 h after solar noon) (Fig. 1). In Dry compared with Wet, FACE caused less of a proportionate reduction in g_s than Control by 2% at mid-morning and midday, but by 25% at mid-afternoon. Full irrigation increased g_s by 66% at mid-morning and midday and 79% at mid-afternoon. Overall, Ψ_W was less negative by 0.16 and 0.33 MPa at midday in FACE compared with Control, and Ψ_W was less negative by 0.25 and 0.59 MPa in Wet compared with Dry during mild (1993) and severe (1994) drought stress years, respectively. Osmotic adjustment ($\Delta\Psi_\pi$) was 0.73 [Ψ_π MPa (Ψ_W MPa)$^{-1}$] for Control and 0.81 [Ψ_π MPa (Ψ_W MPa)$^{-1}$] for FACE (P=0.20), whereas loss of turgor ($\Delta\Psi_P$) was 0.18 [Ψ_P MPa (Ψ_W MPa)$^{-1}$] for Control and 0.16 [Ψ_P MPa (Ψ_T MPa)$^{-1}$] for FACE (P=0.20).

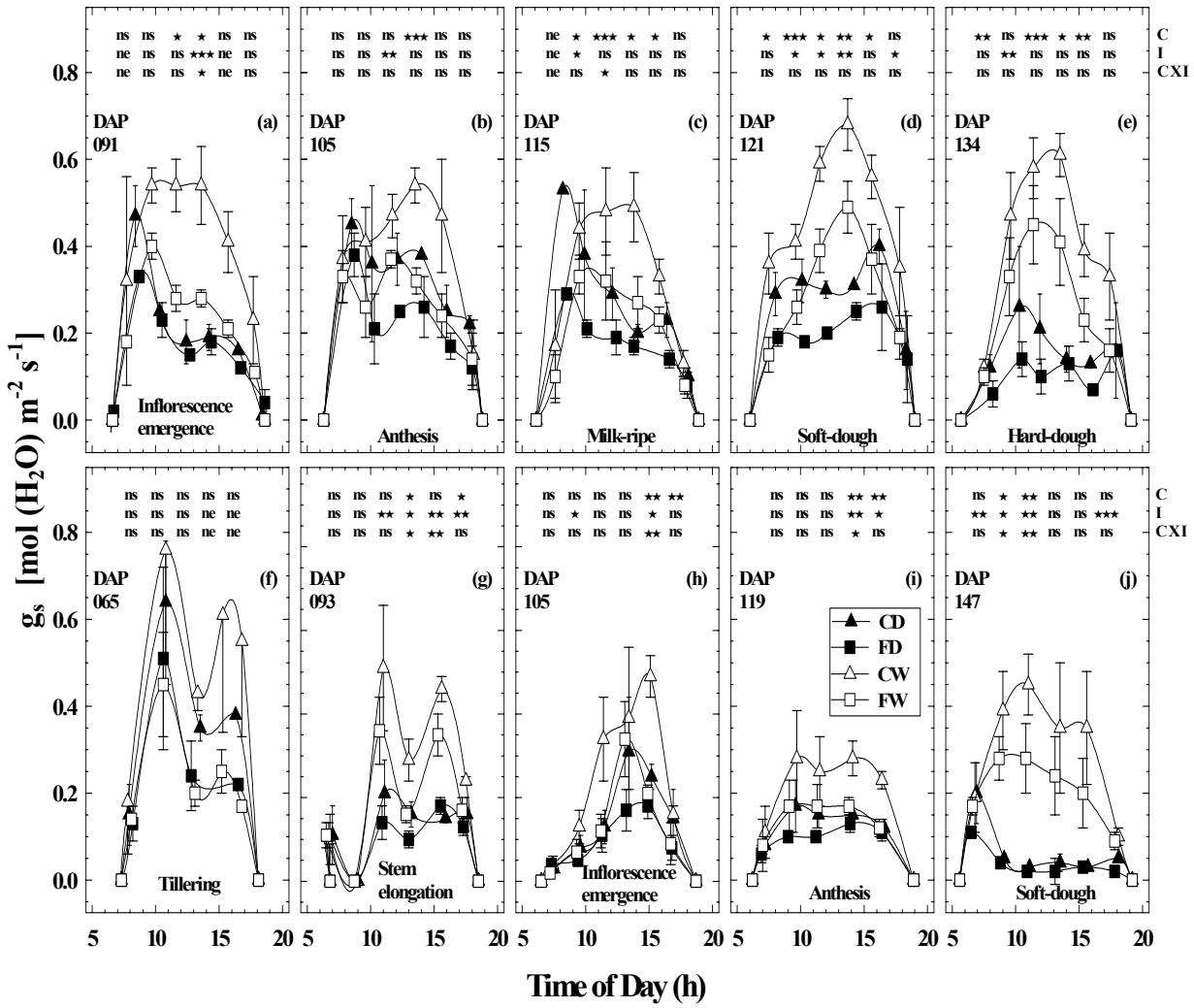

Figure 1. DAWN TO DUSK TRENDS IN STOMATAL CONDUCTANCE (G_S) OF FULLY EXPANDED SUNLIT SPRING WHEAT LEAVES FOR DAY AFTER PLANTING (DAP) AND GROWTH STAGES GIVEN FOR 5 D DURING 1993 (A-E) AND 1994 (F-J). SYMBOLS IN LEGEND REFER TO CONTROL-DRY (CD), CONTROL-WET (CW), FACE-DRY (FD), AND FACE-WET (FW) TREATMENTS. Each vertical bar is one standard error from each datum. Source of variance in ANOVA are carbon dioxide [(C): Control at 370 µmol mol^{-1}, and FACE at 550 µmol mol^{-1}], irrigation effect [(I): Dry at 50% and Wet at 100% replacement of evapotranspiration], and CxI interaction effects. Significance effects given for each growth stage above each datum as ★★★, ★★, ★, and ns for $P \leq 0.01$, $P \leq 0.05$, $P \leq 0.10$, and not significant (ne for effect not estimated), respectively. Actual probability of a greater F-value by chance reported if $P \geq 0.10$ and $P \leq 0.25$.

Compared with Control, FACE stimulated A by 32% at mid-morning, 25% at midday,and 23% at mid-afternoon (Fig. 2). The stimulation of A by FACE was greater by 7% at mid-morning and midday and 35% at mid-afternoon under Dry than Wet. Elevated CO_2, therefore, alleviated water-stress-induced mid-afternoon depressions in wheat carbon gain. Full irrigation increased A by 13, 29, and 28% at mid-morning, midday, and mid-afternoon, respectively.

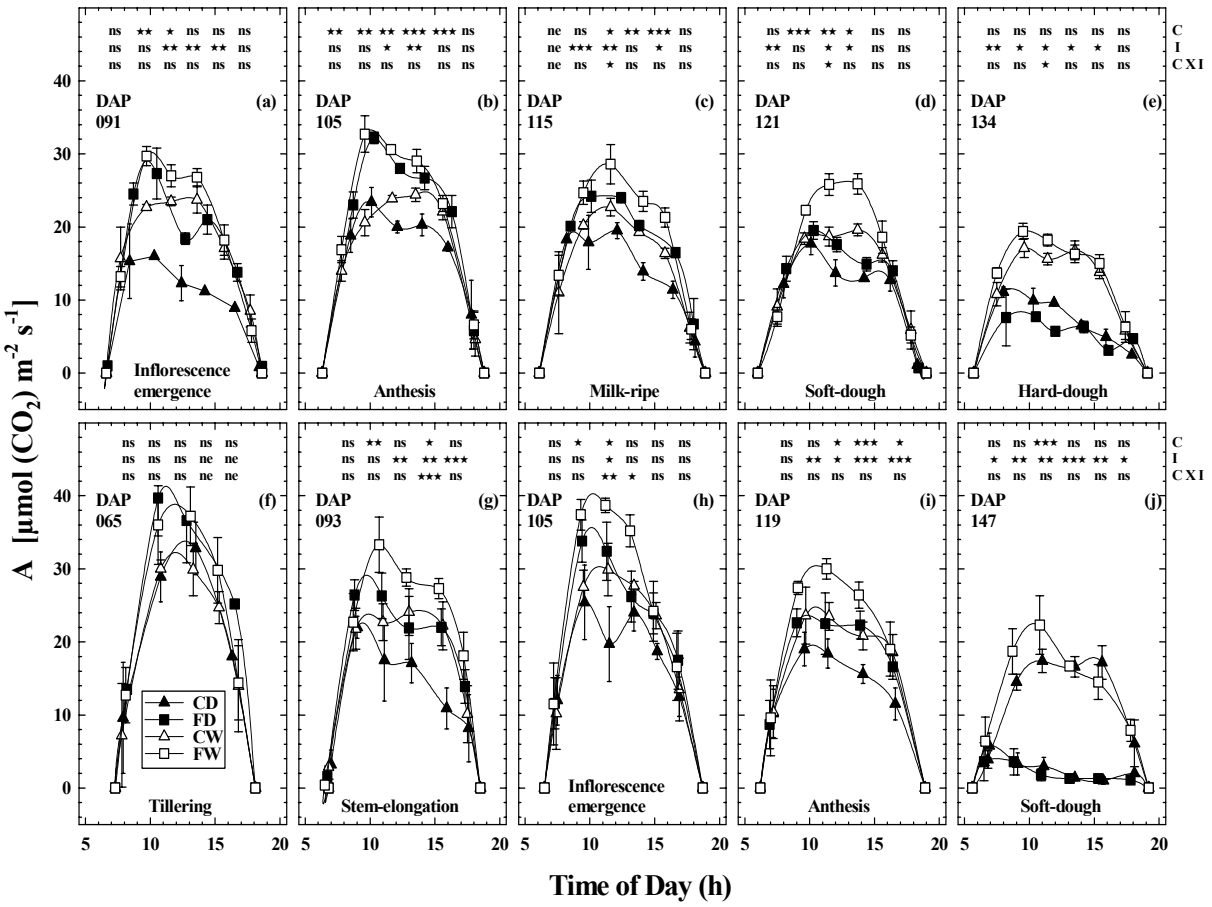

Figure 2. DAWN TO DUSK TRENDS IN LEAF NET ASSIMILATION RATE (A) OF EXPANDED SUNLIT SPRING WHEAT LEAVES FOR DAY AFTER PLANTING (DAP) AND GROWTH STAGES GIVEN FOR 5 D DURING 1993 (A-E) AND 1994 (F-J). SYMBOLS IN LEGEND SAME AS GIVEN IN FIG. 1. SOURCE OF VARIANCE AND RESULTS FROM ANOVA SAME AS DESCRIBED IN FIG. 1.

A hysteresis effect was observed when A vs. photosynthetic photon flux density (PPFD) was plotted from dawn until midday compared to that from midday to dusk (Fig. 3). This hysteresis effect became more pronounced as soil-water content became more depleted. Relationships between A vs. PPFD from dawn to midday and from midday to dusk were normalized (A_n) by dividing each observation of A within a day by the maximum value of A (A_{max}) for that day (usually at mid-morning or midday). Clearly, no hysteresis effect occurred under CW, a slight hysteresis effect was observed under FW, but only a modest increase in this effect was observed for FD. In contrast, a large hysteresis effect was observed for CD (Fig. 3). The smaller the hysteresis effect the greater was daily (A′) and seasonal accumulation (A″) of carbon. Compared with Control, FACE increased A′ and A″ by 25%. However, the stimulation in A″ by FACE over Control was 31% greater under Dry, but only 21% under Wet. This 10% difference in A″ between Dry and Wet can be explained by a proportionate reduction in the hysteresis effect observed for FD compared with CD.

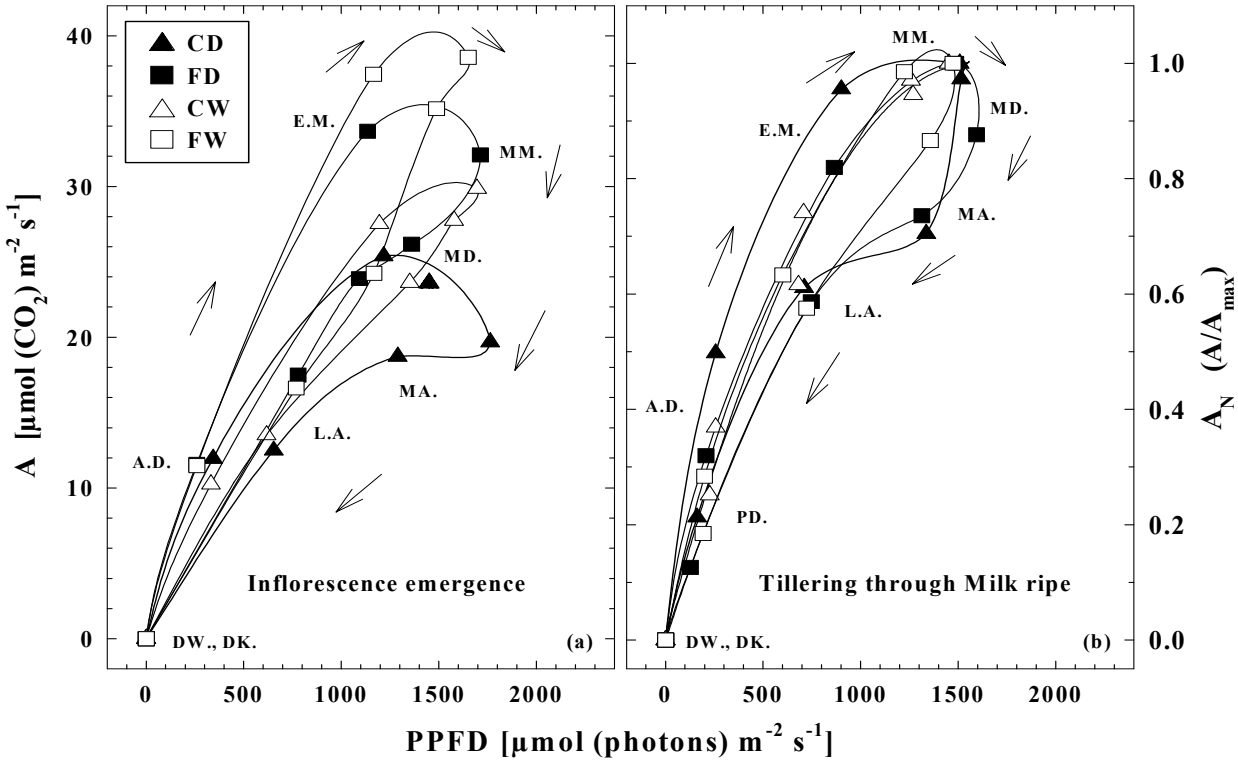

Figure 3: Mean leaf net assimilation rate (A) in response to diurnal course of incident photosynthetic photon flux density (PPFD) during inflorescence emergence on day after planting 105 (replotted from Fig. 2h) (a). Mean normalized leaf net assimilation rate (A_N), across years and growth stages except soft and hard-dough (replotted from Fig. 2a-d and f-i), in response to the diurnal course of incident photosynthetic PPFD (b). Arrows denote direction of hysteresis loop from DW-dawn, AD-after dawn, EM-early morning, MM-mid-morning, MD-midday, MA-mid-afternoon, LA-late-afternoon, PD-pre-dusk, and DK-dusk. Symbol legend same as given in Fig. 1.

INTERPRETATION: Despite the fact that elevated CO_2 directly reduced g_s (Fig. 1), it indirectly increased drought avoidance (reduced evapotranspiration and increased capacity for absorption of water and nutrients by roots), and increased drought tolerance (xeromorphic adaptations and osmoregulation mechanisms). Consequently, as water stress became more severe, the mitigating effect of elevated CO_2 in alleviating drought actually caused a reduction in water-stress-induced stomatal limitation in wheat carbon gain, particularly at mid-afternoon. Nevertheless, these results also demonstrate that although leaves grown under elevated CO_2 will have less stomatal limitations, regardless of water supply, they will still experience some mid-afternoon depression in carbon gain because of non-stomatal limitations (photoinhibition). In a future high-CO_2 world, therefore, additional carbon uptake at mid-afternoon will increase A′ and A″. Presumably, this additional carbon supply will result in an increase in total non-structural carbohydrate pools. Hence, despite any limitations in net primary production because of water deficits, a rise in atmospheric CO_2 will minimize the deleterious effects of drought on physiological function and growth, thereby, expanding the range where a viable crop can be grown, especially under dryland conditions.

FUTURE PLANS: We will continue to analyze, interpret, summarize, and document results from previous FACE experiments on wheat and sorghum. We will also actively plan and seek funding for a FACE alfalfa (*Medicago sativa* L.) experiment (Kimball et al., this volume).

COOPERATORS: See report from Kimball et al., this volume.

QUANTITATIVE REMOTE SENSING APPROACHES FOR MONITORING AND MANAGING AGRICULTURAL RESOURCES

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QUANTITATIVE REMOTE SENSING APPROACHES FOR MONITORING AND MANAGING AGRICULTURAL RESOURCES

MISSION

The ultimate goal of this research is to use remote sensing technology to increase our understanding of processes associated with environmental variability and to provide resource managers with information that will assist them in making tactical and strategic management decisions on farms, rangelands, and natural plant communities. Emphasis will be given to approaches that have potential for operational application, and that also have a strong physical foundation based on quantitative measurements.

REMOTE SENSING CHLOROPHYLL CONTENT IN WHEAT

T. R. Clarke, Physical Scientist; and P. J. Pinter, Jr., Research Biologist

PROBLEM: One of the most important decisions a grower must make is how to manage crop fertilizer inputs in order to assure a profitable yield while preventing groundwater contamination. Variable rate application technology has recently been developed, but the technology for directing applications effectively is less mature. A means of rapidly assessing a wheat crop's fertilizer needs at critical growth stages and at high spatial resolution is needed for this aspect of precision agriculture to succeed.

A second challenge for precision agriculture is a lack of means by which wheat producers can predict grain protein content before harvest with a high degree of spatial resolution. This ability would provide the option of harvesting the highest quality grain separately with the potential of increasing profits.

APPROACH: A Canopy Chlorophyll Content Index (CCCI) that was previously developed for cotton (1999 Annual Report) was tested for wheat using reflectance data collected during the 1997 Free-Air Carbon-dioxide Enrichment (FACE) experiment. The hard red spring wheat *Triticum aestivum* L cv. Yecora Rojo was sown in mid-December 1996 and harvested in late May. Water and fertilizer were applied through a subsurface drip irrigation system. Each of the eight circular experimental plots was split into halves with each half receiving either an ample amount of nitrogen (N) of 383kg/ha/season or a limiting nitrogen treatment of 45 kg/ha/season. Kimball et al. (1999) provide a full description of the FACE experimental design. Hyperspectral canopy reflectance measurements were made at approximately weekly intervals using a field-portable reflectance spectrometer referenced to a $BaSO_4$ panel of known bidirectional reflectance properties. Spectral bands equivalent to those used in the cotton experiment were extracted and used for analysis. These three 10nm-wide bands were Red (R) centered at 670 nm, far-red (F) centered at 720 nm, and near infrared (NIR) centered at 790 nm. Chlorophyll meter (SPAD) measurements of the uppermost fully-expanded leaves were made at about weekly intervals and were used as an estimate of canopy chlorophyll content. Reflectances of the R, F, and NIR bands were combined to produce two variables. The first was the Ratio Vegetation Index (RVI), NIR/R, which is sensitive to the amount of green vegetation present, and the second was the ratio NIR/F, which is sensitive to the chlorophyll content of green vegetation. These two variables were then used as the two dimensions of a plane, and the maximum and minimum measured values for each variable defined the boundaries of a domain within this plane. The position of a coordinate pair within the domain was then correlated to the chlorophyll content as estimated by the SPAD readings.

FINDINGS: A scatter plot of the two variables revealed good separation between low and high N treatments during the early part of the growing season through early stem elongation, and again during early grain filling. During late stem elongation, head emergence, and flowering, there was a distinct drop in far-red reflectance which rendered the planar domain unusable during this period, as seen in Figure 1. Two planar domains were therefore delimited: one for early season through early stem elongation, and a second for milk through early dough stages of grain filling. The early and late planar domains for wheat were used to develop two separate canopy chlorophyll content indices (CCCIwe and CCCIwl). The CCCIwe showed improving

correlation with canopy chlorophyll content, reaching a maximum at early stem elongation (Zadoks 30-34) as seen in Figure 2. The CCCIwl correlated well with final harvested grain quality when two rules were applied to the data. First, phenology must be in the milk to early dough stage (Zadoks 70-85). Second, the RVI must be greater than 8.0 for a datum to be included. With results thus constrained, very good correlation with harvested grain quality as measured by protein content was achieved, as shown in Figure 3.

INTERPRETATION: The three-band, planar domain method of determining canopy chlorophyll content appears effective in this first look. If the technique is verified using existing data from other years, work can proceed on developing nitrogen recommendations based on CCCIwe, and on developing grain quality prediction algorithms based on CCCIwl.

FUTURE PLANS: A second set of wheat data containing nitrogen-limited wheat reflectances from the 1996 FACE experiment will be used to verify the efficacy of both CCCIs. Future experiments in wheat will focus on further verification and development of management tools based on remotely sensed information.

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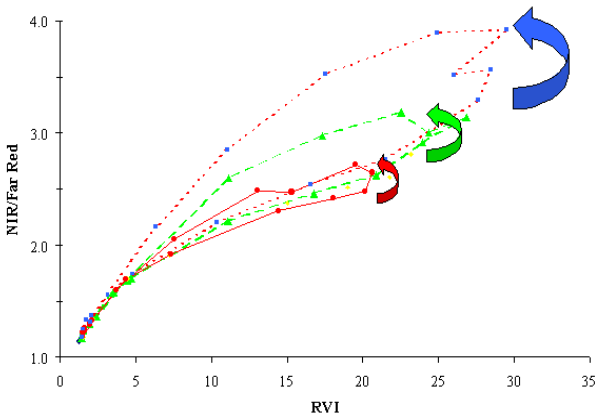

Figure 1. Planar domain of the CCCI applied to wheat. Data from three plots are shown: low nitrogen availability (●), high nitrogen availability (■), and an intermediate level (▲). A shift in the far red band during the late stem elongation and flowering stages of growth resulted in an upward shift in the domain, the location and direction of which is shown by the arrows.

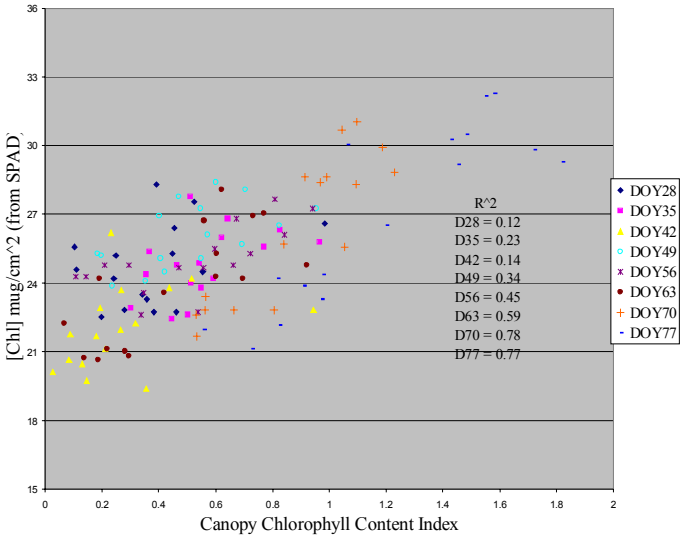

Figure 2. Correlation of chlorophyll content as estimated by SPAD measurement with CCCIwe up to mid stem elongation growth stage.

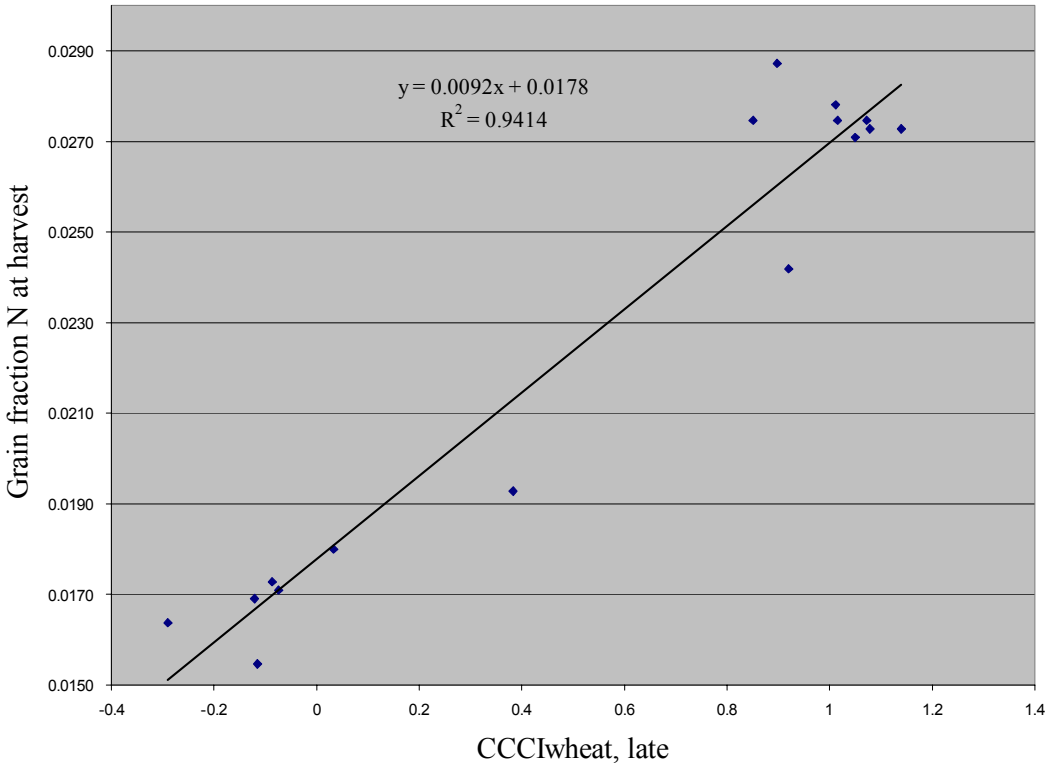

Figure 3. Correlation of grain fraction nitrogen as a measure of protein at final harvest with CCCIwl. Data were constrained to Zadoks growth stage 70 through 85 and Ratio Vegetation Index greater than 8.0.

DEVELOPMENT OF A MODELING AND SENSOR SYSTEM TO PROVIDE INFORMATION FOR PRECISION CROP MANAGEMENT

E.M. Barnes, Agricultural Engineer; T.R. Clarke, Physical Scientist; P.J. Pinter, Jr., Research Biologist; S.E. Richards, Research Lab Assistant; W.E. Luckett, Physical Science Technician

PROBLEM: Precision farm management requires timely, georeferenced information on crop and soil conditions. In this management system, the crop is given what it needs based on the current soil and environmental conditions so that economic return (not necessarily yield) is optimized. Cost efficient methods to provide this information are lacking at the present time. The objective of this project is to provide the tools needed to manage crop inputs economically at a very fine scale (potentially as small as 1 m).

APPROACH: To provide real-time management information, a combined sensor- and modeling-based approach has been under development. This project is part of a cooperative study between the U.S. Water Conservation Laboratory, the University of Arizona, Texas A&M University, and the Idaho National Environmental and Engineering Laboratory (INEEL). The project is also enhanced by the participation of two private companies, Valmont, which is providing a linear move irrigation system for the project, and CDS Ag. Industries, which is providing an injection pump.

The project began in 1998 with cotton and barley field experiments during which agronomic and hand-held radiometer data were collected. These data were used to begin formulation of quantitative relationships between spectral response and crop condition. Concurrent with these experiments, a system was developed to allow the linear move irrigation system to serve as a remote sensing platform (named **Ag**ricultural **I**rrigation **I**maging **S**ystem, AgIIS, i.e., "Ag Eyes"). The AgIIS was completed in time for the 1999 cotton season and was able to provide images in the red, green, red-edge, near infrared (NIR), and thermal portions of the spectrum. During the growing season, the AgIIS was used to obtain images at a minimum of weekly intervals, with as many as three images per week during the period of rapid crop development. The reflective bands were calibrated to units of reflectance by using a plywood panel mounted at the center of the linear move. Periodic radiometer measurements of the panel were taken during the season so that its spectral properties were known.

A Latin square experimental design was used during the 1999 cotton season with four treatments: (1) control (WN, optimal conditions); (2) low nitrogen (Wn, 50% optimal plant requirements); (3) low water (wN, decreased irrigation frequency, allowing the plants to become water stressed five times during the season); and (4) low water and low nitrogen (wn). Soil moisture levels were monitored in every plot using a neutron probe at a minimum of weekly intervals (two access tubes per plot). Additionally, two plots were heavily instrumented with TDR probes in four locations at four depths (5, 10, 15 and 20 cm). The probes were used to determine the soil surface moisture content at hourly intervals using an automated data acquisition system. Stem flow gages were also added to these plots to measure the cotton's daily transpiration rate. The plants were sampled weekly for nitrate status, leaf area index (LAI), leaf, stem, and boll dry weights, plant height and percent canopy cover.

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FINDINGS: The data collected during this study are being used to examine issues related to geostatistical analysis of spatial variability in crop condition (Kostrzewski, 2000), and improvement of remote sensing techniques to infer crop nutrient and water status. In order to present examples of some the progress made in these areas, three spectral indices will be used: the ratio vegetation index (RVI = ratio of NIR to red reflectance), a planar domain version of the crop water stress index (CWSI) similar to the version developed by Clarke (1997), and the Canopy Chlorophyll Content Index (CCCI; Clarke and Barnes, 1999). For a more complete description of these indices, see Barnes et al. (2001).

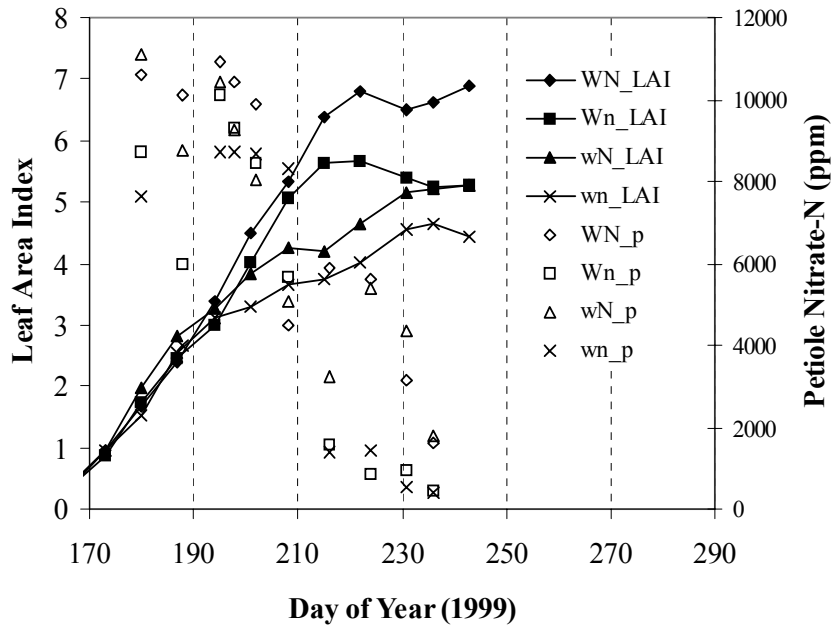

Figure 1. Seasonal trends in treatment average leaf area index (LAI) and petiole Nitrate-N concentration (p).

Figure 1 shows the seasonal trends in leaf area index and petiole nitrate content based on the treatment averages. The difference in water treatments began on DOY 193, after which point there was a definite slowing of LAI accumulation for the low water treatments (wN and wn). By DOY 236 there is little difference in the LAI between the Wn and wN treatments. There was no clear response in LAI to the N treatments until DOY 215 at which point petiole analysis indicate a significant difference between high and low N treatments.

Figure 2 shows the season trends in the stressed treatment averages (Wn, wN, and wn) relative to the control treatment (WN) for the RVI, (1-CWSI) and CCCI. Note that 1-CWSI is used in computing the ratio to the WN treatment in Figure 2c, because under conditions of low water stress, which was common in the WN plots, the CWSI was approximately 0. The relative differences in RVI follow similar trends as LAI with some exceptions (Figure 2a). The sharp relative decrease in RVI on DOYs 194, 202, and 209 was due to a combination of a wet soil background in the high-water treatments (WN and Wn) and some leaf wilting in the low water treatments due to water stress. Note that from DOY 233 to 259 the RVI for the wN treatment becomes higher than the Wn. This illustrates the difficulty in interpreting the differences of simple vegetation indices as a measure of a single stress. Indices based on combinations of the NIR and red areas of the spectrum are strongly correlated with canopy density; therefore, any stress that alters canopy density will impact these indices.

The CCCI begins to show a clear distinction between the low N treatments (Wn, wn) after DOY 214 (Figure 2b), about the same time the petiole data indicated a strong difference between the high and low N treatments (Figure 1). Unlike the RVI, the CCCIs of the low N treatments are consistently less than the control from DOY 214 to 260. While this index does appear to minimize the impact

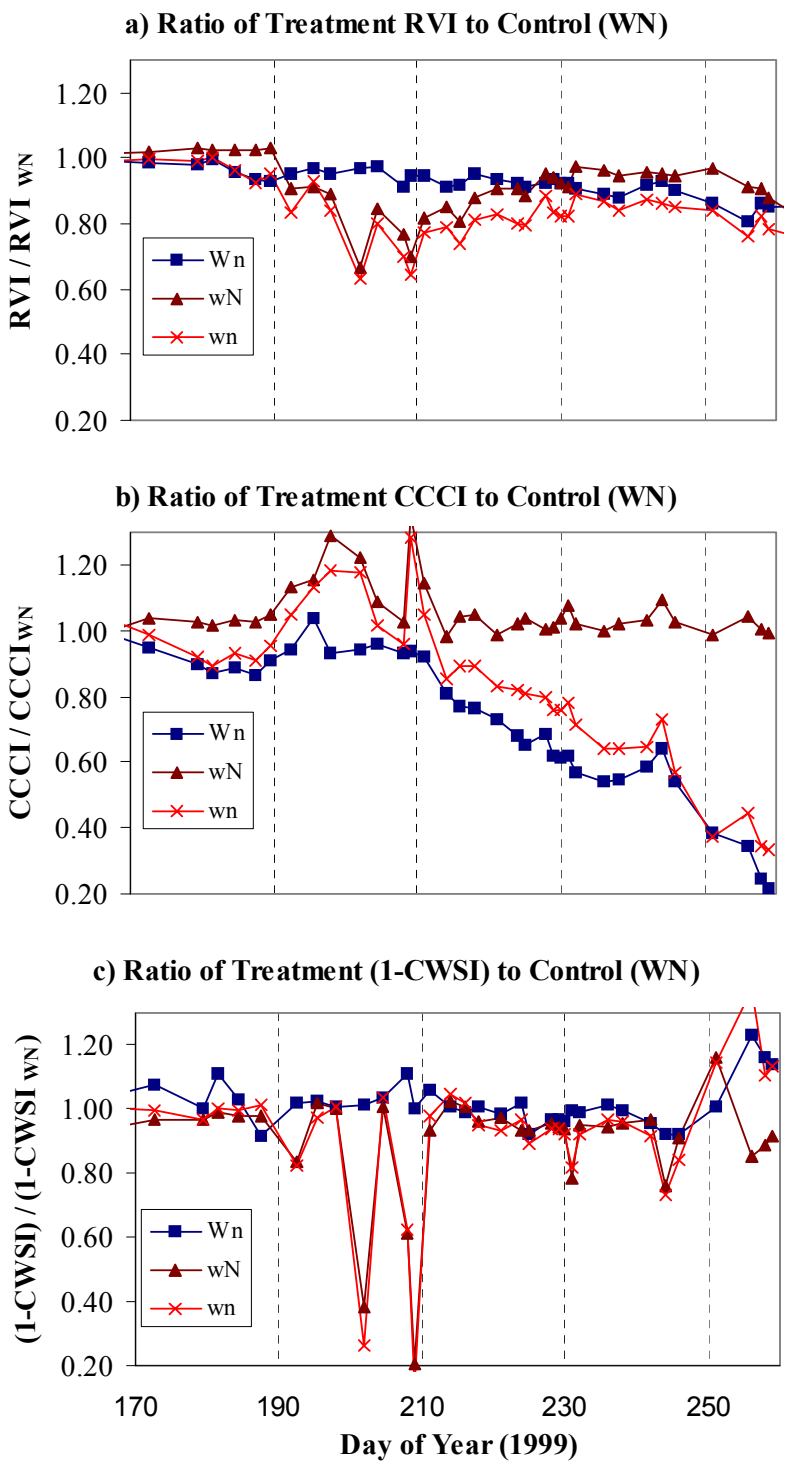

Figure 2. Seasonal trends in the ratio of (a) ratio vegetation index (RVI), (b) 1-crop water stress index (CWSI), and (c) canopy chlorophyll content index (CCCI) in the high water, low N (Wn), low water, high N (wN) and low water, low N (wn) treatments to the respective index in the control treatment (WN).

of canopy density, it was sensitive to changes in the wetness of the soil surface background under partial canopy conditions as indicated by the increase in the index on DOYs 198 and 209. On both of these dates, the soil background in the low water treatments was dry, but wet in the high water plots. This resulted in a false indication that the chlorophyll content was higher in these treatments than the control.

In Figure 2c, the periods after which water was withheld (DOYs 194, 202, 209 and to a lesser extent 231 and 245) are identified by the relative decrease in (1-CWSI) in the low water (wN, wn) treatments. While some of these events also decreased the RVI in the low water plots with respect to the high, the RVI trends are not as related to water treatment levels as those in CWSI later in the season, particularly on DOY 251, after the water treatments were purposely reversed (i.e., water was not applied to the WN and Wn treatments on DOY 250). Also note that on dates after all of the plots were irrigated (e.g., DOY 215), there was little difference in the CWSI between water treatments, unlike the RVI.

INTERPRETATION: The system under development

will provide farmers and agricultural consultants with a simple, cost effective data source to map spatial variations in crop water and nitrogen levels. These data will have the potential to serve as an integral part of a decision support system for precision crop management.

FUTURE PLANS: Work will continue to integrate the sensor information with simulation models to provide decision support in water and nitrogen management. Related studies will begin during the 2000-2001 growing season using AgIIS to determine the feasibility of remote sensing and modeling technologies to provide information relevant to quality management in broccoli.

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INTEGRATION OF REMOTELY SENSED DATA WITH CERES-WHEAT

E.M. Barnes, Agricultural Engineer; P.J. Pinter, Jr., Research Biologist

PROBLEM: The development of crop growth models has been progressing since the 1970s and was originally focused on the prediction of average field conditions. More recently tools have been developed to integrate growth models with geographic information systems (GIS) at either regional or field scales. One limitation to simulating spatial variations in crop production is the large amount of input data necessary to accurately characterize the growth conditions. The objective of this study is to determine how remotely sensed observations can be used to improve a growth model's ability to simulate actual field scale variability.

APPROACH: Wheat was selected as the first crop to test methods to integrate remotely sensed observations with crop models due to the extensive growth and remote sensing data set collected during the FACE wheat experiments (Kimball et al., 1999). CERES-Wheat (Ritchie and Otter, 1985) was selected as the crop model to use in this approach because it is a process oriented model capable of simulating different management practices, while maintaining reasonable input requirements that would not prevent its application by a farm manager. Two potential links between CERES-Wheat and remotely sensed data have been identified: the fractionally absorbed photosynthetically active radiation (fAPAR) and crop water status via the crop water stress index (CWSI).

CERES is essentially a radiation use efficiency model, predicting potential carbon accumulation (PCARB, g plant^{-1}) as a function of solar radiation and leaf area index (LAI):

$$\text{PCARB} = 0.5\ \text{SolarRad RUE}\ [1-\exp(-0.85\ \text{LAI})]\ /\ \text{PlantPop} \qquad (1)$$

where 0.5 is from the assumption that 50% of the total incoming solar radiation (SolarRad, MJ m^{-2} d^{-1}) is PAR, and PlantPop is plant population (plants m^{-2}). The actual amount of carbon accumulation is then decreased if there is water, nitrogen or temperature stress. The model was modified by replacing the [1-exp(-0.85 LAI)] term in the equation 1 with the remotely sensed estimate of fAPAR. Note that by using "observed" fAPAR, the equation provides an estimate of actual carbon production (i.e., it does not need to be modified to account for stress); however, other stress factors in the model that relate to carbon partitioning will not be influenced by these modifications. The remotely sensed estimate of fAPAR was determined as a function of the normalized difference vegetation index (NDVI) using the approach developed by Pinter et al. (1994).

The model predicts transpiration as a function of either the plant available water in the root zone or atmospheric limitations, whichever is smaller. The potential water uptake by the roots (RWU_L, cm^3 water per cm roots per day) in a particular soil layer (L) is calculated by

$$RWU_L = c_1 \exp[c_2 (SW_L - LL_L)]\ /\ [c_3 - \ln(RLV_L)] \qquad (2)$$

where c_x are empirical constants, SW_L is the soil water content (cm^3 cm^{-3}), LL_L is the soil water content at permanent wilting point (cm^3 cm^{-3}) and RLV_L is the root length density (cm root per cm^3 of soil). If the sum of RWU_L across the soil profile (TRWU, cm) is greater than potential

transpiration for the day, RWU_L is decreased so that the water uptake by the plant is equal to potential transpiration. A soil water deficit factor (SWDF1) is then defined as:

$$SWDF1 = TRWU / E_p \quad (3)$$

where E_p is potential transpiration (cm). SWDF1 is essentially equivalent to the definition of (1-CWSI). Therefore, the model was modified when there was a CWSI observation on a given the day and (1-CWSI) was not within 10 percent of SWDF1. The modification was accomplished by solving equation 2 for SW_L and setting the soil water content distribution in the soil profile so that when the total RWU of equation 3 was calculated, SWDF1 would equal (1-CWSI). A limitation to this approach is that when the model is under-predicting plant available water, the amount of water that can be added to the profile will only be sufficient to bring the predicted levels back to the verge of water stress.

FINDINGS: Figure 1 represents the fAPAR determined by remotely sensed estimates and the model's predicted fAPAR for the 1993-94 and 1995-1996 ambient CO2 treatments. The spikes in the model's predicted fAPAR in the 1993-94 season for the dry treatment are due to the water stress factor, which rapidly returns to one after an irrigation. The remotely sensed fAPAR estimates do not respond to rapidly changing stress conditions caused by short-term water stress between irrigations. However, they do reflect the accumulated effects of the water stress in the later half of the season. For the nitrogen stress treatment in the 1995-96 growing season, the nitrogen stress factor does not change as rapidly, indicating the more sustained nature of the stress.

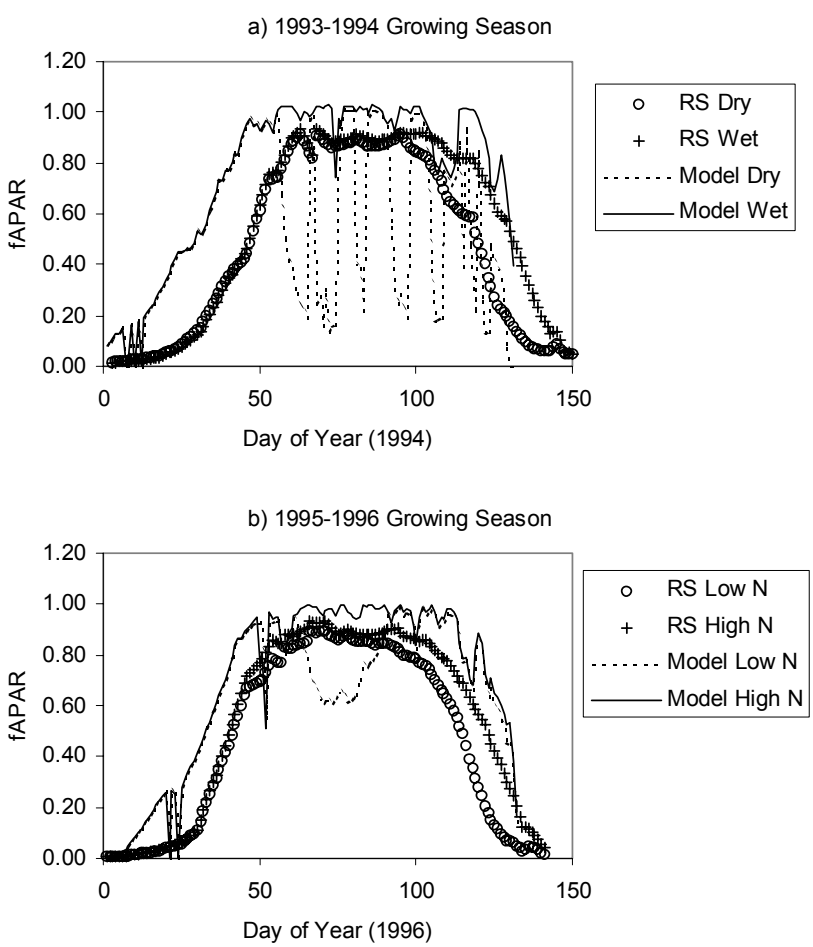

Figure 1. Remotely sensed and model predicted estimates of fAPAR for the 1993-94 (a) and 1995-96 (b) growing seasons.

For water stress, the CWSI is a more appropriate remotely sensed parameter than fAPAR because it is correlated directly with transpiration. In Figure 2, a comparison is shown between observed extractable water in the top

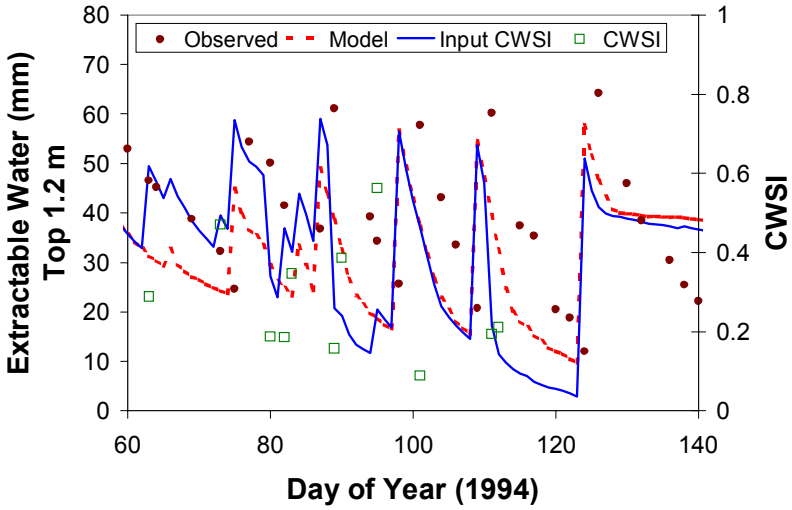

Figure 2. Extractable water in the top 1.2 m of the soil profile as predicted by the model with and without CWSI input. The value of the CWSI input is indicated on the y-axis to the right.

1.2 m of the soil profile with model predictions from CERES with and without CWSI input for the ambient Dry treatment during the 1993-94 season. The squared symbols show when CWSI measurements were made. Early in the season, the CWSI input improved predictions; however, later in the season, CWSI input resulted in under prediction of soil water. This under prediction may have been due to the assumption that the relative distribution of moisture in the soil profile remains unchanged when a CWSI modification is made; however, more investigation is needed. The model alone provided good estimates of soil water content and there were no significant differences in the prediction of yield between the two methods.

Figure 3 shows the results when the 1992-93 ambient Wet treatment was simulated for the first part of the season, and then single CWSI measurements from the Dry treatment were input on selected days and the dry treatment was simulated for the rest of the season. The observed values are for the dry treatment. The different lines indicate the results when the CWSI was input on the day of year indicated. For inputs prior to DOY 90, the CWSI was able to reset soil water to an amount very close to observed. Changes later in the season were not as accurate, but still within 20-mm of the measured value. These test indicate the input of CWSI could be very useful when soil water measurements are not available to initialize the model.

INTERPRETATION: Use of fAPAR estimates from NDVI is best suited for chronic stress conditions (i.e., stress conditions that persist long enough to impact canopy development). Compared to N stress, water stress can develop over much shorter time periods under high evaporative demand, and it can be relieved shortly after an irrigation or rainfall event. Use of the CWSI with the model shows promise as an alternative to measured soil water initial conditions. Dependable methods to forecast yield during the early season would provide a new tool for producers to make informed decisions in the application of precision farming practices. The approaches taken in this study demonstrate that the integration of remotely sensed data and crop models can lead to such a tool; however, improvements in the current methods are needed.

FUTURE PLANS: Further development is need on both approaches, particularly to determine methods to adjust the model's parameters through iteration so that the number of required remotely sensed observations can be minimized.

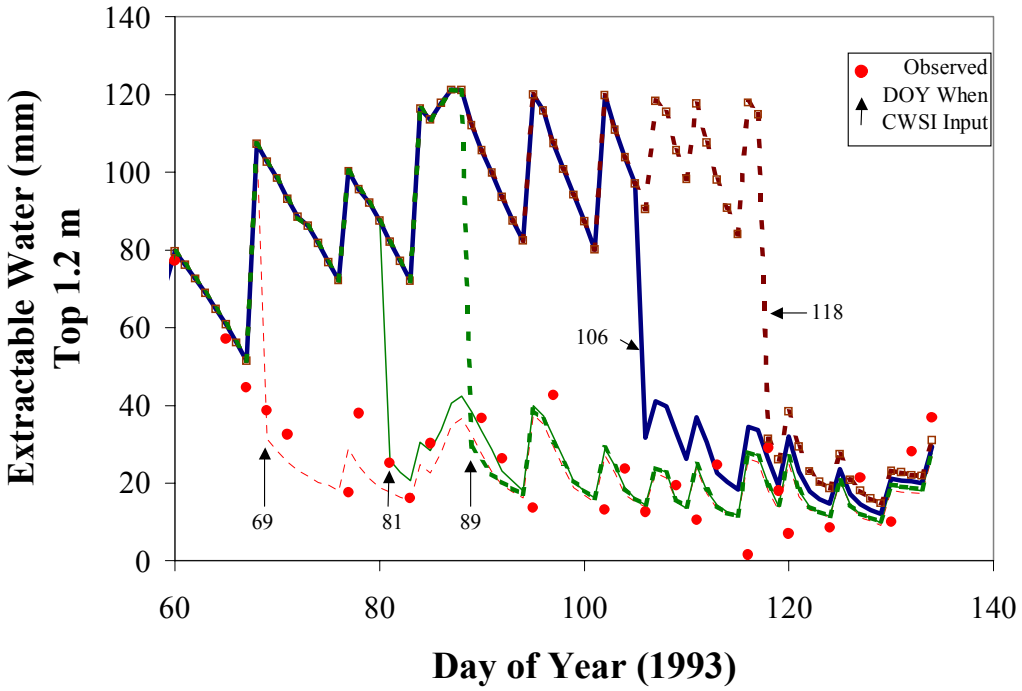

Figure 3. Extractable water in the top 1.2-m of the soil profile predicted by the model when simulating the wet treatment until a day when a CWSI was input and then simulating the dry treatment for the remainder of the season. The observed data points are for the dry treatment.

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GERMPLASM IMPROVEMENT AND AGRONOMIC DEVELOPMENT OF NEW ALTERNATIVE INDUSTRIAL CROPS

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GERMPLASM IMPROVEMENT AND AGRONOMIC DEVELOPMENT OF NEW ALTERNATIVE INDUSTRIAL CROPS

MISSION

To acquire and characterize germplasm of guayule, lesquerella, vernonia, and other promising new, alternative crops. To evaluate and enhance germplasm of new crops for industrial raw materials. To develop knowledge of floral biology and seed production and plant responses to stresses. To develop economical, cultural and seed production systems for new crops under various conditions. To develop methods for efficient guayule latex extraction and seed oil analyses for characterizing latex, resin, and oil properties.

GUAYULE LATEX, RUBBER, AND RESIN

F.S. Nakayama, Research Chemist; T.A. Coffelt and D.A. Dierig, Research Geneticists;
and S.H. Vinyard and A. Faber, Research Technicians

PROBLEM: Termite and wood-rot damage are multibillion dollar problems in the United States. Conventional preservatives used to protect wood from insect and microbial damage are presently of major concern to human health and the environment. Finding alternative and economical preservatives has not been successful. The desert-adapted guayule plant has been observed to have both insect and microbial resistant properties. In addition, the utilization of waste plant material or bagasse, approximately 90% of the total biomass following the extraction of latex rubber from the plant to treat wood, would greatly help the economics of guayule culture.

APPROACH: Wood material was impregnated with the resin that was extracted from the guayule plant. Composite boards were fabricated from guayule bagasse material that remained after the extraction of latex rubber. The binders or adhesives used were high density polyethylene (HDPE) and phenolic resin (PR). The two types of wood prepared from guayule were tested for resistance against termite and wood-rot attacks.

FINDINGS: Impregnation of guayule resin into wood at 50% by weight and higher is needed to make the wood resistant to termite attack (Table 1). When the wood was impregnated at the 97% resin content level, complete termite mortality was achieved.

Table 1. Eastern subterranean termite resistance of southern pine treated with resin extracted from guayule.

Amount of resin extract in wood (%)	Observation (after one week)	Rating (ASTM)[a]
0	Termites still alive	No mortality
10.3	Termites still alive	No mortality
51.8	Mildly termite active	Low mortality
97	All termites dead	High mortality
Guayule particleboard (no adhesive)	Mildly termite active	Low mortality
Guayule composite board with 30% HDPE plastic	All termites dead	High mortality
Guayule branch	All termites dead	High mortality

[a] ASTM D-3345 standard

For the guayule composite board with 30% HDPE, 100% mortality occurred. Thus, fabricating composite board directly from guayule bagasse would be a much simpler approach to take than extracting the guayule resin and then impregnating wood to achieve termite resistance.
The guayule particle board without any adhesive had a mild termite activity. Interestingly, the guayule branch material alone was termite resistant causing high mortality. Bultman et al. (1991) had treated wood with a full-strength resin mixture and obtained 100% and higher resin retention in the

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wood. Their resin-impregnated wood samples were able to resist *Coptotermes Heterotermes* for 71 months in the Panamanian rain forest, and *Reticulitermes* spp. for 62 months in semiarid Arizona.

The decay resistance of composite board made from guayule bagasse is shown in Table 2. Both the composite board with PR and the particleboard without resin were resistant to *G. traveum* and *P. placenta*. The composite board with 30% HDPE as the binder showed resistance to the two fungal organisms. The guayule board made with no adhesive exhibited the largest thickness change for *G. trabeum* (98%) and *P. placenta* (137%). Thus, the guayule bagasse even though it contains resinous material and rubber will need a binder or waterproofing additive to make its wood product water resistant. Unlike the other wood types, the guayule stem alone had moderate resistance to fungi.

Table 2. Decay resistance of guayule composition boards and guayule stems.

Board type and stem	*Gleophyllum traveum*		*Poria placenta*	
	Weight loss (%)	Rating (ASTM)[a]	Weight loss (%)	Rating (ASTM)
PF resin, 10% 14 mm	13.94	Resistant	19.61	Resistant
No adhesive, 8 mm	19.66	Resistant	23.99	Resistant
HDPE, 30% 11 mm	6.46	Highly resistant	5.68	Highly resistant
Guayule stem (with bark & wood core)	30.28	Moderately resistant	12.72	Resistant

[a] ASTM D-2017 standard

The guayule resin extract when impregnated into the southern pine made the treated wood resistant to fungal attack as shown in Table 3. Resistance to *G. traveum* started at 10.3% resin content and 51.8% for *P. placenta*. At resin contents of 51.8% and higher, the treated wood was highly resistant (*G. traveum*). The activities of other types of fungi such as brown-rot (*Gleophyllum traveum*, *Antrodia carbonica*, *Fomitopsis cajanderi,* and *Lentinus ponderosa*) and white-rot (*Dichomitus squalens*, *Trametes versiclor*, and *Ganodermas* sp.) were also observed to be inhibited by the resin extracted from guayule (Bultman et al., 1991).

Table 3. Weight loss and wood-rot rating of southern pine treated with resin extracted from guayule in the presence of fungi.

Amount of resin extract in wood (%)	*Gleophyllum traveum*		*Poria placenta*	
	Weight loss (%)	Rating (ASTM)[a]	Weight loss (%)	Rating (ASTM)
0	58.60	Non-resistant	47.64	Non-resistant
2.6	52.46	Non-resistant	50.98	Non-resistant
10.3	22.42	Resistant	45.00	Non-resistant
51.8	8.69	Highly resistant	36.65	Moderately resistant
97.0	2.95	Highly resistant	11.11	Resistant

[a] ASTM D-2017 standard

The decay resistances of southern pine that was treated with two different types of resin extracts are compared in Table 4.

Table 4. Decay resistance and durability of southern pine treated with two sources of resin extracted from guayule.

Source of resin extract	*Gleophyllum trabeum*		*Poria placenta*	
	Weight loss (%)	Rating (ASTM)[a]	Weight loss (%)	Rating (ASTM)
Acetone extract only	10.48	Highly resistant	4.64	Highly resistant
Bultman resin	12.89	Resistant	8.29	Highly resistant

[a] ASTM D-2017 standard

Our guayule resin extracted with acetone alone showed similar decay resistance as the resin provided by Dr. Bultman, which was extracted from guayule with a solvent system consisting of both acetone and hexane.

INTERPRETATION: The composite board made from 70% guayule fiber and 30% HDPE, the guayule branch, and normally susceptible wood impregnated with 52 to 97% by weight of guayule resin-extract all had antitermitic property. The guayule particleboard with no adhesive showed termite resistant property, but it could not stand high moisture exposure.

The effects of impregnating southern pine wood with the guayule resin-extracts (content of 10.3% and higher) on the decay resistance (10.3% and higher for *Gleophyllum trabeum*, and 51.8% or higher for *Porio placenta*) appeared to be significant. The treated specimens showed greater resistance to *G. traveum* than *P. placenta*. *P. placenta* often causes decay in millworks and in wood situated above ground.

Southern pine wood specimens impregnated with resin derived from either an acetone extract or a combination of acetone and hexane were resistant to the wood-rot fungi. Both the phenolic adhesive bonded and the guayule only particleboard showed good decay resistance property to *P. placenta* and *G. traveum*. The plastic composite board made from 70% guayule and 30% HDPE was highly resistant to both types of wood rotting fungi.

The natural guayule stems and branches with 10 to 15% resin content also exhibited some decay resistance property. Excessive thickness, swelling and specific gravity reduction occurred with the wood made from guayule plant fiber alone where no adhesive was added.

Our preliminary results indicate that the commercial application of the guayule is possible to provide a dependable, renewable, and alternative natural source of wood preservatives. Because the plant is drought tolerant and its derivatives can reduce tree harvest, its cultivation as an alternative crop will help conserve our water and forest resources.

FUTURE PLANS: We will continue to find ways to utilize guayule waste materials for pest control including the fabrication of composite and resin impregnated wood products. We plan to develop cooperative studies with other ARS locations who are working with insect control and to establish Cooperative Research and Development Agreements (CRADA) with private organizations to make use of the waste bagasse for making guayule blends with other waste wood to fabricate high-valued, commercially useful wood products.

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GUAYULE BREEDING AND GERMPLASM EVALUATION

T.A. Coffelt and D.A. Dierig, Research Geneticists; F.S. Nakayama, Research Chemist;
and G. Leake, S. Vinyard, A. Faber, G. Dahlquist, and P. Tomasi, Research Technicians

PROBLEM: Allergies caused by using latex products from the Heavea source are a serious health problem in certain population groups in the United States, such as health care workers and patients who undergo multiple surgeries. Guayule is the best potential source of hypoallergenic latex to solve this problem. Previous work has shown that natural latex from guayule can be used to make medical products, gloves, and condoms that prevent virus and other pathogen transmission. In addition, guayule can be grown in the Southwestern United States to provide a domestic source of this valuable latex. Currently, the United States imports all of its natural rubber of which about 300,000 metric tons is used for natural latex products. The United States consumes about one third of all natural latex in the world. For guayule to be used in the hypoallergenic latex market, lines with higher latex content that can be harvested in less than three years are needed to replace older lines that require three to five years before harvesting. Thus, high yielding, fast growing, and easy to establish germplasm is needed for guayule to be successful as a viable new crop. Recent research results from this program have also shown that maximum benefits for genetic improvements can be made when selections are done when plants are one or two years old rather than four or five years old. The objective of the current studies was to evaluate the genetic variation among 20 new guayule lines for agronomic and latex characteristics compared to two older breeding lines.

APPROACH: Twenty new guayule germplasm lines and two check lines (11591 and N565) were transplanted at The University of Arizona Maricopa Agricultural Center, Maricopa, Arizona, USA on April 6, 1995. Plots were two rows, each 1 m long and 1 m wide. Transplants were spaced 360 mm apart. A randomized complete block design with four replications was used. Plots were irrigated immediately after transplanting and the soil kept moist with frequent irrigations until plants were established. Plants were maintained each year during the active growing season with approximately biweekly irrigations from February through October. Recommended production practices for row crops in Arizona were used to maintain plots during the experiment.

Percentage of plants surviving per plot and plant height were determined one (1996) and two (1997) years after transplanting. Plant width was determined two years after transplanting. All surviving plants in each plot were measured for plant height and width and the mean for each replicate used for statistical analyses. Changes in survival rate of each plot were calculated by subtracting the survival rates for 1997 from the 1996 survival rates. Changes in plant height of each plot from 1996 to 1997 were determined by subtracting the plot mean heights in 1996 from the plot mean heights in 1997. A plant height to width ratio was calculated in 1997. Two representative plants from each plot were selected in 1998 for determining latex content per plant, wet and dry plant weight, chipping losses, latex yield per plant, and latex and plant biomass yields per m^2. Wet plant weights were taken before and after chipping to obtain plant biomass yields at harvest and plant weight losses during chipping. The weight loss during chipping was converted to a percent loss by dividing the after chipping weight by the before chipping weight to standardize data across plots. Dry plant weight was determined by oven drying a sample of the chipped plant material at 60^0C. The weight of latex per plant, latex yield per m^2, and plant biomass yield per m^2 were calculated for each plot. Data were analyzed using the Proc GLM procedure in SAS (1988) for a randomized complete block

design. Replications were considered fixed effects and genotypes random effects. Significance among genotype means was determined by analyses of variance and LSD at the P=0.05 level.

FINDINGS: Significant variation was found among the 20 lines and two checks for all characteristics (Tables 1 and 2). None of the lines were significantly better than the checks for all characteristics, but five lines were identified that merit further study. G7-14 was the fastest growing line (406 mm tall) the first year and had the best survival rate (99%). A higher latex content per plant occurred in two lines, P3-11 (4.1%) and P10-4 (4.2%), than 11591 (2.4%) or N565 (3.1%). G1-16 had the highest wet plant weight (2.7 kg), and was one of the highest for dry plant weight (1.6 kg) and total biomass yield (2214g/m^2). The most promising line was N9-3, which had the highest plant dry weight (1.6 kg), weight after chipping (2.3 kg), latex weight per plant (60.3 g), latex yield per m^2 (83 g) , and total plant biomass yield per m^2 (2259 g), as well as the lowest percentage loss during chipping (14.8%).

INTERPRETATION: Results from this study indicate that variation is available in this germplasm for significant improvements in all characteristics studied through a breeding and selection program. This is the first study on variation present in germplasm lines for chipping loss. Chipping losses can be associated with several factors, but the amount of leaf material in the sample may be the most important. Leaves tend to be lost during the handling and chipping process. Those plants that were higher in percentage of leaf weight may be the ones with higher chipping losses. Another observation that supports this theory is that samples with less leaf material tend to pass more completely through the chipper, thus leaving less residue in the chipper. Data were not collected in this study on leaf weights or residue left in the chipper. Therefore, further tests will be necessary to identify the specific trait(s) responsible for the significant differences in chipping loss observed in this study. Leaves contain very little or no latex. Thus, removal of leaves prior to chipping and/or harvesting may be desirable for a more efficient chipping process that would require less cleaning of equipment. Latex yields reported in this study should be helpful to industry in order to plan the number of hectares to plant for meeting latex production needs.

FUTURE PLANS: An Initiative for Future Agriculture and Food Systems grant was obtained by the USWCL as part of a consortium grant to the University of Arizona. Tests will be initiated in the fall of 2000 and spring of 2001 to study the effects of planting date, plant population, irrigation level, fertility, harvest time, post-harvest/pre-chipping storage conditions, cutting height, and regrowth on latex, seed, and plant growth traits. A cooperative yield test will be established for evaluating promising lines from the breeding program for release and new populations for making selections will be developed. These studies will involve close cooperative work with scientists at the various locations involved in guayule research.

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Table 1. Plant stands, height, width, and height/width ratio for 22 guayule lines grown at Maricopa Agricultural Center, Arizona, USA in 1996 and 1997.

Line	Stand 1996 (%)	Stand 1997 (%)	Stand (1996-1997)	Height 1996 (mm)	Height 1997 (mm)	Height (1997-1996)	Width (1997) (mm)	Height/ Width
11591	97.00	96.25	0.75	336.50	**547.30**	**210.80**	461.00	1.19
N565	80.00	79.25	0.75	250.50	418.00	167.50	436.80	*0.96*
G1-10	91.25	89.75	1.50	*239.50*	371.30	131.80	313.50	**1.20**
G1-16	86.00	79.00	7.00	290.80	431.30	140.50	398.30	1.09
G7-14	**99.25** [1]	**98.50**	0.75	**405.80**	520.50	114.80	458.80	1.13
N6-2	84.50	77.50	7.00	243.00	352.50	109.50	312.50	1.14
N6-3	91.50	86.25	5.25	269.50	388.80	119.30	347.50	1.12
N7-2	75.00	72.50	2.50	311.80	489.30	177.50	444.80	1.10
N7-5	79.75	72.75	7.00	240.30	*339.50*	99.20	315.50	1.12
N8-1	61.00	58.00	3.00	261.80	427.30	165.50	389.80	1.10
N8-5	75.25	74.50	0.75	*238.30*	351.30	113.00	321.80	1.10
N8-10	90.75	88.50	2.25	270.30	422.00	151.80	387.00	1.09
N9-3	94.00	90.00	4.00	292.50	465.30	172.80	415.00	1.12
N9-4	61.00	61.75	*-0.75*	319.30	510.80	191.50	**473.00**	1.08
N12-18	87.75	80.50	**7.25**	274.00	402.80	129.50	355.80	1.14
N13-1	83.00	82.25	0.75	299.80	468.00	168.30	420.80	1.11
O16-1	82.25	80.50	1.75	291.50	480.50	189.00	431.80	1.11
P3-11	95.50	92.50	3.00	306.00	430.00	124.00	380.80	1.13
P10-3	*56.50*	*56.50*	0.00	250.30	404.80	154.50	371.00	1.09
P10-4	97.00	91.00	6.00	275.50	345.50	*70.00*	*288.50*	**1.20**
P10-5	86.00	82.25	3.75	242.00	367.80	125.80	308.50	**1.20**
P11-1	84.50	78.25	6.25	247.30	380.80	133.50	337.80	1.13
LSD	16.65	18.34	6.00	51.20	63.70	45.90	62.50	0.10

[1] Numbers in bold are the largest values for the column and numbers in italics are the lowest values for the column.

Table 2. Latex content, latex yield, plant wet weight, plant dry weight, chipping loss, and plant biomass of 22 guayule lines grown at Maricopa Agricultural Center, Arizona, USA in 1998.

Line	Wet weight (kg)	Dry weight (kg)	Chip weight (kg)	Chip loss (%)	Plant biomass (g/m^2)	Latex content (%)	Latex plant (g)	Latex Yield (g/m^2)
11591	1.91	1.18	1.57	19.48	1626	2.36	27.95	38.68
N565	2.00	1.23	1.66	15.90	1706	3.09	35.03	48.47
G1-10	1.32	1.02	0.97	27.55	1408	3.24	33.41	46.24
G1-16	**2.70**	1.60	2.18	20.23	2214	*2.19*	35.81	49.56
G7-14	1.72	0.99	1.25	**28.03**	1363	2.26	*22.16*	*30.66*
N6-2	1.31	0.77	1.05	20.43	1059	3.49	27.45	38.00
N6-3	1.55	0.91	1.19	24.10	1263	3.54	31.82	44.04
N7-2	1.85	1.06	1.55	16.68	1470	2.88	29.85	41.31
N7-5	1.33	0.76	1.02	23.65	1052	3.55	26.79	37.07
N8-1	2.20	1.28	1.83	17.25	1775	3.47	45.80	63.38
N8-5	1.36	0.82	1.00	26.63	1128	4.01	32.74	45.30
N8-10	2.43	1.51	1.98	18.10	2090	3.41	49.93	69.09
N9-3	2.64	**1.63**	**2.27**	14.83	**2259**	3.57	**60.34**	**83.08**
N9-4	1.59	0.97	1.22	23.48	1339	3.91	37.67	52.13
N12-18	1.41	0.83	1.10	22.35	1149	3.46	28.74	39.78
N13-1	2.29	1.38	1.84	*14.80*	1910	3.30	44.49	61.57
O16-1	1.76	1.00	1.33	24.80	1387	3.26	32.30	44.69
P3-11	1.48	0.92	1.09	27.58	1270	4.07	38.28	52.98
P10-3	2.13	1.30	1.80	15.80	1799	3.01	39.20	54.25
P10-4	*1.00*	*0.60*	*0.73*	26.08	*830*	**4.22**	25.28	34.99
P10-5	1.44	0.88	1.17	19.60	1221	2.84	26.23	36.30
P11-1	1.58	0.93	1.17	24.89	1287	3.68	34.34	47.52
LSD	0.75	0.50	0.68	9.21	686	0.98	19.03	26.34

[1] Numbers in bold are the largest values for the column and numbers in italics are the lowest values for the column.

BREEDING IMPROVEMENTS OF LESQUERELLA

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PROBLEM: Seed oil yields of *Lesquerella fendleri* (Gray) Wats., *Brassicaceae*, a potential oilseed crop native to the Southwestern United States, cannot yet compete with similar imported oilseeds. The seed oil from lesquerella contains hydroxy fatty acids (HFA), comparable to castor. Imports of castor oil and derivatives amount to more than 65,000 tons per year at a value exceeding $100 million per year. Unique properties of lesquerella oil along with coproducts are promising for commercialization if yields are improved.

Other species of *Lesquerella* produce higher quantities of HFA than *L. fendleri,* but none are as productive in seed yield. There are more than 70 species from the western half of the U.S. that do not cross-pollinate in the wild. Traits from these species could be incorporated into *L. fendleri* to improve yields. There is also a high amount of diversity within *L. fendleri* that could be utilized for plant improvements. Public releases of seed have been made in the past by this laboratory with higher oil and lesquerolic acid contents and reduced oil pigmentation.

Only limited amounts of seed from germplasm collections are able to be obtained from the wild. Seed increases, evaluation, and passport information are necessary to utilize these accessions successfully in our breeding program. It is also necessary to make seed available to other researchers through the National Plant Germplasm System.

APPROACH: Hybrids were produced between *L. fendleri* and one of four other species from greenhouse controlled crosses (Table 1). These species were chosen based on a higher HFA content compared to *L. fendleri*. Embryo rescue was necessary since seeds did not form from the crosses. Some plants resulting from these crosses were sterile, possibly due to some incongruity between the two species. Fertility was restored by chromosome doubling using the chemical colchichine. The order of our procedures to obtain plants for greenhouse crossing was embryo rescue, shoot culture, colchicine treatment, and root culture.

Half-sib selections for high oil content were planted at Maricopa Agricultural Center and re-selected for both high oil and seed yield. The number of mass selected individuals was increased this year from 500 to 1300 plants to obtain more variability. The selections originated from a previously released line, WCL-LY2.

Seeds originating from a past collection trip in Mexico were field or greenhouse grown at the U. S. Water Conservation Laboratory (USWCL), Phoenix, Arizona, for seed increase and evaluation. When only limited seed quantities were available, seeds were started in the greenhouse in October and transplanted into the field in November and December. When plants began to flower, screen cages were placed over individual field plots and supplied with housefly larvae which then emerged for pollination. The pollinators within cages prevented cross pollination with other accessions. Plants were also grown in greenhouses if the accession of a species was not adaptable to an Arizona climate. Flies were also placed in the greenhouses on a weekly basis during the flowering period.

Plant growth measurements were taken throughout the season; and after harvest, seeds from each accession were analyzed for oil content and composition.

FINDINGS: F_1 interspecific hybrids had traits such as leaf shape, trichomes, stem thickness, and flower color that were intermediate of the parental plants. Although parental crosses were done via bud pollinations and siliques formed, less than 0.1% seed amounts were produced. Embryo rescue increased the number to 75%, depending on the cross. Colchicine applied at a rate of 0.1% to shoot meristems and cultured for two days was successful in producing fertile hybrids. When *L. fendleri* was crossed to one of the four species, the F_1 hybrid had the same HFA content as *L. fendleri* maternal parent instead of a mid-parent value. When the reciprocal was crossed, the hybrid had the same HFA content as the other species used as the maternal parent. This indicated that HFA content is maternally inherited.

Table 1. Chromosome number, oil, lesquerolic, and auricolic acids of *L. fendleri* and four other species used for interspecific crosses.

Lesquerella species	*n* = x	Oil content (%)	Lesquerolic acid (C20-1OH) (%)	Auricolic acid (C20-2OH) (%)
fendleri	6	23.6	50.2	trace
lindheimeri	6	21.6	81.7	0.35
pallida	6	na	81.4	3.68
gracillis	6	28.9	68.8	trace
auriculata	8	33	8.1	17.4

Seed oil content averaged 29.0% for the 1300 randomly selected plants. Unselected plants averaged 23.6% for oil contents. The top 150 plants were selected from this population and bulked together for next season planting. This population averaged 34.09% for oil content.

Two new germplasm lines developed here were released to the public this year. WCL-LY2 has high oil content and seed yield compared to the previous released line and WCL-SL1is salt tolerant.

Forty-eight accessions from 12 species were increased this year at the USWCL. Some of these are shown in Table 2. A single plant of *L. fendleri* growing in the evaluation plots, A4005, segregated for cream-colored flowers. This has never been reported for this species. All other flowers are yellow. It could be a useful phenotypic marker for breeding.

Following harvest, seeds of increased accessions were sent to the ARS Curator in Parlier, California, to be entered in the National Plant Germplasm System. A service contract with a cooperator in Mexico provided more accessions from areas that were not able to be collected last year because of lack of seed on plants, or there was not enough rain for plants to reach the full flowering cycle. Seed was obtained from these accessions and other localities not previously visited.

Table 2. Results of evaluation of some of the *Lesquerella* species increased and evaluated at the

USWCL, 1999-2000.

	Collection number	Species	Date of 1st flower	1000 Seed wt. (g)	Oil (%)	Hydroxy fatty acid (%)	Seeds per silique	Plant height (cm)	Plant width (cm)
1	A3342	*argyraea*	12/09	1.30	18.39	57.84	17.75		
2	A4004	*argyraea*	01/16	0.51	19.29	55.48	18.7	18.4	43.5
3	A4014	*argyraea*	01/25	0.50	20.26	53.18	8.7	26.2	51.6
4	A4030	*argyraea*	12/27	1.05	17.19	59.05	10.7	10.7	57.1
5	A863	*argyraea*	03/30	1.00	26.44	24.64			
6	A2401	*douglasii*	01/18	1.38	19.73	43	2.16		
7	A2402	*douglasii*	01/19	1.20	9.83	38.05	1.12		
8	A2403	*douglasii*	01/18			48.04	1.16		
9	A3343	*fendleri*	01/31		17.64	51.54	3.12		
10	A4001	*fendleri*	01/12	0.69	21.61	55.48	11.7	22.8	46
11	A4002	*fendleri*	01/31	0.56	24.82	54.47	17.7	23.9	45.5
12	A4003	*fendleri*	02/11	0.58		55.07	13.7	23.8	65.1
13	A4005	*fendleri*	01/30	0.71	22.7	55.33	13.4	25.9	51.7
14	A4006	*fendleri*	01/31	0.57	22.69	55.86	14.3	29.3	73
15	A4007	*fendleri*	01/19	0.53	25.1	55.7	16.2	26.5	59.4
16	A4015	*fendleri*	03/09	0.70	21.93	52.28	7.6	18.6	27
17	A4016	*fendleri*	02/17	0.61	20.23	53.05	8.7	18.1	22.7
18	A4024	*fendleri*	01/27	0.63	22.49	56.58	16.7	13.4	33.6
19	A4027	*fendleri*	03/20	0.67	19.99		7	14.3	22.4
20	A2217	*lasiocarpa*	12/10	0.69	27.51	31.51	20.4		
21	A2217	*lasiocarpa*	01/31	0.81	26.10	53.25	22.5		
22	A2228	*lasiocarpa*	01/25	0.46	23.61	50.26	20.7		
23	A2232	*lindheimeri*	12/21	1.00		83.24	12.1		
24	A3344	*mexicana*	03/20	1.22	12.89	56.21			
25	A1859	*pinetorum*	01/31			9.53	1.9		

26	A1853	*purpurea*	01/08			27.21	1.98		
27	A4020	*schaffneri*	02/24					6.7	18.4
28	A640	*wardii*	01/06	1.58	18.08	33.11	1.98		
29	AS741	*wardii*	12/20	1.58	16.21	19.19	4.74		

INTERPRETATION: These are the first confirmed interspecific hybrids of *Lesquerella* species native to the Western U.S. Since hybrids had the same oil profile as the maternal parent, species other than *L. fendleri* with high HFA contents were used. Hybrids have HFA contents of more than 80%. Many of the other traits from these species are not desirable and will need to be bred out. This will be done through backcrossing the hybrid with *L. fendleri* over several generations until the hybrid has most characteristics of *L. fendleri* except for the HFA contents. This has the potential to reduce the cost of the seed oil drastically from about $3 to $1 per pound and to compete with castor effectively in the marketplace.

The 150 selected plants for oil content and seed yields had some plants with oil contents above 39%. This is the first year we have seen plants above 37%. This may be due to recombination of favorable alleles and/or a higher probability of finding those plants with the larger selection of plants. Over an eight-year period, there has been a 10% increase in oil content. With larger sample sizes, we may be able to reach 40% within five years.

Breeding *L. fendleri* with wild relatives may yield offspring that bear bigger seeds with more oil and higher amounts of hydroxy fatty acid. It may also expand the growing region outside the Southwest U.S. Special care must be taken to assure that seed is increased without contamination from other accessions, evaluated to obtain usable information about the accession, and properly handled from harvest to storage. The seed deposited into National Plant Germplasm System benefits researchers nationally and internationally. It also has a long-term benefit to our breeding program.

FUTURE PLANS: Interspecific hybrids are being backcrossed to *L. fendleri*. High oil lines will be used as the donor pollen parents. We have some hybrid plants that have already been backcrossed once (BC_1F_1) and will be backcrossed again this season. Others will be backcrossed for the first time. Internal transcribed spacers (ITS) molecular markers are being used to confirm hybridity and are especially useful to distinguish at the species level. These utilize the 18S - 26S nuclear ribosomal DNA region. Taxonomic information, such as leaf trichome descriptions, number of ovules, and flower petal length, is also being collected. Chromosome counts of colchicine hybrids have started.

The selection within *L. fendleri* for high oil will continue with increased sample numbers. Further germplasm releases are anticipated. Seed increase and evaluation of seed sent this year from Mexico will be evaluated this coming year.

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