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FACE: Free-Air CO_2 Enrichment for Plant Research in the Field

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Chapter 15

Evaluating Cotton Response to Free-Air Carbon Dioxide Enrichment with Canopy Reflectance Observations

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I. INTRODUCTION

Most approaches for monitoring plant growth and development have serious drawbacks which limit their use in agricultural research programs. First of all, traditional methods for measuring above-ground green leaf area index (LAI) and biomass are very labor intensive, tedious and time consuming to execute. The size, number and frequency of samples required to characterize the plant canopy and detect statistically significant differences between treatments are usually large and often not practicable because of constraints on labor and space for cold storage and oven drying. Destructive sampling techniques are usually not appropriate in small research plots because the removal of some individuals from the population alters the response of remaining plants. Furthermore, the time required for physically separating leaves from stems and measuring them with a leaf area meter or waiting for tissues to dry in an oven precludes the use of these data in real-time decision making processes. Thus, important information that has immediate utility for assessing the efficacy of different treatments, identifying incipient problems, or guiding future sampling efforts is typically not available until several days or weeks after the samples have been collected.

Non-invasive, remote sensing techniques which use plant canopy reflectance to monitor changes in canopy condition avoid these problems while providing scientists with an efficient tool for research and management of their projects. The physical basis for this approach derives from the contrasting spectral reflectance properties of plant canopies and the underlying soil. Healthy green plant foliage typically has a very low reflectance (2–5%) in the visible portion of the electromagnetic spectrum and high reflectance (50–60%) in the near-infrared (NIR). By contrast reflectance of visible light from many soils may be an order of magnitude greater than that from green plants, while in the NIR it might be only half that of the plants.

Differences in reflectance characteristics of plants and soils can be enhanced by computing spectral vegetation indices (VIs) which are combinations of reflectances in two or more waveband intervals. VIs can assume the form of simple band ratios, normalized differences between bands or linear combinations of bands which have been optimized to yield specific information about a plant canopy (Tucker 1979, Richardson and Wiegand 1977, Jackson 1983). The ratio of NIR/Red reflectances or a normalized difference between these bands [(NIR-Red)/(NIR+Red)] are common examples of VIs that are responsive to canopy LAI. Although band ratio VIs are particularly sensitive to changes in the angle of solar irradiance and view angle of the sensor (Pinter *et al.* 1990), they retain much of their information about the plant canopy under conditions of varying surface soil moisture, topography, illumination intensity, and intermittent cloud cover (Jackson *et al.* 1980, Pinter *et al.* 1987). These advantages are particularly important if remote measurements of canopy reflectance are to be used routinely as reliable estimates of plant response throughout the growing season.

Several spectrophotometric studies have examined the relationship between reflectance in visible and NIR wavelengths and the water relations, chloride concentration and pigment content of individual detached, cotton leaves (Thomas *et al.* 1967, 1971; Thomas and Gausman, 1977; Bowman, 1989.) Significant correlations were usually found when extreme conditions were included in the analysis. These authors point out that natural variation caused by other factors such as leaf age, interactions of light with multiple leaves within a canopy, etc. will prevent the use of detailed single leaf reflectance *per se* for detecting stress under more moderate and physiologically meaningful conditions.

Subtle differences in daily plant water status however, are ultimately expressed as relatively large differences in plant growth, ground cover, canopy architecture and development of leaf area. Thus at the level of the entire canopy, agronomic parameters are more amenable to detection by remote means. Thomas *et al.* (1967) for example, successfully used aerial infrared photography to estimate the yield of cotton subjected to varying levels of moisture stress during growth. Henneberry *et al.* (1979) demonstrated that aerial color infrared photography could detect effects of growth regulators, fertilizer treatment and disease in cotton. In 1984, Kimes *et al.*, characterized the directional reflectance characteristics of a long staple cotton canopy, *Gossypium barbadense* L., showing the importance of solar illumination angles and viewing direction on measured values. Jackson and Ezra (1985) made frequent reflectance measurements over a cotton canopy which was abruptly deprived of water. They attributed the significant changes in spectral response to changes in canopy architecture caused by severe wilting of the leaves. Huete (1985) then examined the reflectance of a developing upland cotton canopy (*G. hirsutum* L.) and related it to the spectral response of background soil conditions. Results of these investigations form the basis for using canopy reflectance for non-destructive monitoring of cotton growth and development during the season.

The experiment we describe here was designed to evaluate multispectral reflectance techniques for monitoring season-long cotton plant response to ambient and enriched carbon dioxide concentrations during 1989. Observations were made using a handheld radiometer configured to measure canopy reflectances in three visible and one NIR wavelength interval. Our objectives were: 1) to determine the feasibility of using a ground-based, handheld system for measuring spectral reflectance factors of a moderately tall, row crop; 2) establish relationships between plant characteristics and VIs calculated from canopy reflectances of CO_2 enriched and ambient cotton; and 3) to monitor the temporal response of cotton to CO_2 enrichment via multispectral VIs.

II. METHODS

The reflectance measurements were carried out within the experimental framework provided by the 1989 large scale FACE Experiment (*F*ree-*A*ir *C*arbon dioxide *E*nrichment). The experiment was conducted on upland cotton (*G. hirsutum* L. cv Delta Pine 77) grown in a 37 ha field on the demonstration farm at the University of Arizona's Maricopa Agricultural Center (MAC) located approximately 40 km south of Phoenix, AZ (33.075°N 111.983°W). The surface soils were classified as a reclaimed Trix clay-loam (fine-loamy, mixed (calcareous), hyperthermic Typic Torrifluvents). The field had been fallow for the previous 3 years and was in cotton production for approximately 10 years prior to that. Seeds were planted on 17 April 1989 in east/west oriented rows spaced at 1-m intervals. Plants within measured areas were thinned to approximately 100,000 ha^{-1} on 1 May. Water application to the entire field was by subsurface micro-irrigation (drip irrigation). Other cultural operations and agrichemical treatments [including aerial application of Pix (a commercial growth regulator), insecticides and defoliants] were applied according to local practices by farm personnel. Reflectance observations and plant samples were taken from within 20 m-diameter, open field, circular arrays (enriched during daylight hours with 550 µmol mol^{-1} CO_2) and paired, open field, control canopy areas (with ambient CO_2 levels). Additional details on experimental design, CO_2 delivery system and actual CO_2 levels during the season are provided by Lipfert *et al.* (1991) and in companion chapters in this book.

A. Destructive Plant Sampling

Agronomic characteristics were estimated from 7 sets of destructive plant samples, beginning 30 May 89 and subsequently obtained at biweekly intervals until 23 August 89. The sampling routine consisted of removing every third cotton plant from a 3-m section of row in a predetermined location within the 550 µmol mol^{-1} treated arrays and ambient controls. This was equivalent to sampling all the plants in 1.0 m^2 area, but did not leave exaggerated bare spots that would seriously impact aerodynamics of CO_2 flow in the canopy. This procedure usually yielded 9 or 10 plants per sample on each sampling date. The number of fruiting structures and the dry biomass of leaves, stems, fruit and roots were determined on all plants. Before drying, a subsample, consisting of all the green leaves from one of the plants, was measured using an optical leaf area meter. The total leaf area of the entire sample was then estimated using the area from the single plant subsample, it's associated wet biomass, and the wet biomass of the other plants in the sample. LAI was computed as the total leaf area of the sample (m^2) divided by the ground surface area (m^2) subtended by the sample.

B. Reflectance Measurements

Canopy reflectances were measured using an Exotech Model 100A[1] portable radiometer equipped with 15° field-of-view optics and spectral bandpass filters spanning 3 visible and one NIR wavelength intervals. Spectral filters were similar to the blue channel (0.45 to 0.52 µm) on LANDSAT 5 and the green (0.50 to 0.59 µm), red (0.61 to 0.68 µm), and NIR (0.79 to 0.89 µm) channels on SPOT satellite platforms. The radiometer was deployed along a permanent north-south transect which crossed 12 cotton rows in four replicate plots having the 550 µmol mol^{-1} treatment and 4 replicates of the ambient CO_2 control. The radiometer was extended at arm's length towards the east and held approximately 1.5 m above the soil surface. Data were collected at a rate of four, evenly-spaced measurements per row of plants. The sensor was always pointed in a nadir direction. Thus each lens viewed an area that was approximately 0.26 m in diameter when the plants were 0.5 m in height.

Multispectral observations were made 2 to 4 times each week from 17 May until 20 September 89 (40 data sets). Forty eight measurements in each wavelength interval were combined to yield an average reflectance for each transect. The entire measurement sequence over 8 transects (4-550 µmol mol^{-1} arrays and 4-ambient controls) required approximately 25-35 minutes to complete. To minimize the effects of changing solar zenith angle as the season progressed, observations were centered on a morning time period corresponding to a solar zenith angle of 45°. The solar azimuth during measurements varied from a minimum of 87° in June to a maximum of 134° in September; the mean azimuth was 100°. Qualitative observations of weather, sky conditions and canopy appearance were recorded at time of data collection.

Analog signals from the radiometer were recorded on a portable data acquisition system (Polycorder[1], Omnidata International, Inc.) which also registered the time when measurements were taken. Single band reflectance factors were calculated as the ratio of radiant excitance measured over each cotton target to irradiance inferred from a time-based linear interpolation of data collected at 15-minute intervals over a 0.6 by 0.6 m, horizontally positioned, calibrated, painted $BaSO_4$ reference panel. Correction factors were applied

[1] Trade names and company names are included for the benefit of the reader and do not constitute an endorsement by the U. S. Department of Agriculture.

to the $BaSO_4$ data to compensate for its non-lambertian reflectance properties at a solar zenith of 45° (Jackson *et al.* 1987). The NIR/Red spectral vegetation index was computed as the NIR reflectance divided by red reflectance. The normalized difference (ND) was computed as the difference of NIR and red reflectances divided by their sum.

III. RESULTS AND DISCUSSION

A. Agronomic Characteristics

Above-ground, dry biomass accumulated in a fairly linear fashion from 30 May (DOY150) until 23 August (DOY235; Figure 15-1), increasing more rapidly in the 550 μmol mol^{-1} CO_2 treated cotton than the ambient controls. Most of this difference was caused by larger boll numbers and heavier bolls and stems in the CO_2 enriched treatment (data not shown). Green LAI increased in a typical manner during the first part of the season. In direct conflict with destructive samples however, our visual observations from mid-July until late-August suggested that there were large differences between LAI of enriched and ambient cotton. During this period, spider mite populations (*Tetranychus* sp.) built to economically damaging levels in the higher CO_2 treatment (see chapter by Akey in this book). We believe this caused the extensive, atypical leaf shedding and slowing of new growth which appears in LAI data shown in Figure 15-1. Spider mites did not reach damaging levels in the ambient controls. Indeed, our visual assessment of the controls revealed those plants were still growing rapidly and leaf area continued to increase on DOY208 and 221 instead of declining as the data in Figure 15-1 indicate. The discrepancy between visual observations and destructive samples in the ambient CO_2 treatment may have been caused by the relatively small number of plants in each sample and the fact that they may not have been representative of the control area as a whole.

B. Soil Reflectance

Bare soil reflectances were measured routinely in the experimental field on a 1 by 1 m patch of sunlit soil adjacent to the cotton canopy. Reflectances for each waveband are shown in Figures 15-2, 15-3, 15-4, and 15-5 for 23 mostly clear days when cloud cover was less than 20% and not present within 20–30° of the sun. Reflectance factors of the clay loam soil increased with wavelength, averaging about 7-8% in the blue, 10-11% in the green, 15-16% in the red and 20-25% in the NIR. The day-to-day variation displayed in each waveband was caused principally by differences in surface moisture conditions. In the red, for example, reflectances dropped abruptly to 12% on DOY181 (Figure 15-4) when capillary action moistened the soil surface above the subsurface drip line. Reflectances in all wavelengths increased gradually after DOY212 as the surface soils became progressively drier.

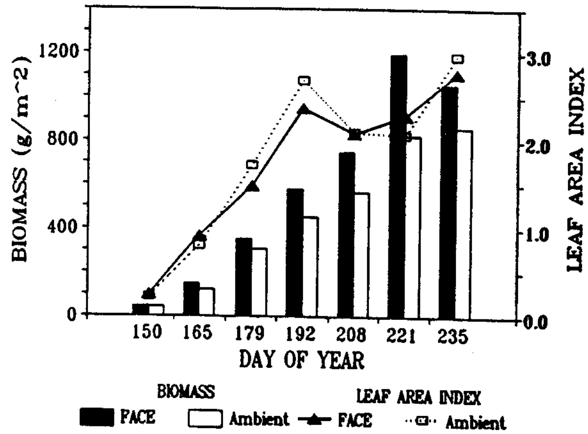

FIGURE 15-1. Agronomic data from destructive cotton samples in the 1989 FACE experiment.

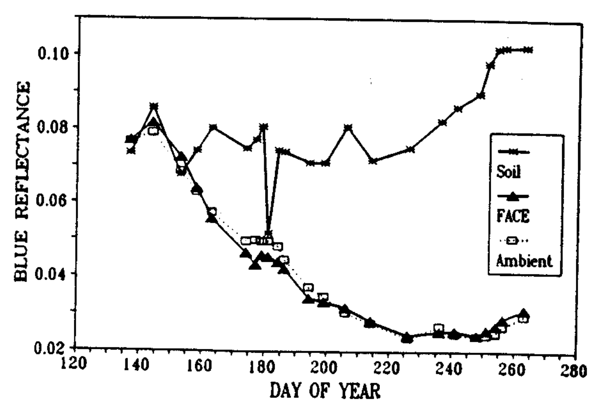

FIGURE 15-2. Reflectance factors from soil and cotton in the blue (0.45 to 0.52 μm) wavelength interval. Data are for clear days only.

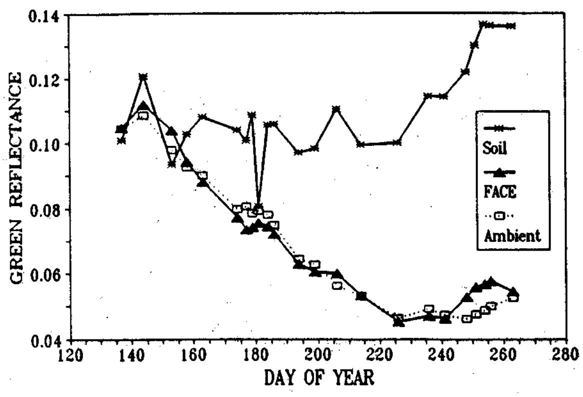

FIGURE 15-3. Reflectance factors from soil and cotton in the green (0.50 to 0.59 μm) wavelength interval. Data are for clear days only.

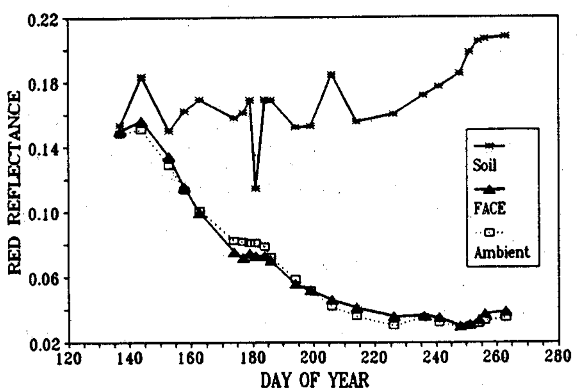

FIGURE 15-4. Reflectance factors from soil and cotton in the red (0.61 to 0.68 μm) wavelength interval. Data are for clear days only.

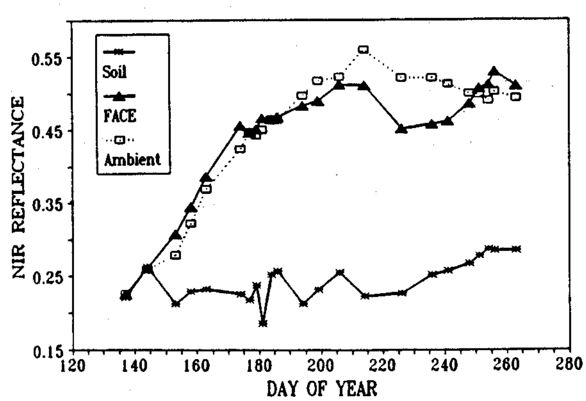

FIGURE 15-5. Reflectance factors from soil and cotton in the NIR (0.79 to 0.89 μm) wavelength interval. Data are for clear days only.

C. Canopy Reflectance

Reflectance factors for 550 μmol mol^{-1} and ambient CO_2 cotton are also shown in Figures 15-2, 15-3, 15-4, and 15-5 for clear days. Data in these and subsequent figures represent mean values from the three replicate treatments having similar CO_2 enrichment regimes and means from three matched ambient CO_2 controls. Reflectances from replicate 4 were not included because the CO_2 in that array was not turned on until several weeks after measurements had begun. Initially, reflectances from the treated and ambient cotton were very similar to the bare soil. Then as the canopy developed and covered more of the soil, the spectral characteristics of the soil were dominated by reflectances typical of vigorous green foliage. By early August, reflectance in the visible portion of the spectrum decreased to between 2 and 5%, while in the NIR it increased to more than 50%.

CO_2 treatment differences were apparent in each wavelength interval. In the three visible wavebands (Figures 15-2 to 15-4), cotton in the enriched treatment displayed slightly higher reflectances until the second week of June (DOY160). From then until mid-July (DOY200), visible reflectances from the enriched cotton were consistently lower but the differences were relatively small. Rather large differences between CO_2 treatments became evident in green wavelengths during September (Figure 15-3, after DOY248). This may have been caused by late season changes in pigments of individual leaves. During that time, large numbers of adult whiteflies, *Bemisia* sp., were also present in the upper portions of the canopy; the shiny honeydew they deposited on the upper surfaces of leaves may have caused a specular increase in visible reflectance.

In the NIR (Figure 15-5), differences between CO_2 treatments were more conspicuous and opposite from those observed in the visible wavelengths (Figures 15-2 to 15-4). NIR reflectances from the enriched plants were several percent higher than ambient controls during three weeks in June (DOY153 until DOY175). These data were consistent with larger and more vigorous plants in the enriched treatment during that period. For several weeks, NIR reflectances of both treatments were similar. Then, after mid-July

(DOY193) enriched plants displayed lower NIR reflectance factors. By mid-August (DOY226), NIR measured in the enriched canopy was fully 7% lower than the ambient controls, supporting the visual impression of substantial canopy decline in the 550 μmol mol^{-1} treatment.

D. Spectral Vegetation Indices

The season long trajectories of the NIR/Red and ND are shown in Figures 15-6 and 15-7 for the bare soil, enriched and ambient cotton targets. These data are smoother than the reflectance factor data (Figures 15-2 to 15-5) because band ratioing techniques suppress variation in single band reflectances caused by day-to-day changes in illumination intensity and cloudy skies (Pinter *et al.* 1987). As a result all 40 days of spectral data are shown in Figures 15-6 and 15-7. While both VIs enhanced the contrast between bare soil and the cotton canopy, the NIR/Red ratio was particularly effective at minimizing variation in soil reflectance caused by changes in surface moisture content on DOY181.

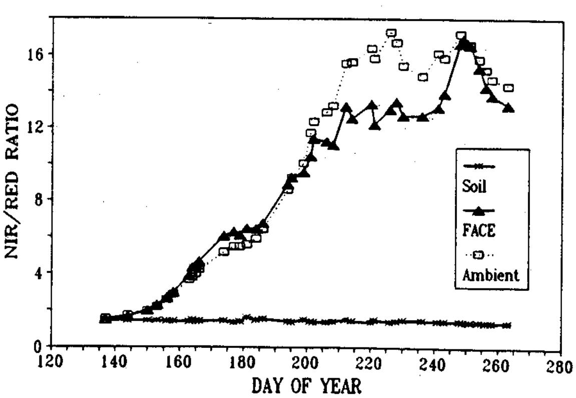

FIGURE 15-6. Season-long NIR/Red vegetation index measured during the 1989 FACE experiment. This figure also includes data collected on cloudy days.

The NIR/Red and ND differed in their ability to portray changes in canopy density at various times of the season. The NIR/Red (Figure 15-6) increased slowly with the gradual increase of ground cover by the developing canopy. By the beginning of July, the NIR/Red in the ambient

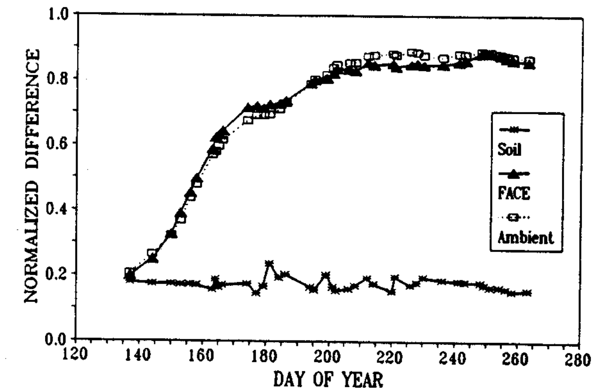

FIGURE 15-7. Season-long normalized difference vegetation index measured during the 1989 FACE experiment. This figure also includes data collected on cloudy days.

CO_2 canopy was between 5 and 6 units, only one third of the maximum value of 17 units observed six weeks later in mid-August (DOY228). The ND however, increased more rapidly at the beginning of the season (Figure 15-7). By early July, the ND had already attained approximately 75% of its maximum value. The asymptotic nature of ND relationship with canopy development was evident in data collected after the last week in July (DOY210). The ND did not change much from that time until measurements were terminated in mid-September. The NIR/Red however, showed distinct differences between CO_2 treatments from DOY210 to 248 and then declined sharply after DOY249. We suspect that this late-season decline was caused by increases in red reflectance due to pigment changes associated with leaf senescence or the shiny honeydew residue mentioned earlier.

The NIR/Red VI appeared more useful than the ND when related to experimental variables during mid- and late-season. To further emphasize the effect of CO_2 treatment, the NIR/Red measured over the ambient cotton was subtracted from the NIR/Red from the enriched cotton and graphed versus day of the year (Figure 15-8). Large positive values were apparent during June and early July (DOY165 to 185), when the 550 μmol mol^{-1} CO_2 treatment was enhancing growth. Later, during most of July and August when leaf drop and spider mite infestations precipitated a dramatic decline in the visual appearance of the enriched cotton, large negative values were observed.

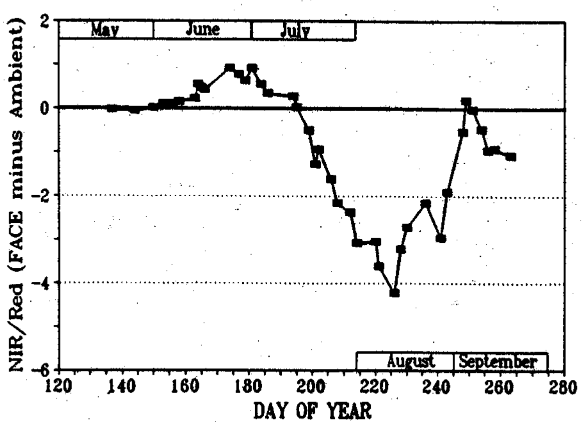

FIGURE 15-8. The difference between the NIR/Red vegetation index measured in the FACE (550 µmol mol^{-1} CO_2) treatment and the ambient CO_2 controls versus day of the year. This figure also includes data collected on cloudy days.

The NIR/Red difference (Figure 15-8) was also very responsive to canopy regrowth following various management procedures applied to the cotton (see chapter by Mauney *et al.* in this book). As an example, foliar nutrients were sprayed on both the enriched and ambient cotton on 9 August (DOY221) after tissue sampling confirmed plants in the enriched treatment had suboptimum nutrient status. At about the same time, the micro irrigation system was changed from subsurface to surface lines capable of delivering a more reliable supply of water to the plants. The plants in the enriched treatment responded almost immediately. The sharp reversal in the NIR/Red difference after 14 August (DOY226) was associated with observations of marked regrowth in the canopy.

By the first week in September (DOY249) both the enriched and ambient cotton canopies were similar from a spectral VI standpoint, then they began to diverge again. Assuming that the general decrease in NIR/Red during this time was caused either by pigment changes or honeydew deposits, then the faster decrease in the NIR/Red suggests that these changes or damage were greater in the enriched treatment. More research is needed to clarify these late season spectral phenomena.

Finally, there appears to be a correlation between daily changes in VIs and spraying of Pix, a commercial growth regulator intended to suppress vegetative canopy development and enhance reproductive development. Close inspection of Figures 15-6 and 15-7 reveals two transient plateaus in the overall increase of NIR/Red and ND during the early and middle portion of the season. The first, between DOY174 and DOY186 is most conspicuous. It follows the aerial application of Pix (1,1-dimethyl-piperidinium chloride; 0.58 l ha^{-1}) to the entire field on DOY173. The second plateau is just a momentary blip (DOY195 to 198) which can only be detected in the enriched cotton, but it also follows a second application of Pix on DOY192. The timing of the two Pix applications and subsequent changes in VIs may be coincidental. However, if a growth regulator acts as intended, spectral vegetation indices should be capable of monitoring its effect.

E. Predicting Biomass and LAI from VIs

Biweekly samples of cotton plants from enriched and ambient CO_2 treatments provided an opportunity to test the hypothesis that spectral vegetation indices were a function of agronomic characteristics of the canopy. Average biomass and LAI data for cotton within each array and ambient control were paired with spectral VIs measured in the same replicate on the same day. Spectral data were interpolated linearly for two plant sampling dates when reflectance data were not taken. Results for the NIR/Red versus biomass are shown in Figure 15-9. They suggest that the relationship between NIR/Red and biomass was dependent upon CO_2 treatment during the 1989 FACE experiment. This is because the ratio of leaf area to total biomass was different in the two treatments, especially during the middle portion of the growing season when the larger plant samples were obtained (Figure 15-1, DOY208 to 235). A nadir viewing radiometer viewed mostly leaves in the ambient canopy during this time and a higher vegetation index was obtained. In the enriched cotton however, many of the leaves had been shed and the index was more strongly influenced by the spectral characteristics of the stems and bolls. A similar phenomena was observed in alfalfa, where selective grazing by sheep stripped leaves from plant stems and decreased the NIR/Red index without appreciably reducing biomass (Mitchell *et al.*, 1990).

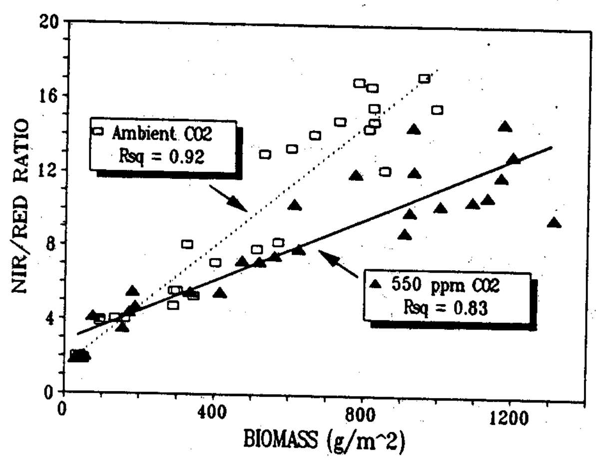

FIGURE 15-9. Least squares linear regression between NIR/Red vegetation index and biomass of FACE (550 µmol mol⁻¹ CO_2) and ambient CO_2 controls.

The relationship between green LAI and NIR/Red measured during the experiment yielded results that were much more variable than those for biomass (Figure 15-10). They imply that similar relationships exist for both CO_2 enriched and ambient cotton plants. Because of the discrepancy between visual observations of the canopy and the destructive plant samples discussed earlier in this chapter, the data of both Figures 15-9 and 15-10 should be viewed as preliminary until verified with additional measurements.

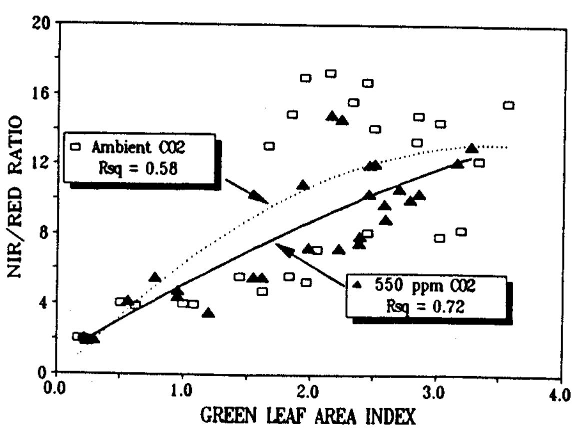

FIGURE 15-10. Least squares regression between NIR/Red vegetation index and green leaf area index of FACE (550 µmol mol⁻¹ CO_2) and ambient CO_2 controls.

IV. CONCLUDING REMARKS AND NEEDS FOR ADDITIONAL RESEARCH

Canopy level observations of single band reflectance factors and multispectral vegetation indices were used to monitor the response of cotton to elevated CO_2 concentrations during the 1989 FACE experiment. These data were sensitive to the amount and condition of green plant material viewed by the radiometer, revealing foliage response to mite and insect pressure, growth regulating chemicals and nutrient conditions. Differences in plant growth, leaf area and biomass between cotton in enriched and ambient CO_2 treatments were evident in season-long vegetation indices. This information was not degraded appreciably when measurements were taken under cloudy sky conditions. Because of the speed and facility with which measurements could be taken and processed, we found them useful for augmenting less frequent destructive plant sampling procedures and very practical for monitoring and supervising the experiment in a near-real time capacity.

Faced with the prospect of rising levels of atmospheric CO_2, additional research is needed to verify relationships between spectral data and agronomic parameters of CO_2 enriched plants. We found significant correlations between the NIR/Red index and cotton biomass; its relation with leaf area index was weaker but still significant. Our preliminary data suggest that the relationship between biomass and spectral vegetation indices is a function of CO_2 concentration in the plant environment. Such findings will affect interpretation of VIs acquired via satellite sensors and used to estimate carbon balance on a global scale in the future (*eg.* see Tucker, *et al.* 1986). Upcoming research in FACE experiments will address the relationship between spectral VIs and photosynthetically active radiation absorbed by the canopy and examine the effect of CO_2 on high resolution reflectance and absorption spectra of single leaves.

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