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Effect of free-air CO_2 enrichment on the chlorophyll content of cotton leaves

P.J. Pinter, Jr*[,a], S.B. Idso[a], D.L. Hendrix[b], R.R. Rokey[a], R.S. Rauschkolb[c], J.R. Mauney[b], B.A. Kimball[a], G.R. Hendrey[d], K.F. Lewin[d], J. Nagy[d]

[a] *US Water Conservation Laboratory, 4331 East Broadway Road, Phoenix, AZ 85040, USA*
[b] *Western Cotton Research Laboratory, Phoenix, AZ 85040, USA*
[c] *University of Arizona, Maricopa Agricultural Center, Maricopa, AZ 85239, USA*
[d] *Brookhaven National Laboratory, Bldg. 318, Upton, NY 11973, USA*

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Abstract

In vivo chlorophyll concentrations were estimated using a Minolta SPAD 502 meter on upper-canopy leaves of cotton plants exposed to air enriched to an atmospheric CO_2 concentration of approximately 550 μmol mol^{-1} in a free-air CO_2 enrichment (FACE) study. Measurements were made on 27 days during the final 90 days of the 1991 growing season. In both well-watered and moderately water-stressed plants, leaves in the FACE plots had greater chlorophyll *a* concentrations than leaves in the ambient air control plots (about 370 μmol CO_2 mol^{-1}): season-long chlorophyll *a* averages were 7.1% greater in the 'wet' treatment and 8.2% greater in the 'dry' treatment. This finding differs from what has been observed in a number of studies where experimental plants were grown in small pots. It is, however, typical of what has been observed in studies employing larger pots and open fields, and is a compelling rationale for conducting additional studies of this nature in FACE projects.

1. Introduction

A number of studies have shown that the leaf nitrogen (N) concentrations of plants grown at elevated atmospheric CO_2 concentrations are often lower than those of plants grown in ambient air (Wong, 1979; Hocking and Meyer, 1985, 1991). A common explanation for this observation is that the increased carbon assimilation resulting from enhanced growth at high CO_2 simply dilutes plant N (Hilbert et al.,

* Corresponding author.

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1991), a phenomenon that has been observed to occur when other factors enhance plant growth as well (Greenwood et al., 1990). Consequently, as leaf chlorophyll content is tightly coupled to leaf N status (Evans, 1989), one would also expect leaf chlorophyll concentration to decline with atmospheric CO_2 enrichment, as has indeed been observed (Wulff and Strain, 1982; Houpis et al., 1988). In fact, one would expect the phenomenon to be universal. However, in the present study to investigate the effect of CO_2 enrichment on cotton leaf chlorophyll in a realistic field setting without microclimate artifacts imposed by chamber walls or limited rooting volumes, we observed just the opposite, i.e. leaf chlorophyll concentrations increased with atmospheric CO_2 enrichment.

2. Materials and methods

The experiment was conducted on cotton (*Gossypium hirsutum* L. cv. 'Deltapine 77') at the Maricopa Agricultural Center of the University of Arizona in a rural agricultural setting south of Phoenix, Arizona. An overview of the free-air CO_2 enrichment (FACE) experiments has been provided by Hendrey and Kimball (1994). In brief, CO_2 concentrations of approximately 550 μmol mol^{-1} were maintained from sunrise to sunset within the center sections (20 m in diameter) of four circular FACE manifolds (of 25 m diameter), with the plants being returned to ambient conditions at night. Four ambient CO_2 (approximately 370 μmol mol^{-1}) control areas of identical size were also maintained within the field. Each of these eight plots was split into two irrigation treatments: well-watered or 'wet' (typically provided with water on Monday, Wednesday and Friday to replenish measured potential evaporation since the last application of water) and moderately stressed or 'dry' (also provided with water on Monday, Wednesday and Friday, but with only two-thirds of that given the wet plots). Further details concerning CO_2 and water treatments and overall plot layout have been given by Lewin et al. (1994) and Mauney et al. (1994).

Leaf chlorophyll measurements of 36 upper-canopy leaves in each of the 16 plots were made on 27 mornings between 19 June and 16 September 1991 (day of year (DOY) 170–259) with a hand-held Minolta SPAD 502 chlorophyll meter (Minolta Corporation, Ramsey, NJ). This device measures the differential attenuation by leaves of light in wavebands centered near 650 nm (red) and 940 nm (near-IR) where absorption by chlorophyll is different. It has been found to be an excellent indicator of leaf N and chlorophyll concentrations when properly calibrated (Yadava, 1986; Marquard and Tipton, 1987; Schaper and Chacko, 1991; Monje and Bugbee, 1992; Wood et al. 1992). In fact, it has been found to work so well that Tenga et al. (1989) have suggested that its unitless readings may themselves constitute a useful response variable. Nevertheless, we performed a careful calibration of the meter on the crop we studied. Specifically, on 8 August 1990 (DOY 220), approximately halfway through our measurement program, six SPAD meter readings were obtained for each of six control leaves and six FACE leaves selected from both irrigation treatments and all four replicates in a similar manner to season-long morning readings. Six disks (of 0.95 cm diameter) were then removed from each of these leaves and extracted in 3 ml of 80% acetone containing 200 mg l^{-1} butylated hydroxytoluene. Disks were stored in this mixture for several weeks at −19°C. At the end of this period, the leaf tissues were pigment-free. Aliquots were then removed, and chlorophyll *a*, chlorophyll *b* and accessory pigments (carotenoids and xanthophylls) were determined according to the procedures of Lichtenthaler and Wellburn (1983). Least-squares regression techniques were used to develop predictive relations between pigment concentrations and SPAD meter readings (General Linear Models, GLM (Statistical Analysis Systems (SAS) Institute, Inc., 1985)).

3. Results

A single, highly significant ($P = 0.001$), linear model accounted for 69% of the variation in chlorophyll *a* in our SPAD calibration data set (Fig. 1). Differences in this relationship resulting from CO_2 and irrigation treatments were not statistically significant ($P > 0.05$). The R^2 for chlorophyll *b* vs SPAD readings (adjusted $R^2 = 0.58$; $Y = -3.94 + 0.191X$) and combined carotenoids and xanthophylls vs SPAD readings (adjusted $R^2 = 0.18$; $Y = 547 + 14.2X$) were lower, albeit still significant ($P = 0.01$).

Inasmuch as our calibration data set also spanned the range of average meter

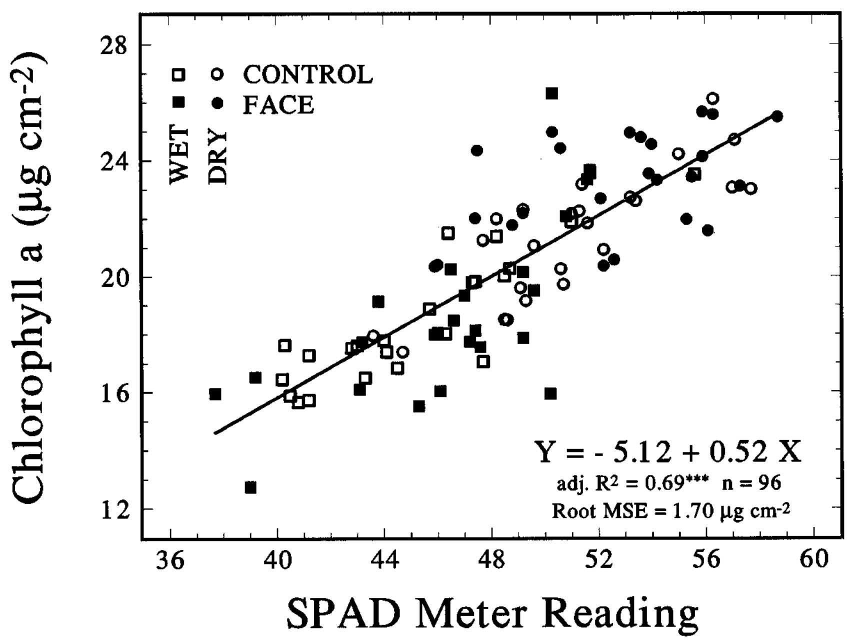

Fig. 1. Relationship between in vivo measurements of cotton leaves using the Minolta SPAD 502e meter and chlorophyll *a* concentrations determined from acetone extracts of disks from the same leaves. (*** indicates model significance at $P = 0.001$).

readings obtained in the field, we used the predictive equation of Fig. 1 to estimate chlorophyll *a* for the cotton plants during the season. Almost without exception, the dry irrigation treatment displayed higher chlorophyll *a* concentrations than the wet treatment (Fig. 2). In fact, the sharp decline in chlorophyll *a* between 2 July (DOY 183) and 16 July (DOY 193) reflects an increase in irrigation amounts delivered to the wet treatment during that time frame (Fig. 2). The difference related to irrigation was highly significant ($P = 0.01$) for 25 of the 27 measurement days (GLM procedure, SAS Institute Inc., 1985).

The effect of CO_2 on chlorophyll *a* concentration was more variable, and was perhaps confounded by cycling water status between irrigations. Nevertheless, leaves from FACE plants nearly always had higher concentrations than the leaves of control plants, and these differences related to CO_2 were significant ($P = 0.05$) on 11 days. When averaged over the entire period of measurement, the CO_2-induced enhancement of chlorophyll *a* as estimated with the SPAD meter was significant ($P = 0.05$): +7.1% in the wet and +8.2% in the dry treatment.

4. Discussion

How are these results to be explained? One possibility may be differential avail-

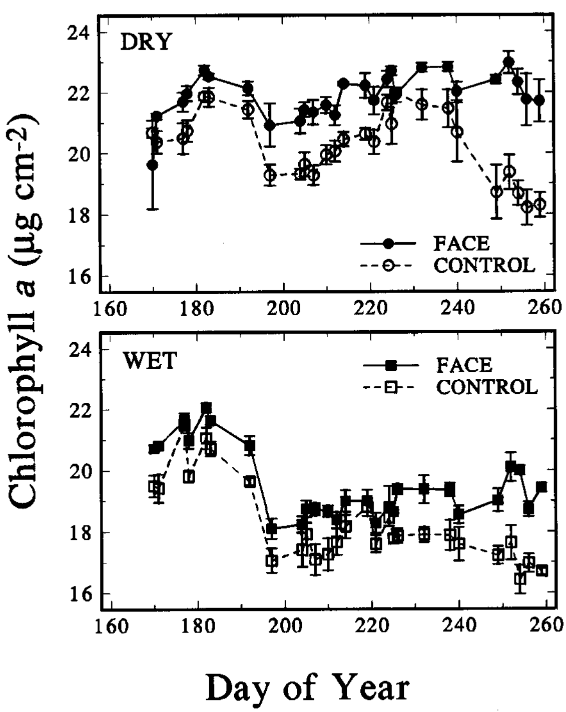

Fig. 2. SPAD-Predicted concentrations of chlorophyll *a* vs day of year for control and FACE cotton leaves in dry and wet irrigation treatments. Vertical bars depict ±SEM from four replicated subplots.

ability of soil N between the control and FACE plots caused by the previous (1990) season's crop N extraction patterns. However, to a depth of 120 cm, the beds of the control plots had more than three times the extractable nitrate-N available to them than did the FACE plots at pre-planting, whereas in the furrows they had twice as much (R. Rauschkolb, unpublished data, 1992). At the conclusion of the growing season, these ratios were 1.2 and 3.6. Hence, soil N status would have favored the development of more chlorophyll in the control plants and probably acted to reduce the differences we observed.

Another possible explanation is that the CO_2-enriched plants were more efficient at acquiring N and incorporating it into their chloroplasts, allowing them to meet the full potential of the opportunity for enhanced productivity provided by the elevated atmospheric CO_2 concentrations of the FACE plots (Pinter et al., 1994). Such an efficiency increase has been noted in several CO_2 enrichment experiments (Sionit, 1983; Goudriaan and De Ruiter, 1983; Hocking and Meyer, 1991), and there are even a few cases where the result has been a CO_2-induced increase in N concentration per unit leaf area (Lincoln et al., 1984; Overdieck et al., 1988; Peñuelas and Matamala, 1990), which would imply a similar increase in chlorophyll, owing to the tight coupling between these two factors (Evans, 1989). Although we did not specifically test N concentrations to verify this hypothesis, nutrient analysis was performed on upper-canopy cotton leaf blades on 17 July and 21 August 1991 (J.R. Mauney, unpublished data, 1992), during which period we also have specific leaf weights for representative, upper-canopy leaves. Results confirmed that N was not limiting in any treatment. Expressed in terms of unit leaf area, total N levels of the FACE treatment averaged 10% higher than controls, a result which is totally consistent with our hypothesis.

Arp (1991) has produced an analysis that helps to make sense of the wide range of results reported in the literature. On the basis of previously published studies, he plotted the CO_2-enriched/ambient ratio of leaf chlorophyll per unit area against the size of the pots in which the experimental plants were grown, and showed that this ratio rose from something considerably less than unity for pot volumes of the order of 1 l to one or more at a pot volume of approximately 6 l. His analysis further showed that for larger pot volumes or open field conditions, all of the ratios were either equal to or greater than unity. Our results are fully compatible with Arp's review of this subject, and lend additional support to the growing body of experimental evidence that indicates that when roots are restricted by physical barriers such as the walls of small pots, growth responses to atmospheric CO_2 or other growth-promoting factors may not be representative of what would occur with increases in these factors in real-world agriculture or natural ecosystems (Ruff et al., 1987; Thomas and Strain, 1991).

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