

# Correlation of Stomatal Conductance with Photosynthetic Capacity of Cotton Only in a CO<sub>2</sub>-Enriched Atmosphere: Mediation by Abscisic Acid?<sup>1</sup>

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JOHN W. RADIN\*, WOLFRAM HARTUNG<sup>2</sup>, BRUCE A. KIMBALL, AND JACK R. MAUNEY  
U.S. Department of Agriculture, Agricultural Research Service, Western Cotton Research Laboratory  
(J. W. R., W. H., J. R. M.) and U.S. Water Conservation Laboratory (B. A. K.), Phoenix, Arizona 85040

## ABSTRACT

Some evidence indicates that photosynthetic rate ( $A$ ) and stomatal conductance ( $g$ ) of leaves are correlated across diverse environments. The correlation between  $A$  and  $g$  has led to the postulation of a "messenger" from the mesophyll that directs stomatal behavior. Because  $A$  is a function of intercellular CO<sub>2</sub> concentration ( $c_i$ ), which is in turn a function of  $g$ , such a correlation may be partially mediated by  $c_i$  if  $g$  is to some degree an independent variable. Among individual sunlit leaves in a cotton (*Gossypium hirsutum* L.) canopy in the field,  $A$  was significantly correlated with  $g$  ( $r^2 = 0.41$ ,  $n = 63$ ). The relative photosynthetic capacity of each leaf was calculated as a measure of mesophyll properties independent of  $c_i$ . This approach revealed that, in the absence of  $c_i$  effects, mesophyll photosynthetic capacity was unrelated to  $g$  ( $r^2 = 0.06$ ). When plants were grown in an atmosphere enriched to about 650 microliters per liter of CO<sub>2</sub>, however, photosynthetic capacity remained strongly correlated with  $g$  even though the procedure discounted any effect of variable  $c_i$ . This "residual" correlation implies the existence of a messenger in CO<sub>2</sub>-enriched plants. Enriched CO<sub>2</sub> also greatly increased stomatal response to abscisic acid (ABA) injected into intact leaves. The data provide no evidence for a messenger to coordinate  $g$  with  $A$  at ambient levels of CO<sub>2</sub>. In a CO<sub>2</sub>-enriched atmosphere, though, ABA may function as such a messenger because the sensitivity of the system to ABA is enhanced.

elusive, however. Wong *et al.* (28) originally envisioned that a mesophyll metabolite other than CO<sub>2</sub> might regulate stomatal behavior, because the sensitivity of stomata to  $c_i$  is typically too low to account for such a correlation (7, 10). Cowan *et al.* (5) suggested ABA as that metabolite based upon theoretical considerations, but no evidence has been reported to confirm a role for this or any other metabolite.

The linear correlation between  $g$  and  $A$  has been the basis for models of leaf gas exchange (11, 27), but a stable  $c_i$  is not observed in all plants under all cultural conditions. Recent demonstrations of large variations in <sup>13</sup>C:<sup>12</sup>C ratios of plant tissues indicate differences in water use efficiency and  $c_i$  (8, 9). In field-grown cotton in particular, water stress caused a large decrease in  $c_i$  and an increase in the stomatal limitation to photosynthesis (24). In earlier experiments at the same location, chronic water stress increased seasonal water use efficiency of N-deficient cotton by over 20%, clearly implying a lowered average  $c_i$  over the season (23). The variation in  $c_i$  apparently arose because stomata were capable of responding directly to the environment independently of the mesophyll. Jones (15) also reported that in this species, unlike some others to which it was compared, the stomatal limitation to photosynthesis markedly increased during drought. Again, this illustrates the lack of correlation between  $A$  and  $g$ , and the instability of  $c_i$ . In a comprehensive study (14),  $A$  and  $g$  were correlated in field-grown cotton ( $r^2 = 0.42$ – $0.55$ ), but less so than reported by Wong *et al.* (28).

Both  $A$  and  $g$  may vary with  $c_i$ , and depending upon the actual value of  $c_i$  relative to the value for saturation of photosynthesis,  $A$  can be much more sensitive to  $c_i$  than is  $g$  (7), *i.e.*  $1/A \, dA/dc_i > -1/g \, dg/dc_i$ . Recent data (24) reveal that in field-grown cotton a change in external CO<sub>2</sub> level sufficient to decrease steady-state  $c_i$  by 10% would cause a 13% decrease in  $A$  but only a 3% increase in  $g$ . Clearly, in such a species CO<sub>2</sub> is a potential messenger carrying information from stomata to mesophyll, because (a) stomatal response to the environment is to some degree independent of the mesophyll; and (b) the variation in  $c_i$  thus produced can greatly affect  $A$ . As a result, correlations may devolve in part from effects of  $c_i$  on  $A$ . Studies of mechanisms of coordination must be able to distinguish between CO<sub>2</sub> and other compounds as messengers.

In this paper we describe experiments to test for the existence of a factor, other than CO<sub>2</sub>, coordinating variability in photosynthesis with variability in stomatal conductance of field-grown cotton. Curves relating  $A$  to  $c_i$  were established using large numbers of plants. Measured values of  $A$  were compared to population-based estimates of  $A$  ( $A_e$ ) at the  $c_i$  of each individual leaf from the  $A(c_i)$  curve. Deviations of measured  $A$  from  $A_e$  were then correlated with deviations of measured  $g$  from estimated  $g$  ( $g_e$ ) in the same leaves.

In recent years the concept of coordination between stomatal and mesophyll activities has been of great interest (10, 12, 28, 29). The hypothesis of optimization of gas exchange (4) implies that stomata must respond dynamically to mesophyll activities. Wong *et al.* (28) originally reported that  $g^3$  is linearly related to  $A$ ; Field (12) has more recently summarized the evidence in favor of this relationship. This finding underlies the related observation that  $c_i$  of leaves is regulated at a constant or near-constant level presumed to be optimum for integrated water use efficiency (12, 28). A mechanism of stomatal-mesophyll coordination has been

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<sup>2</sup> Permanent address: Lehrstuhl Botanik I der Universität Würzburg, Mittlerer Dallenbergweg 64, D 8700 Würzburg, West Germany.

<sup>3</sup> Abbreviations:  $g$ , stomatal conductance;  $g_e$ , estimated stomatal conductance;  $c_i$ , intercellular CO<sub>2</sub> concentration;  $A$ , photosynthetic rate;  $A_e$ , estimated photosynthetic rate;  $r$ , correlation coefficient.

## MATERIALS AND METHODS

Cotton (*Gossypium hirsutum* L. cv Deltapine 61) was seeded on March 31, 1988, in rows spaced 1 m apart on a clay loam soil. Emergence was mostly complete by April 10. Early in May plants were thinned to a density of 10/m<sup>2</sup>. Insecticides were applied as needed throughout the season. A drip irrigation system was installed April 11, and open-top transparent plastic chambers were installed in the field by April 22. These chambers, 3 m × 3 m × 2 m high, were situated to enclose sections of three rows of plants. Air containing either ambient or enriched levels of CO<sub>2</sub> was injected into the chambers as described in greater detail elsewhere (19, 24). The average CO<sub>2</sub> concentrations in ambient and enriched chambers were 356 and 643 μL/L during daytime, and 389 and 657 μL/L at night, respectively. The coefficients of variation for these determinations over 1 d were 10 to 13%.

Water was applied weekly through the drip irrigation system. The well-watered plots received an amount of water equal to the previous week's open-pan evaporation with an adjustment for leaf area, and the water-stressed plots received 2/3 as much water as the well-watered plots. Rainfall was subtracted from the calculated irrigation requirements. Each irrigation treatment was replicated twice, with four chambers on each plot. Leaf water potentials were monitored season-long with a pressure chamber by standard procedures (23), but predawn water potentials were not significantly affected by irrigation treatment.

The four chambers on each plot were split into two nitrogen treatments and two CO<sub>2</sub> levels. Urea was added weekly to half the chambers through the irrigation system (seasonal total urea-N = 96 kg/ha). The other half of the chambers received no added N. Overall, there were 16 chambers (2 levels of water, N, and CO<sub>2</sub> in all combinations, each combination replicated twice). Because the effect of added N was not significant in these experiments, results were combined across N levels.

Curves relating  $A$  to  $c_i$  were constructed by methods similar to those described earlier (24). Air containing about 2000 μL/L CO<sub>2</sub> was passed from a high-pressure cylinder through a flowmeter and a valve to control flow rate. The air stream was then split into two parts, with the ratio between the two controlled by another valve. One part was passed through soda lime to remove CO<sub>2</sub>. The two parts of the stream were recombined, passed through MgClO<sub>4</sub> to remove water vapor, and then directed into the cuvette and analyzer of an ADC portable photosynthesis system (Analytical Development Co., Hoddesdon, England).<sup>4</sup> The CO<sub>2</sub> concentration in the cuvette was controlled by the ratio of flows in the two parts of the split air stream. Photosynthesis, leaf temperature, stomatal conductance, and  $c_i$  were calculated by the DL2 microprocessor attachment for the system from measured parameters and boundary layer resistance (20). The CO<sub>2</sub> concentration was varied in 12 to 20 steps from 0 to 1000 μL/L, and photosynthesis rate was determined on a different leaf at each step. All leaves selected were in full sunlight (PAR > 1700 μmol m<sup>-2</sup> s<sup>-1</sup>) and were fully expanded near the top of the canopy. Leaf temperatures within the cuvette remained between 30 and 34°C. Measurements were made between 0930 and 1200 h on several clear days beginning September 19 and ending October 1. A single operator could normally complete a 15-step  $A(c_i)$  curve in 30 to 40 min.

Correlations between photosynthetic capacity and stomatal conductance were based upon deviations of individual leaves from population behavior. The measured  $A$  of a leaf was expressed as a fraction of  $A_e$  determined from the  $A(c_i)$  curve, using the  $c_i$  of that leaf (Fig. 1). The measured  $g$  of the same leaf was

similarly expressed as a fraction of the  $g_e$ . In this case the mean  $g$  of the population was suitable because the effect of  $c_i$  was much smaller than the effect on  $A$  (24). With the experimental protocol outlined here, tests showed no significant effect of CO<sub>2</sub> concentration in the cuvette on  $g$ . (Presumably this resulted in part from the very rapid stabilization of the system after the cuvette was closed on a leaf. A reading typically required only about 20 s.)  $A(c_i)$  curves, and the associated values of  $A/A_e$  and  $g/g_e$ , were obtained on at least 3 d for each treatment. Data were pooled across days before correlation analysis. The paired ratios of  $A/A_e$  and  $g/g_e$  were then the basis for calculating correlations for all four treatment combinations. To minimize errors inherent in measurements at low  $A$ , only values obtained at cuvette CO<sub>2</sub> concentrations above 300 μL/L were included in the calculations.

Correlations were also calculated for direct comparisons of  $A$  and  $g$ . The steady-state  $A$  and  $c_i$  of each leaf were estimated from external CO<sub>2</sub> concentration in the chamber, from measured  $g$ , and from  $A(c_i)$  curves fitted to each leaf's gas exchange rates. The fitted curves for leaves of each treatment were similar in form to the  $A(c_i)$  curves from pooled data within the same treatment.

Stomatal responses to ABA were assessed by injection of (±)-ABA into leaves of intact plants (3). The ABA (Sigma Chemical Co., St. Louis, MO) was dissolved in a drop of ethanol and diluted with water to 5 × 10<sup>-4</sup> M. A mature healthy leaf was selected near the top of the plant in a chamber, and 2 μL of the ABA solution was injected into one of the major lateral veins of the leaf near its junction with the petiole. In a rapidly transpiring leaf, the injected volume was very rapidly taken up into the lamina. After injection,  $g$  of both the treated and untreated halves of the leaf were followed with a LiCor LI-1600 steady state porometer (LiCor Instruments, Lincoln, NE). Leaf conductances were calculated as the sums of the conductances of the abaxial and adaxial surfaces. Within each treatment combination, 12 trials were run on 6 d beginning August 6 and ending September 29.

## RESULTS AND DISCUSSION

Relationships between  $A$  and  $c_i$  were established on 3 separate days for plants in each of the treatments. A representative  $A(c_i)$  curve is shown in Figure 1. This curve is characterized by an almost linear response to  $c_i$  up to about 400 μL/L, with the slope slowly decreasing above that value. Each datum for this curve

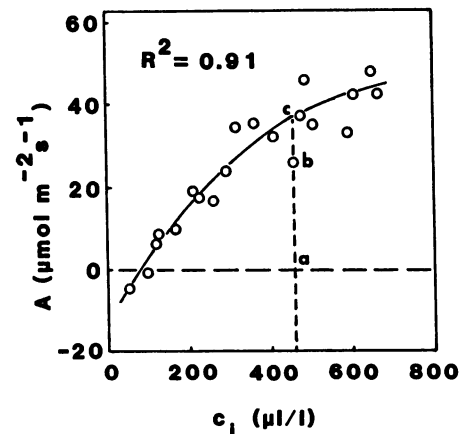


FIG. 1. Representative  $A(c_i)$  curve for leaves of field-grown cotton. These data are from well-watered plants grown at ambient CO<sub>2</sub>. Each point is derived from data collected with a different leaf. The relationship between  $A$  and  $c_i$  was estimated as a quadratic regression for which the  $r^2$  value is shown. The vertical dashed line illustrates the calculation of  $A/A_e$  when  $A$  was at point  $b$  along the line, then  $A_e$  was at point  $c$ , and  $A/A_e$  was the ratio of distances  $ab/ac$ .

<sup>4</sup> Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

represents a separate leaf. The scatter around the regression line indicates variability among individual leaves for photosynthetic capacity. For each leaf,  $A_e$  was obtained from the curve at the  $c_i$  of that leaf. Figure 1 illustrates the calculation: when  $A$  was at point  $b$  along the vertical dashed line,  $A_e$  was at point  $c$ . The ratio  $A/A_e$  then is represented by the ratio of distances  $ab/ac$ .

Within each of the four treatment combinations, steady-state  $A$  was significantly correlated with  $g$  (Table I). In each case, though, steady-state gas exchange parameters, including  $c_i$ , differed widely among leaves (Table I). Thus the origins of the correlations (*i.e.*  $\text{CO}_2$  or some other factor) could not be distinguished. This problem was approached by use of  $A/A_e$  instead of  $A$ . This quantity represents the relative photosynthetic capacity of the mesophyll and is essentially independent of  $c_i$ . In plants grown at ambient  $\text{CO}_2$ , discounting the effects of variable  $c_i$  in this fashion reduced the correlations to nonsignificance (Table I). Thus in these plants most of the correlation between  $A$  and  $g$  appeared to be mediated by a variable  $\text{CO}_2$  supply.

Plants grown in enriched  $\text{CO}_2$  behaved very differently. Regardless of water status of the plants,  $A/A_e$  was significantly correlated with  $g/g_e$  at the higher  $\text{CO}_2$  level (Table I). In the well-watered treatment, the  $r^2$  for  $A/A_e$  versus  $g/g_e$  was about 30% of the  $r^2$  for  $A$  versus  $g$ . This implies that in the regression of  $A$  on  $g$ , about 70% of the variation due to regression was dependent upon  $\text{CO}_2$ , and about 30% was dependent upon some other factor. By the same reasoning, in the water-stressed plants at elevated  $\text{CO}_2$  very little of the coordination between  $A$  and  $g$  was dependent upon  $\text{CO}_2$  (Table I). The correlations between  $A/A_e$  and  $g/g_e$  for water-stressed plants are illustrated in Figure 2 to show the effect of  $\text{CO}_2$  enrichment on this relationship.

To some extent it is predictable that enriched external  $\text{CO}_2$  would lessen the role of  $c_i$  in coordinating  $A$  with  $g$ . The  $A(c_i)$  curves (Fig. 1) indicate a decreasing photosynthetic response to increasing  $\text{CO}_2$  in the  $c_i$  range maintained by leaves in  $\text{CO}_2$ -enriched air (Table I). The tendency to saturation at high  $c_i$  was slightly greater in the  $A(c_i)$  curves from enriched- $\text{CO}_2$  plants than in those from ambient- $\text{CO}_2$  plants (data not presented). Similar results were reported earlier (24). Nonetheless, the coordination of  $A/A_e$  with  $g/g_e$  in these plants must be independent of such considerations, because  $A_e$  incorporates the effect of variable  $c_i$  on photosynthetic rate. The ratio  $A/A_e$  thus is not confounded by  $c_i$  and represents a "pure" mesophyll property. The improved correlation between  $A/A_e$  and  $g/g_e$  in plants grown at high  $\text{CO}_2$  clearly implies that  $\text{CO}_2$  enrichment enhances the transfer or recognition of a messenger (other than  $\text{CO}_2$  itself) from mesophyll to guard cells or vice versa. Because the number and size of mesophyll cells greatly exceed those of the guard cells, it seems most likely that the messenger in  $\text{CO}_2$ -enriched plants originates in the mesophyll and is transferred to the guard cells as postulated by Wong *et al.* (28) earlier.

The effects of  $\text{CO}_2$  enrichment and water stress are consistent

with a possible role of ABA in the coordination of  $A/A_e$  and  $g/g_e$ . Both  $\text{CO}_2$  enrichment and water stress are known to enhance stomatal closure in response to ABA (22, 26). The applicability of these concepts was tested by injecting ABA into leaves of intact plants in the field. The ABA was introduced into one of the major lateral veins of the transpiring leaf; it was then distributed to that half of the lamina. The other half of the lamina served as a control to monitor  $g$  in the absence of ABA. Conductance began to decrease almost immediately after injection of ABA on the treated side of the leaf, but was almost unaffected on the untreated side (Fig. 3). The ratio of  $g$  10 min after injection to  $g$  at the time of injection was taken as a measure of stomatal response to ABA. Enrichment with  $\text{CO}_2$  strongly promoted stomatal closure in the presence of ABA (Table II). Analysis of variance reveals that of the two main effects ( $\text{CO}_2$ , water) and their interaction on responsiveness to ABA, the  $\text{CO}_2$  effect was highly significant ( $P = 0.01$ ) but neither the effect of water stress nor its interaction with  $\text{CO}_2$  was significant ( $P = 0.05$ ). Nonetheless, water stress tended to increase responsiveness to ABA, especially at ambient  $\text{CO}_2$ , and also to increase sensitivity to  $\text{CO}_2$  itself (Table II). This behavior is similar to that reported earlier (22). The conductances in these experiments tended to exceed those reported in Table I. This discrepancy probably results from differences in the time of year when the data were collected. Injections of ABA were carried out over a 2-month period that included substantial hot weather. High temperatures are known to increase stomatal conductance in cotton (24, 25).

Recently, it has been reported that stomata in heterobaric leaves may respond to ABA unevenly, giving rise to "patchiness" (K Raschke, personal communication). When excised grape leaves were treated with ABA and then presented with  $^{14}\text{CO}_2$ , the radioactivity was irregularly distributed among areoles in the lamina, indicating zero gas exchange in some areoles (6). ABA also caused apparent patchy closure in sunflower as indicated by fluorescence quenching (6). If ABA causes a similar patchy closure in stomata of cotton leaves, then the apparent sensitization by  $\text{CO}_2$  becomes much more difficult to interpret in terms of coordination with mesophyll photosynthetic capacity. Although we have no direct evidence, a patchy response to ABA, leading to zero gas exchange in some heterobaric areoles of the leaf, seems unlikely in cotton. First, exogenous ABA had little effect on  $A(c_i)$  curves in cotton (22). Second, in the present work the dose of ABA injected into leaves was only 1 nmol. This is 1 to 2 orders of magnitude less than the dose typically delivered to excised leaves through the transpiration stream. Third, the cotton leaf is amphistomatous, unlike the grape leaf. Under the conditions of our tests, the ABA exerted an effect primarily on the adaxial stomata, leaving the abaxial stomata relatively less affected. In the experiment depicted in Figure 3, the ratio of abaxial conductance to adaxial conductance ( $g_{ab}/g_{ad}$ ) remained at 2 or

Table I. Variability and Correlations among Gas-Exchange Properties of Cotton Leaves

The ambient and enriched  $\text{CO}_2$  concentrations were approximately 350 and 650  $\mu\text{L/L}$ , respectively. The number of individual leaves included within each survey varied from 55 to 63 for the different treatments.

Treatment	Range of Leaf Properties			$r^{2a}$	
	$A$ $\mu\text{mol m}^{-2}\text{s}^{-1}$	$g$ $\text{mol m}^{-2}\text{s}^{-1}$	$c_i$ $\mu\text{L/L}$	$A$ versus $g$	$A/A_e$ versus $g/g_e$
Ambient $\text{CO}_2$					
Well-watered	13.7–29.7	0.20–0.75	184–305	0.41 **	0.06 NS
Water-stressed	10.3–30.0	0.17–0.77	164–304	0.45 **	0.04 NS
Enriched $\text{CO}_2$					
Well-watered	16.9–44.9	0.13–0.58	337–552	0.49 **	0.15 **
Water-stressed	16.4–61.8	0.15–0.56	377–552	0.46 **	0.40 **

<sup>a</sup> NS, not significantly different from zero ( $P = 0.05$ ); \*\*, highly significantly different from zero ( $P = 0.01$ ).

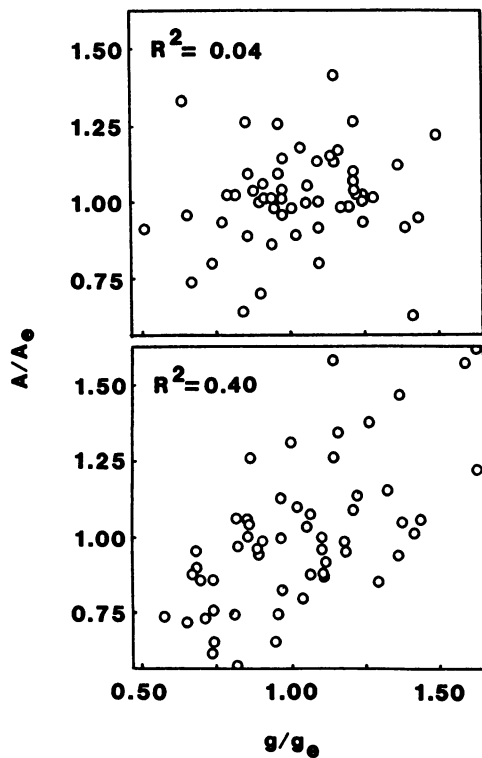


FIG. 2. Plots showing the relationships between  $A/A_e$  and  $g/g_e$  in plants from the two water-stressed treatments (see Table I). Upper panel, ambient  $\text{CO}_2$ ; lower panel, enriched  $\text{CO}_2$ .

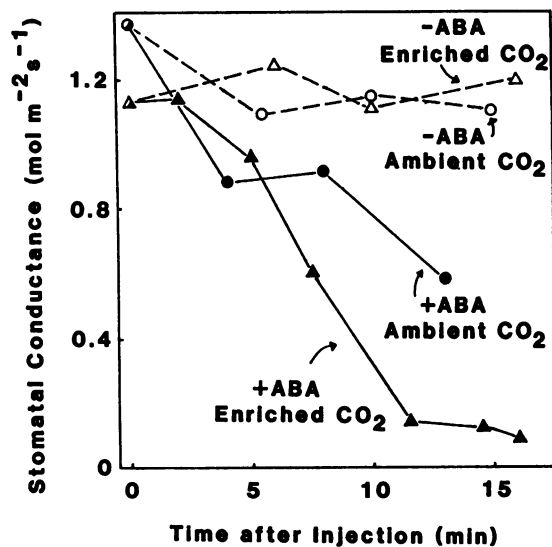


FIG. 3. Effect of ABA on stomatal conductances of leaves in ambient- $\text{CO}_2$  and enriched- $\text{CO}_2$  atmospheres. ABA was injected into a lateral vein at time zero. Conductance was determined separately on the injected half of the leaf (+ABA) and on the uninjected half which served as a control (-ABA).

less throughout the measurement period in the controls. After ABA injection, though,  $g_{ab}/g_{ad}$  increased rapidly to greater than 5. This differential effect on the two surfaces makes it unlikely that an areole can become completely sealed, because even if patchy closure were to occur on the adaxial surface, gas exchange could continue through the abaxial stomata.

Raschke (26) first reported that  $\text{CO}_2$  and ABA act synergistically to close stomata. The demonstration has since been repeated with cotton (22) but data in the present paper are the first

Table II. Stomatal Conductances of Leaves before and 10 min after Injection of ABA ( $g_0$  and  $g_{10}$ , Respectively)

The relative response to ABA is shown as the ratio  $g_{10}/g_0$ .

Treatment	Stomatal Conductance		$g_{10}/g_0$
	$g_0$	$g_{10}$	
	$\text{mol m}^{-2}\text{s}^{-1}$		
Ambient $\text{CO}_2$			
Well-watered	$1.02 \pm 0.14^a$	$0.53 \pm 0.09$	$0.57 \pm 0.11$
Water-stressed	$0.80 \pm 0.12$	$0.33 \pm 0.07$	$0.39 \pm 0.07$
Enriched $\text{CO}_2$			
Well-watered	$0.77 \pm 0.10$	$0.14 \pm 0.03$	$0.28 \pm 0.09$
Water-stressed	$0.44 \pm 0.08$	$0.11 \pm 0.03$	$0.24 \pm 0.07$

<sup>a</sup> Values are means  $\pm$  SE of 12 trials.

to demonstrate this interaction in field plants. The existence of this interaction strongly implicates ABA as a messenger coordinating stomatal conductance with photosynthetic capacity of the mesophyll, as the two characters were correlated only in plants grown in a  $\text{CO}_2$ -enriched atmosphere. It remains to be shown how photosynthetic capacity might regulate the rate of ABA release from chloroplasts, where it is present in high concentration (13). However, a relationship between photosynthetic capacity and stromal pH might account for the participation of ABA in stomatal-mesophyll coordination. ABA accumulates in the alkaline plastid as the impermeant  $\text{ABA}^-$  anion (13). If decreased photosynthetic rate is associated with decreased stromal pH (as has been suggested for osmotically stressed chloroplasts [2]), then increased release of ABA would follow. Cowan *et al.* (5) calculated that the stromal acidification resulting from a light-dark transition should greatly increase the release of ABA from chloroplasts, ultimately doubling the apoplastic pool of ABA to which guard cells are exposed.

The failure to find a correlation between  $A/A_e$  and  $g/g_e$  at ambient  $\text{CO}_2$  is consistent with previous demonstrations that cotton stomata are rather unresponsive to  $c_i$  or to ABA under many conditions (7, 14, 22–25), and with the lack of stomatal control of water loss from this crop (1, 17, 24). Indeed, Hutmacher and Krieg (14) showed that the  $g$  and  $c_i$  of irrigated cotton are considerably too high for optimum water use efficiency. On the other hand, the establishment of a correlation between  $A/A_e$  and  $g/g_e$  at elevated  $\text{CO}_2$  implies the existence of functional "optimization" mechanisms, at least in water-stressed plants which showed the highest degree of correlation (Table I). Further studies of the conditions promoting this correlation, the role of ABA in establishing it, and its effect on water use efficiency seem warranted.

Enrichment of  $\text{CO}_2$  in the atmosphere increases plant water use efficiency by increasing photosynthesis rate and often by decreasing transpiration rate (10, 14, 19, 22, 24). Our data indicate a third possible mechanism for increasing water use efficiency:  $\text{CO}_2$  enrichment couples stomatal behavior more closely to mesophyll photosynthetic capacity, thereby decreasing the chance of a leaf displaying a low  $A:g$  ratio. In these populations, the very substantial variability in leaf photosynthetic capacity and in stomatal conductance (Table I, Fig. 2) implies that the impact of this "optimization" may be large. We have not evaluated whether the variability in  $A:g$  ratio is from environmental and developmental differences, or is partially genetically controlled. The latter possibility seems unlikely, considering that the data were acquired from a single well-established cultivar. If microenvironmental differences can account for the variability, though, the factors must be subtle and at this point are unidentified.

It is clear from this study that the  $A:g$  ratios of populations of cotton plants are not optimized. Successful selection of heritable

improvements in gas exchange characteristics in cotton (21) and other crops (16, 18) indicates that a genetic approach to gas exchange efficiency is feasible. The portable photosynthesis system allows fairly rapid nondestructive identification of individuals with a high *A:g* ratio, and it may be useful in a screening or breeding program based upon gas exchange.

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#### LITERATURE CITED

- ACKERSON RC, DR KRIEG 1977 Stomatal and nonstomatal regulation of water use in cotton, corn, and sorghum. *Plant Physiol* 60: 850–853
- BERKOWITZ GA, M GIBBS 1983 Reduced osmotic potential effects on photosynthesis. Identification of stromal acidification as a mediating factor. *Plant Physiol* 71: 905–911
- BURSCHKA C, JD TENHUNEN, W HARTUNG 1983 Diurnal variations in abscisic acid content and stomatal response to abscisic acid in leaves of irrigated and non-irrigated *Arbutus unedo* plants under naturally fluctuating environmental conditions. *Oecologia* 58: 128–131
- COWAN IR, GD FARQUHAR 1977 Stomatal function in relation to leaf metabolism and environment. In Jennings DH, ed, *Integration of Activity in the Higher Plant*. Cambridge University Press, Cambridge, pp 471–505
- COWAN IR, JA RAVEN, W HARTUNG, GD FARQUHAR 1982 A possible role for abscisic acid in coupling stomatal conductance and photosynthetic carbon metabolism in leaves. *Aust J Plant Physiol* 9: 489–498
- DOWNTON WJS, BR LOVEYS, WJR GRANT 1988 Stomatal closure fully accounts for the inhibition of photosynthesis by abscisic acid. *New Phytol* 108: 263–266
- DUBBE DR, GD FARQUHAR, K RASCHKE 1978 Effect of abscisic acid on the gain of the feedback loop involving carbon dioxide and stomata. *Plant Physiol* 62: 413–417
- EHLERINGER JR, ED SCHULZE, H ZIEGLER, OL LANGE, GD FARQUHAR, IR COWAN 1985 Xylem-tapping mistletoes: water or nutrient parasites? *Science* 227: 1479–1481
- FARQUHAR GD, RA RICHARDS 1984 Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Aust J Plant Physiol* 11: 539–552
- FARQUHAR GD, TD SHARKEY 1982 Stomatal conductance and photosynthesis. *Annu Rev Plant Physiol* 33: 317–345
- FARQUHAR GD, SC WONG 1984 An empirical model of stomatal conductance. *Aust J Plant Physiol* 11: 191–210
- FIELD CB 1987 Leaf-age effects on stomatal conductance. In Zeiger E, GD Farquhar, IR Cowan, eds, *Stomatal Function*. Stanford University Press, Stanford, CA, pp 367–384
- HARTUNG W, B HEILMANN, H GIMMLER 1981 Do chloroplasts play a role in abscisic acid biosynthesis? *Plant Sci Lett* 22: 235–242
- HUTMACHER RB, DR KRIEG 1983 Photosynthetic rate control in cotton. Stomatal and nonstomatal factors. *Plant Physiol* 73: 658–661
- JONES HG 1985 Partitioning stomatal and non-stomatal limitations to photosynthesis. *Plant Cell Environ* 8: 95–104
- JONES HG 1987 Breeding for stomatal characters. In Zeiger E, GD Farquhar, IR Cowan, eds, *Stomatal Function*. Stanford University Press, Stanford, CA, pp 431–443
- JORDAN WR, JT RITCHIE 1971 Influence of soil water stress on evaporation, root absorption, and internal water status of cotton. *Plant Physiol* 48: 783–788
- KIDAMBI SP, DR KRIEG, FS GIRMA 1986 Genetic control of gas exchange rates in grain sorghum. 1986 *Agronomy Abstracts*: 68
- KIMBALL BA, JR MAUNEY, JW RADIN, FS NAKAYAMA, SB IDSO, DL HENDRIX, DH AKEY, SG ALLEN, MG ANDERSON, W HARTUNG 1986 Effects of increasing atmospheric CO<sub>2</sub> on the growth, water relations, and physiology of plants grown under optimal and limiting levels of water and nitrogen. Number 039, *Response of Vegetation to Carbon Dioxide*. US Department of Energy, Carbon Dioxide Research Division, Washington, DC
- PARKINSON KJ 1985 A simple method for determining the boundary layer resistance in leaf cuvettes. *Plant Cell Environ* 8: 223–226
- QUISENBERRY JE, CW WENDT, JD BERLIN, BL MCMICHAEL 1985 Potential for using leaf turgidity to select drought tolerance in cotton. *Crop Sci* 25: 294–299
- RADIN JW, RC ACKERSON 1981 Water relations of cotton plants under nitrogen deficiency. III. Stomatal conductance, photosynthesis, and abscisic acid accumulation during drought. *Plant Physiol* 67: 115–119
- RADIN JW, JR MAUNEY, G GUINN 1985 Effects of N fertility on plant water relations and stomatal responses to water stress in irrigated cotton. *Crop Sci* 25: 110–115
- RADIN JW, BA KIMBALL, DL HENDRIX, JR MAUNEY 1987 Photosynthesis of cotton plants exposed to elevated levels of carbon dioxide in the field. *Photosynth Res* 12: 191–203
- RADIN JW, LL PARKER, G GUINN 1982 Water relations of cotton plants under nitrogen deficiency. V. Environmental control of abscisic acid accumulation and stomatal sensitivity to abscisic acid. *Plant Physiol* 70: 1066–1070
- RASCHKE K 1975 Simultaneous requirement of carbon dioxide and abscisic acid for stomatal closing in *Xanthium strumarium* L. *Planta* 125: 243–259
- REYNOLDS JF, RL DOUGHERTY, JD TENHUNEN, PC HARLEY 1988 PRECO: a model for the simulation of plant response to elevated CO<sub>2</sub>. Number 042, *Response of Vegetation to Carbon Dioxide*. US Department of Energy, Carbon Dioxide Research Division, Washington, DC
- WONG SC, IR COWAN, GD FARQUHAR 1979 Stomatal conductance correlates with photosynthetic capacity. *Nature* 282: 424–426
- WONG SC, IR COWAN, GD FARQUHAR 1985 Leaf conductance in relation to rate of CO<sub>2</sub> assimilation. I. Influence of nitrogen nutrition, phosphorus nutrition, photon flux density, and ambient partial pressure of CO<sub>2</sub> during ontogeny. *Plant Physiol* 78: 821–825