Photosynthetic Rate Control in Cotton

STOMATAL AND NONSTOMATAL FACTORS

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ABSTRACT

The relationship between single leaf photosynthesis and conductance was examined in cotton (Gossypium hirsutum L.) across a range of environmental conditions. The purpose of this research was to separate and define the degree of stomatal and nonstomatal limitations in the photosynthetic process of field-grown cotton.

Photosynthetic rates were related to leaf conductance of upper canopy leaves in a curvilinear manner. Increases in leaf conductance of CO2 in excess of 0.3 to 0.4 mole per square meter per second did not result in significant increases in gross or net photosynthetic rates. No tight coupling between environmental influences on photosynthetic rates and those affecting conductance levels was evident, since photosynthesis per unit leaf conductance did not remain constant. Slowly developing water stress caused greater reductions in photosynthesis than in leaf conductance, indicating nonstomatal limitations of photosynthesis.

Increases in external CO2 concentration to levels above ambient did not produce proportional increases in photosynthesis even though stomatal or intercellular CO2 concentration increased. The lack of a linear increase in photosynthetic rate in response to increases in leaf conductance and in response to increases in external CO2 concentration demonstrated that nonstomatal factors are major photosynthetic rate determinants of cotton under field conditions.

Definition of the order of limitations in the photosynthetic process is a prerequisite to genetically increasing the photosynthetic rate of crop species. Although stomatal conductance has often been proclaimed as the principal limitation to photosynthetic CO2 assimilation (1, 5, 7, 8, 16), it is doubtful that stomatal conductance alone would be rate-limiting across all conditions. Previous demonstrations of nonstomatal factors limiting photosynthesis have often been associated with atypical environmental conditions. Nonstomatal limitations have been considered secondary to stomatal conductance within the range of normal environmental conditions for crop species (5). However, experiments separating stomatal and nonstomatal photosynthetic limitations on plants grown in and acclimated to field environmental conditions are almost nonexistent.

The CO2 concentration required to maximize photosynthesis varies with species and environmental conditions (10, 13, 18, 19). A curvilinear photosynthesis: external CO2 supply (Cₐ) relationship would be expected, but the intercellular CO2 concentration (Cₐ) at atmospheric CO2 and its effect on photosynthesis is not well defined for most species, including cotton. Farquhar and Sharkey (5), in summarizing much of their own research and reanalyses of existing literature, have concluded that stomatal conductance may respond to changes in photosynthetic rate to maintain Cₐ near saturation. Photosynthetic rate is related to CO2 concentration and leaf conductance via A = (Cₐ - Cₐ) conductance. Recent evidence, involving both C₃ and C₄ species, indicated that at moderate PPFD (700 μmol photons m⁻² s⁻¹) and atmospheric Cₐ, Cₐ existed near the saturation level of the net photosynthesis: Cₐ response curve (13). Under conditions of nonlimiting PPFD, the range of Cₐ levels at normal atmospheric Cₐ and the relative stomatal limitation of photosynthesis has not been well defined.

If one accepts the premise that stomatal conductance is the major limitation to photosynthesis, then attempts to increase photosynthesis and crop productivity will occur with concomitant increases in transpirational water use. Under semi-arid conditions, this response will probably be more detrimental than beneficial since crop development is largely time dependent and water use efficiency is of utmost concern. If stomatal conductance responds to photosynthetic rate changes which are largely biochemical in nature, then opportunity exists to increase photosynthesis genetically within a species and possibly to increase water use efficiency if differences in the photosynthesis: Cₐ relationship exist within the germplasm. The experiments reported here were designed to analyze the relationship between stomatal conductance and photosynthesis in cotton under field conditions, and to assess the relative importance of stomatal and nonstomatal control of photosynthesis under both irrigated and water-stressed conditions.

MATERIALS AND METHODS

Photosynthetic rates and leaf conductances of cotton (Gossypium hirsutum L.) were determined under field conditions over a 2-year period. Eight commercial cultivars, four near isogenic strains differing in leaf morphology, and six photoperiod-sensitive strains which do not flower during the normal growing season were utilized in this experiment to provide a wide range of plant characteristics which may influence plant performance.

The cultivars were grown under field conditions in the 1980 and 1981 growing seasons. Seeding occurred about May 20 in each year. The 1980 growing season was characterized by pro-

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2 Abbreviations: Cₐ, CO2 concentration external to the leaf; Cₐ, stomatal or intercellular CO2 concentration; ψₑ, leaf water potential; PPFD, photosynthetic photon flux density; PMA, phenyl mercuric acetate; VPFD, leaf to air vapor pressure difference.
longed periods of hot days (37–42°C maximum), low RH, and relatively low rainfall. The 1981 growing season was not as extreme in terms of temperature (34–38°C maximum) or vapor pressure deficit.

All plots received preplant irrigation adequate to bring the upper 1.5 m to field capacity. The soil type was a Pullman clay loam soil (fine, mixed, thermic family of Torreric Paleustoll) approximately 1 to 1.5 m deep underlain by a calcic horizon. The experimental design was a randomized complete block with three replicate plantings of each cultivar within each irrigation level. Established plant populations were 11 to 14 plants m⁻².

Irrigation water was supplied to one set of plots when the leaf water potential (Ψₛ) of the uppermost fully expanded leaf declined to a midday minimum of ~1.8 MPa. Hereafter, plants in plots receiving supplemental water will be referred to as 'irrigated.' The second set of plots received no supplemental irrigation beyond preplant irrigation, and will be referred to as 'dryland.' Xylem water potential (Ψₓ) was determined on upper canopy leaves using a pressure chamber.

Leaf conductance of water vapor was determined by measuring abaxial and adaxial stomatal conductance with a Li-Cor steady state diffusion porometer and assuming the two surface resistances act in parallel. Boundary layer resistance was estimated with wet filter paper and included in calculations of total leaf conductance. Leaf conductance of CO₂ was calculated as 0.625 times leaf conductance of water vapor.

Gross photosynthetic rates of individual leaves were determined using a ¹⁴CO₂ fixation technique (9, 14). The same leaf was utilized for concurrent determinations of conductance and photosynthesis. All determinations were made on the most recent fully expanded main stem leaf. Net carbon exchange rate and transpiration rate were determined on single leaves enclosed in a 25- × 25- × 6-cm flow-through chamber equipped with a Teflon FEP film top and bottom to allow transmission of longwave radiation. Air temperatures within the chambers were within 1 to 3°C of external air temperature. Individual measurements were usually completed within a 15- to 20-min equilibration period. IR gas analysis of CO₂ concentration changes and capacitance measurements of humidity changes were utilized to determine carbon exchange rate and transpiration rate, respectively. Leaf and chamber temperatures were measured with thermocouples and were used with transpiration rate determinations to calculate leaf conductance. The internal or substomatal CO₂ concentrations (C) were calculated using the model A = (Cₑ-Cₑ) conductance with allowances for the influence of transpiration included in the calculation as described by Farquhar and Sharkey (5).

A series of short term experiments were conducted with the purpose of determining the potential to influence leaf conductance or photosynthetic rates independently. Mesh screen shading material was utilized to provide reduced PPFD from ambient. Whole canopies were shaded beginning at dawn on the day of measurements. The influences of antitranspirant-type chemicals on photosynthesis versus leaf conductance were evaluated using irrigated plants. Applications of PMA at concentrations ranging from 1.0 × 10⁻⁵ to 5.0 × 10⁻⁴ m were made by immersing leaves in solutions in the early morning hours of days on which measurements were made. Solutions of ABA at concentrations ranging from 1.0 × 10⁻⁵ to 1.0 × 10⁻⁴ m were applied by spraying leaves to wetting. Photosynthesis and leaf conductance measurements were made beginning 30 min after application of ABA treatments.

The effects of four different external CO₂ concentrations on net photosynthesis and leaf conductance were evaluated using gas mixtures containing 125, 267, 345, and 590 μl CO₂ l⁻¹ of air. All four gas mixtures contained 20 per cent O₂ (v/v). During these specific experiments, air temperatures ranged from 37 to 40°C and VPD ranged from 4.2 to 5.3 kPa. Data were analyzed using various statistical methods including analysis of variance, Duncan's Multiple Range Test, and regression analyses.

RESULTS AND DISCUSSION

Regression analyses of single leaf gross photosynthesis with leaf conductance of CO₂ was best described by a quadratic function across all genetic materials, sampling times, and environmental conditions (Fig. 1). Data were pooled for all treatments (genetic and environment) in order to establish the existence of any generalized photosynthesis:leaf conductance relationship for cotton. The quadratic nature of the relationship indicated that the rate of CO₂ assimilation increased as stomatal conductance increased to 0.3 mol m⁻² s⁻¹. As leaf conductance exceeded 0.3 mol m⁻² s⁻¹, gross photosynthetic rate per unit leaf conductance declined. Low r² values for the gross photosynthesis:leaf conductance relationship demonstrated that even at leaf conductances less than 0.4 mol m⁻² s⁻¹, a large degree of variability existed in the relationship. Differences in gross photosynthesis per unit leaf conductance were also apparent between irrigated and dryland plants, with 10 per cent to 20 per cent lower ratios in dryland plants at leaf conductances in excess of approximately 0.3 mol m⁻² s⁻¹ (Fig. 1). The degree of reduction in photosynthesis per unit conductance increased with the severity of water stress. Leaf water potentials reached a late season minimum of ~3.1 MPa in dryland plants in 1980 and ~2.3 MPa in 1981. Depending on the period of measurement during the growing season and the season (1980 or 1981), the minimum daily leaf water potential differential between nonirrigated and irrigated plants was between 0.2 and 1.0 MPa.

Several short term experiments were conducted to further elucidate the cause of the nonlinear response of photosynthesis to increasing leaf conductance. Reductions in PPFD of 30 per cent and 60 per cent from ambient (1700–1900 μmol photons m⁻² s⁻¹) were achieved with polypropylene shading material suspended over the canopy. Reductions in PPFD to less than 1200 μmol m⁻² s⁻¹ were required to alter either photosynthesis or leaf conductance (data not shown). Further reductions in PPFD resulted in greater reductions in photosynthesis than in leaf conductance. The nonlinear photosynthesis:leaf conductance relationship (Fig. 1) does not represent nonstomatal limitations to photosynthesis related to PPFD levels.

The use of chemical antitranspirants to alter either photosynthesis or conductance independently was attempted. Increasing PMA applications from 1 × 10⁻⁵ m produced significant reductions in leaf conductance without concurrent reductions in gross photosynthesis.

FIG. 1. Gross photosynthetic rates as a function of conductance of CO₂ across all cultivars under irrigated and nonirrigated conditions for 1980 and 1981. The r² values are for quadratic regressions of the photosynthetic:conductance relationships, with n = number of observations.
photosynthetic rate (Table I). These evaluations were conducted during periods when leaf conductances were at the highest levels of the diurnal cycle. The effects lasted a minimum of 3 d following applications. ABA treatments resulted in a similar trend but the effects were of shorter duration (less than 2 h) and more variable (Table I). At the highest PMA and ABA concentrations used, leaf conductances were reduced approximately 50 per cent from levels in control leaves, whereas photosynthetic rates were reduced by only 40 per cent. The relatively high conductances in the nontreated control leaves did not contribute substantially to evaporative cooling and temperature moderation, as leaf temperatures did not increase significantly in treated leaves until conductances were reduced by more than 20 per cent (Table I). Air temperatures during the antitranspirant studies were in the range of 31 to 34°C.

It has been assumed that if guard cells respond to CO₂ levels, they will respond to Cᵢ rather than Cₑ. Separation of data according to temperature ranges demonstrated that Cᵢ increased as leaf temperature increased across the range from 24 to 40°C (Table II). Both gross and net photosynthetic rates declined rapidly as leaf temperature increased beyond 30°C (Table II). However, leaf conductance did not decline in proportion to the reductions in net photosynthesis, resulting in lower net photosynthesis:leaf conductance ratios and higher Cᵢ at elevated temperatures. Several studies have suggested that many species maintain a nearly constant Cᵢ across varying environmental conditions (2, 15, 20, 21). Increasing VPD also has been reported to have greater effects on leaf conductance than on photosynthesis (4, 5, 13, 17). In contrast, photosynthetic rates of field-grown cotton exhibited greater sensitivity than leaf conductance to increases in leaf temperature or VPD resulting in an increase in Cᵢ. No attempt was made to separate the effects of temperature from VPD in this study; however, the major cause of the change in VPD was increasing temperature.

To verify the concept that the rate of CO₂ diffusion through the stomata was nonlimiting, net photosynthetic rates of individual leaves were measured at external CO₂ concentrations of 125 through 590 µl CO₂ l⁻¹ air during the fruit development stage in irrigated and dryland plants. Air temperatures during the periods of measurement were 36 to 40°C. Increasing Cᵢ from 125 µl CO₂ l⁻¹ air resulted in increased photosynthesis and reductions in leaf conductance (Fig. 2) resulting in large increases in the net photosynthesis:conductance ratios. The highest leaf conductance was observed at low Cᵢ. Increasing Cₑ caused a consistent reduction in leaf conductance and a significant increase in net photosynthesis. The leaf conductance response to Cᵢ and Cₑ would suggest stomata respond to Cᵢ. Water-stressed plants (−3.1 MPa Ψₛ) had lower leaf conductance and net photosynthesis at every Cᵢ compared with irrigated plants (−2.1 MPa Ψₛ). Lower photosynthesis:conductance ratios and higher Cᵢ:Cₑ ratios in the water-stressed plants indicated a nonstomatal inhibition of photosynthesis. Differences in leaf temperature and resultant effects on photosynthesis were not large enough to account for the reduction in the photosynthesis:conductance ratios in the dryland plants. Rapid imposition of water stress causing stomatal closure has resulted in initial increases in the photosynthesis:conductance ratio in some species (1, 11, 12, 15).

However, in Sorghum and Eucalyptus species subjected to a slowly developing water stress, comparable to our experiment, net photosynthesis:conductance ratios and Cᵢ did not change as stress intensified (2, 15). In this experiment, water stress was severe enough to reduce photosynthesis:conductance ratios and increase Cₑ, indicating a proportionally greater nonstomatal inhibition of photosynthesis in water-stressed plants (Fig. 2).

Farquhar and Sharkey (5) have suggested that an estimate of the degree of stomatal limitation of photosynthesis can be obtained by comparing the photosynthetic rate that would occur if conductance of CO₂ was infinite (Cᵢ equals Cₑ) to actual photosynthetic rates at ambient atmospheric Cₑ. By such an analysis, the stomatal limitation was generally less than 25 per cent under irrigated conditions and approximately 15 per cent under water-stressed (dryland) conditions (Fig. 2B).

At ambient Cᵢ of 340 to 345 µl CO₂ l⁻¹ air, calculated Cᵢ levels of both irrigated and nonirrigated plants were within the range from 265 to 295 µl CO₂ l⁻¹ air across most conditions. This range of Cᵢ was beyond the linear phase of the photosynthesis:Cᵢ relationship and therefore was near the Cᵢ required to maximize net photosynthesis (Fig. 2B).

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**Table I. Influences of PMA and ABA Applications on Single Leaf Gross Photosynthesis and Leaf Conductance**

<table>
<thead>
<tr>
<th>Chemical Treatment</th>
<th>Conc. × 10⁻⁵ M</th>
<th>Gross Photosynthesis</th>
<th>Leaf Conductance</th>
<th>Leaf Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>µmol CO₂ m⁻² s⁻¹</td>
<td>mol m⁻² s⁻¹</td>
<td>°C</td>
</tr>
<tr>
<td>PMA</td>
<td>0.0 (control)</td>
<td>18.3 a</td>
<td>0.62 a</td>
<td>30.5 b</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>17.1 a</td>
<td>0.56 b</td>
<td>30.6 b</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>17.7 a</td>
<td>0.54 b</td>
<td>31.0 b</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>17.6 a</td>
<td>0.56 b</td>
<td>31.1 b</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>16.4 a</td>
<td>0.47 c</td>
<td>31.4 ab</td>
</tr>
<tr>
<td></td>
<td>50.0</td>
<td>11.4 b</td>
<td>0.31 d</td>
<td>32.7 a</td>
</tr>
<tr>
<td>ABA</td>
<td>0.0 (control)</td>
<td>18.9 a</td>
<td>0.59 a</td>
<td>30.1 c</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>19.6 a</td>
<td>0.58 a</td>
<td>30.4 bc</td>
</tr>
<tr>
<td></td>
<td>3.3</td>
<td>18.3 a</td>
<td>0.55 ab</td>
<td>30.2 bc</td>
</tr>
<tr>
<td></td>
<td>6.7</td>
<td>17.2 a</td>
<td>0.51 b</td>
<td>31.2 ab</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>12.6 b</td>
<td>0.36 c</td>
<td>32.2 a</td>
</tr>
</tbody>
</table>

* Means within a column followed by different letters were significantly different at the P < 0.05 level by Duncan's Multiple Range Test.

**Table II. Photosynthesis, Leaf Conductance, and Intercellular CO₂ Concentration of Cotton at Different Temperature Ranges and Corresponding VPD under Irrigated (Irr) and Dryland (Dry) Conditions**

**Data for this analysis were collected throughout the 1980 season for commercial cotton cultivars.**

<table>
<thead>
<tr>
<th>Leaf Temperature Range</th>
<th>Gross Photosynthesis</th>
<th>Net Photosynthesis</th>
<th>Leaf Conductance</th>
<th>Intercellular CO₂ Conc.</th>
<th>VPD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol CO₂ m⁻² s⁻¹</td>
<td>µmol CO₂ m⁻² s⁻¹</td>
<td>mol m⁻² s⁻¹</td>
<td>µl l⁻¹</td>
<td>kPa</td>
</tr>
<tr>
<td>24–29</td>
<td>18.3ab</td>
<td>16.6bc</td>
<td>15.9a</td>
<td>14.6a</td>
<td>0.40a</td>
</tr>
<tr>
<td>29–32</td>
<td>18.8a</td>
<td>14.9cd</td>
<td>15.6a</td>
<td>11.9b</td>
<td>0.43a</td>
</tr>
<tr>
<td>32–36</td>
<td>15.7c</td>
<td>13.6de</td>
<td>11.5b</td>
<td>10.0c</td>
<td>0.37ab</td>
</tr>
<tr>
<td>36–40</td>
<td>14.1de</td>
<td>12.5e</td>
<td>9.4c</td>
<td>8.3e</td>
<td>0.33b</td>
</tr>
</tbody>
</table>

* Means within a physiological parameter are significantly different at the P < 0.05 level by Duncan's Multiple Range Test if followed by different letters.
PHOTOSYNTHETIC RATE CONTROL IN COTTON I

CONCLUSIONS

These data were collected across a wide range of genetic materials and environmental conditions and pooled for these analyses. Some responses evidently were common to most cultivars, such as reductions in gross and net photosynthesis per unit leaf conductance with increases in temperature and VPD and with increasing water stress. The low $r^2$ values for the overall photosynthesis-leaf conductance relationship in the pooled analyses, however, indicated the strong possibility of genetic variability in photosynthesis-conductance ratios. The opportunity for genetic selection of higher photosynthesis per unit leaf conductance is viable and should be explored, including the determination of whether improvements can be made through increases in photosynthesis or reductions in stomatal conductance.

LITERATURE CITED


Fig. 2. The response of leaf conductance (A) and net photosynthesis (B) to intercellular CO2 concentration under irrigated (O, A) and dryland (O, △) conditions. Arrows denote applied CO2 concentrations. The vertical dashed line indicates the ambient CO2 concentration. Lines a and b represent the net photosynthesis - C1 relations at C2 = 345 μl CO2 I-1 air. The shaded areas represent the degree of stomatal limitation of net photosynthesis.

If the rate of diffusion and availability of CO2 were the major limitation to photosynthesis, a marked increase in photosynthetic rates should have been evident at CO2 levels in excess of typical C1 under ambient atmospheric conditions. No large increases in net photosynthetic rates were observed; therefore, nons stomatal factors must exert a major influence on photosynthetic rates of field-grown cotton under both irrigated and water-stressed conditions.