Photosynthesis in Relation to Leaf Characteristics of Cotton from Controlled and Field Environments

DAVID T. PATTERSON, JAMES A. BUNCE, RANDALL S. ALBERTE,2,3 AND ELIZABETH VAN VOLKENBURGH4
Department of Botany, Duke University, Durham, North Carolina 27706

ABSTRACT

In situ and light-saturated net photosynthetic rates per unit leaf area were greater in cotton (Gossypium hirsutum L.) plants grown in pots in the field than in similar plants from a phytotron growth chamber. Light-saturated stomatal resistances did not differ in leaves of similar age and exposure on field and chamber plants; lower photosynthetic rates in chamber leaves were associated with greater mesophyll resistance. Differences in net photosynthetic rates were related to differences in leaf thickness. When the photosynthetic rates were expressed per unit of mesophyll volume or per unit chlorophyll differences between field and chamber plants were much less when rates were expressed per unit leaf area. Characterization of the chloroplast lamellar proteins showed that the field leaves had smaller photosynthetic units than the chamber leaves. Since the field leaves also contained more chlorophyll per unit area, this resulted in a much larger number of photosynthetic units per unit area in the field leaves.

With the increasing use of controlled environment facilities in research in the plant sciences have come questions concerning the comparability of plants grown in controlled and field environments and the use of physiological data obtained in controlled environments to predict plant responses under field conditions. An investigation directed at answering some of these questions is now underway as a cooperative effort between the Duke University and North Carolina State University units of the Southeastern Plant Environment Laboratories (16). As part of this investigation, we studied photosynthetic characteristics of cotton grown in a controlled environment room in the Duke phytotron and in the field.

Previous studies have shown that maximum photosynthetic rates are often greater in field-grown plants than in greenhouse-grown plants (5, 11, 12), but comparisons with plants from conventional growth chambers have seldom been made. Hesketh (13) reported that cotton plants grown in a growth chamber at 3,000 ft-c of fluorescent light had photosynthetic rates similar to winter-grown greenhouse plants. When the chamber-grown plants were given supplemental higher intensity incandescent light during growth, the photosynthetic rate was increased to the level of summer-grown greenhouse plants. These studies indicate that differences in light intensity and light quality during growth have considerable influence on measured photosynthetic rates and illustrate the difficulty of using photosynthesis data obtained from plants grown in different environments to compare the photosynthetic efficiencies of different species and varieties.

In the present study, we found differences in in situ photosynthetic rates under ambient conditions in the field and chamber environments. Measurements of the response of photosynthetic rate to changes in light intensity were then made under standard conditions in the laboratory to determine whether the differences observed in situ could be related to the plant material itself rather than to differences in the ambient light environments. Stomatal diffusive resistance, leaf anatomy, and chloroplast lamellar characteristics were also studied as possible explanations for the observed differences in photosynthetic rates.

MATERIALS AND METHODS

Growing Conditions. Cotton (Gossypium hirsutum L. var. McNair 612) was grown in a growth chamber and out-of-doors in 25-cm plastic pots containing a 1:1 gravel-vermiculite mixture. All of the plants were watered to excess, morning and afternoon, with modified half-strength Hoagland solution. Plants were grown in a chamber programed for a 12-hr thermoperiod with a 26 C/20 C day/night temperature. The chamber photoperiod was 14 hr, comprised of 12 hr fluorescent and incandescent lighting (650 μeinstein m⁻² sec⁻¹ photosynthetically active radiation, 400–700 nm) with 1-hr extensions by the incandescent lamps (50 μeinstein m⁻² sec⁻¹ PAR) preceding and following the 12-hr full light. Daytime relative humidity was maintained at 70% and an automatic CO₂ supplementation system (18) maintained the chamber CO₂ concentration at or above 300 ppm.

The field plants received higher daily total radiation as well as higher peak radiation than the chamber plants (Table 1). The average relative humidity in the field was 60%. For the period of vegetative growth (May through early August), the average daily maximum and minimum air temperatures were 29 C and 18 C, respectively. From these values, average day and night temperatures were calculated by the method of Went (22) to be 26 C and 21 C, respectively, indicating that the temperature regime chosen for the growth chamber was reasonably representative of the field regime.

Photosynthesis Measurements. In situ measurements were made with a small hand-held pincer cuvette similar to one described by Catsky (8). A coated polypropylene film (Propafilm from Imperial Chemical Industries) served as the cuvette win-

---

1 This research was supported by National Science Foundation Grant GI-39229 (RANN).
2 Supported by a National Science Foundation Energy-Related Postdoctoral Fellowship.
3 Permanent address: Department of Biology, University of California, Los Angeles, Calif. 90024
4 Present address: Department of Botany, University of Washington, Seattle, Wash. 98105

5 Abbreviations: PAR: photosynthetically active radiation; PSU: photosynthetic unit.
dow. Air was pumped through both the upper and lower compartments of the cuvette simultaneously, through a mixing chamber on the outlet side of the cuvette, and then to the measurement cell of the Beckman 865 differential IR gas analyzer. A Lambda quantum sensor was attached to the cuvette so that values for CO₂ uptake and incident radiation could be obtained simultaneously. Measurements were made on 20 well exposed canopy leaves in each of the two environments.

Laboratory determinations of the response of photosynthetic rate to changes in irradiance were made with water-jacketed Plexiglas cuvettes enclosing whole attached leaves. A bank of four 150 W GE "cool beam" incandescent flood lamps served as the light source for each cuvette. Irradiance was controlled by a rheostat and was measured with Lambda quantum sensors. The rate of CO₂ uptake was determined on the third fully expanded leaf on four different plants from each of the two environments, at a range of irradiance from 50 to 1750 μeinstein m⁻² sec⁻¹ PAR. Equilibration at each irradiance generally required 20 min or less. Cuvette temperature was maintained at 28 ± 1 °C during the measurements to provide leaf tissue temperatures comparable to those measured in the growth chamber. Cuvette vapor pressure deficit was maintained at 8 ± 1 mmHg by bubbling incoming air through water.

Leaf Resistances. Light-saturated stomatal resistances were determined by the method of Kanemasu et al. (15) for both leaf surfaces on well exposed canopy leaves in the two environments. Resistances to H₂O flux were converted to resistances to CO₂ flux by multiplying by 1.605 (14).

Total resistance to CO₂ flux was calculated using light-saturated photosynthetic rate as the flux and the external CO₂ concentration as the CO₂ concentration gradient (23). From wind speed and leaf dimensions we estimated that the boundary layer resistances of both field and chamber leaves were about 1 sec cm⁻¹ to CO₂ (21). Stomatal and boundary layer resistances to CO₂ flux were subtracted from total resistance to yield mesophyll resistance.

Leaf Anatomy. Stomatal frequency was determined from leaf impressions of upper canopy leaves. A plastic film (automotive ignition sealer) was sprayed onto the leaves, allowed to dry, removed, and observed under magnification. Leaf size did not change with the drying of the plastic spray, indicating that no shrinkage occurred. Peels were removed from leaves using a clear plastic tape which prevented shrinkage or stretching. Counts were made for both surfaces of three leaves from different plants from each environment. The area sampled was intervascular tissue near the center of the leaf. Stomatal counts were made on five randomly chosen spots (area of .063 mm²) on each surface.

Leaf thicknesses were determined by microscopic observation of leaf cross-sections. Samples about 1 × 3 cm were cut from intervascular tissue near the center of upper canopy leaves from each environment. Five fully expanded upper canopy leaves were sampled from each environment. Sectioning was done under water with a model G Oxford vibratome on samples mounted in pith. Five sections were made per leaf sample, and five observations of total, palisade, spongy mesophyll, and epidermal thickness were made per sample using an ocular micrometer.

Specific leaf weight was determined from discs (1.13 cm² area) obtained from exposed canopy leaves. Weights were determined after oven-drying at 65°C.

Characterization of Chloroplast Lamellae. Chloroplast lamellae were prepared from leaves by methods described previously (3). The washed lamellae were then solubilized in Triton X-100 following the procedures of Shiozawa et al. (20). The light-induced oxidation of P700 was measured in the Triton extracts (20), and the PSU size was calculated from the ratio of total Chl to P700 in the samples (2). The density of PSUs per unit leaf area was calculated from the Chl content per unit area and the PSU size. Leaf Chl content and Chl a/b ratios were determined from 80% acetone extracts by the method of Arnon (4).

RESULTS

Exposed canopy leaves on field-grown cotton plants had in situ net photosynthetic rates per unit leaf area approximately twice as great as rates determined in situ for similar leaves on chamber-grown plants (Table I). Average PAR intensities during the field measurements were about three times greater than in the chamber.

When measured in the laboratory under a series of irradiances, the field plants had higher net photosynthetic rates per unit leaf area throughout the range of irradiances used, but the differences were greatest above 750 μeinstein m⁻² sec⁻¹ (Fig. 1). Light saturation occurred at about 750 μeinstens m⁻² sec⁻¹ in the chamber plants and 1500 μeinstens m⁻² sec⁻¹ in the field plants. Net photosynthetic rates at light saturation averaged 43 mg CO₂ dm⁻² hr⁻¹ in the field plants and 28 mg CO₂ dm⁻² hr⁻¹ in the chamber plants (Table II). Light-saturated stomatal resistance to CO₂ flux was about 2.4 cm sec⁻¹ in both field and chamber leaves (Table III). Resistances on the lower surface were less than upper surface, corresponding to the pattern of stomatal frequencies (Table III). Stomatal frequencies (stomates/mm²) were greater in the field plants for both leaf surfaces (Table III). Pore sizes were not measured, but chamber leaves can be deduced to have less resistance per pore than field leaves.

### Table 1. Summary of Environmental Conditions in the Growth Chamber and Field Flot.

<table>
<thead>
<tr>
<th></th>
<th>Chamber</th>
<th>Field</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>26/20</td>
<td>29/18</td>
</tr>
<tr>
<td>Avg day/night</td>
<td>26/20</td>
<td>26/21</td>
</tr>
<tr>
<td>Avg total daily radiation (cal cm⁻²)</td>
<td>240</td>
<td>450</td>
</tr>
<tr>
<td>(μE m⁻²) (PAR)</td>
<td>2.48 × 10⁷</td>
<td>4.20 × 10⁷</td>
</tr>
<tr>
<td>Peak radiation</td>
<td>600–700</td>
<td>2000–2200</td>
</tr>
</tbody>
</table>

### Table 2. Net Photosynthetic Rates and Calculated Light-saturated Mesophyll Resistance for Field and Chamber Cotton Leaves. Values in Parentheses are Standard Errors of the Means. PAR = Photosynthetically Active Radiation, 600–700 nm.

<table>
<thead>
<tr>
<th></th>
<th>Chamber</th>
<th>Field</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum photosynthetic rates of exposed canopy leaves in situ</td>
<td>15.0 (0.4)</td>
<td>32.5 (1.1)</td>
</tr>
<tr>
<td>(mg CO₂ hr⁻¹ dm⁻² leaf area)</td>
<td>8.8 (0.2)</td>
<td>14.5 (0.5)</td>
</tr>
<tr>
<td>(mg CO₂ hr⁻¹ cm⁻² mesophyll)</td>
<td>580 (11)</td>
<td>1803 (30)</td>
</tr>
<tr>
<td>Light-saturated photosynthetic rates</td>
<td>28.1 (0.8)</td>
<td>43.0 (1.7)</td>
</tr>
<tr>
<td>(mg CO₂ hr⁻¹ dm⁻² leaf area)</td>
<td>16.5 (0.5)</td>
<td>19.2 (0.8)</td>
</tr>
<tr>
<td>(mg CO₂ hr⁻¹ cm⁻² mesophyll)</td>
<td>8.0 (0.3)</td>
<td>7.7 (0.3)</td>
</tr>
<tr>
<td>Mesophyll resistance</td>
<td>4.5</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Copyright © 1977 American Society of Plant Biologists. All rights reserved.
Calculated light-saturated mesophyll resistances to CO₂ flux were 4.5 sec cm⁻¹ for the chamber leaves and 1.8 sec cm⁻¹ for the field leaves.

The greater total thickness of the field leaves was due to thicker palisade and spongy mesophyll layers (Table III). The total epidermal thickness was greater in the chamber leaves. Thus, total mesophyll volume per unit area was considerably greater in the field leaves (2.24 cm³ dm⁻²) than in the chamber leaves (1.70 cm³ dm⁻²). The greater thickness of the field leaves was also reflected in their higher specific leaf weights (0.534 g dm⁻² compared to 0.348 g dm⁻² for the chamber leaves).

The thicker field leaves contained more Chl per unit area than the chamber leaves (Table III). The greater Chl content and smaller PSU size in the field leaves can explain the presence of more PSUs per unit leaf area in the field leaves. On a mesophyll volume basis, the difference in the number of PSUs in the two types of leaves is much smaller. The differences in the PSU size and the Chl a/b ratios in the two leaf types are fully accounted for by differences in the lamellar content of the light-harvesting Chl a/b protein (Table III) which has been shown to vary in response to irradiance during growth and to be reflected in the content of Chl b present (1, 7).

**DISCUSSION**

The differences in the maximum photosynthetic rates observed *in situ* were greater than the differences in light-saturated rates because of the higher irradiance in the field during the *in situ* measurements. These greater differences would be predicted from the shapes of the photosynthesis-irradiance response curves obtained under laboratory conditions. The photosynthetic response curves for the chamber and field leaves (Fig. 1) indicate that under the ambient chamber irradiance during the *in situ* measurements (500–700 μeinstein m⁻² sec⁻¹ PAR), the chamber leaves would be operating at about 80% of their rate at light saturation, whereas the field leaves would be at light saturation at the irradiance levels in the field during the *in situ* measurements.

Light-saturated stomatal resistances were similar in field and chamber leaves and the higher photosynthetic rates of field leaves under standard conditions were due to lower mesophyll resistances. Our estimate of mesophyll resistance reflects the total nonstomatal limitation to CO₂ uptake, and does not differentiate between possible limitations due to diffusive transfer within the leaf, photochemical reactions, biochemical reactions, or respiration. Nobel et al. (17) found thicker leaves to have lower mesophyll resistances (per leaf area) because of increased mesophyll surface area. If, as the data of Nobel et al. (17) suggest, leaf thickness differences directly reflect differences in mesophyll surface area, then we might expect differences in leaf thickness to account for differences in mesophyll resistance. In the present study, the differences in mesophyll resistance were greater than the differences in either leaf or mesophyll thickness (ratios of chamber to field were 2.50, 0.83, and 0.76 respectively). It seems that differences in the diffusive pathways of CO₂ do not completely account for the observed differences in photosynthetic rates in the field and chamber leaves.

The field plants had more mesophyll volume per area, and when photosynthesis was expressed on a mesophyll volume basis (Fig. 2 and Table II), the rates of chamber and field leaves were more alike. Nobel et al. (17) found that the higher light-saturated photosynthetic rates of *Plectranthus parviflorus* Henckel grown in high light were due to the increase in mesophyll surface area per unit leaf area rather than to changes in the photosynthetic activity per unit of mesophyll area. Similarly, Charles-Edwards and Ludwig (9) reported that in *Lycopersicon esculentum* Miller grown in different light environments, light-saturated photosynthetic rates per unit leaf volume differed only slightly, whereas rates per unit leaf area differed by a factor of 1.9 when plants grown at 20 and 80 w m⁻² were compared.

Other studies have reported strong correlations between light-saturated photosynthetic rates and carboxylation enzyme activities when both are expressed on a leaf area basis (6, 10). Such studies typically have not taken into account differences in leaf thickness or volume per unit area. It now appears that when plants from different light environments are compared, changes in leaf tissue volume are likely to be more important than changes in activity per unit of volume.

The amounts of Chl per unit area, the numbers of PSUs per unit area, and, presumably, the amount of carboxylation enzyme per unit area, and mesophyll surface area per unit leaf area are...
all closely correlated with leaf mesophyll volume. All of these factors may contribute to the differences in light-saturated photosynthetic rates between the field and chamber leaves. It is interesting to note that the ratio of Chl content per unit area in the chamber leaves to Chl content per unit area in the field leaves (0.62) is almost identical to the ratio of light-saturated photosynthetic rates per unit area in the same leaves (0.65). Consequently, the light-saturated rates per mg Chl are quite similar (Table II), suggesting that total Chl content may be a better indicator than leaf area of the photosynthetic potential (see also Sestak [19]).

In summary, when photosynthetic rates are expressed on mesophyll volume basis, plants from controlled and field environments can be shown to be more alike than when rates are expressed on an area basis (compare Figs. 1 and 2). The choice of the basis for the expression of photosynthetic rates can aid considerably in the extrapolation of results from phytotron studies to the field. This is particularly important in the construction and application of models of the photosynthetic process.

Acknowledgments—We thank P. J. Kramer and M. M. Peet for their help in the preparation of this manuscript.

LITERATURE CITED