1984

Leaf physiology and anatomy of soybean genotypes differing in leaf photosynthetic ability

Duane Merlin Ford
Iowa State University

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LEAF PHYSIOLOGY AND ANATOMY OF SOYBEAN GENOTYPES DIFFERING IN LEAF PHOTOSYNTHETIC ABILITY

Iowa State University

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Ph.D. 1984
Leaf physiology and anatomy of soybean genotypes differing in leaf photosynthetic ability

by

Duane Merlin Ford

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

Department: Agronomy
Major: Crop Production and Physiology

Approved:

Signature was redacted for privacy.

In/Charge of Major Work

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa
1984
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>iii</td>
</tr>
<tr>
<td>GENERAL INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>SECTION I: CAUSES OF DIFFERENCES AMONG SOYBEAN LINES BRED FOR DIVERGENT LEAF PHOTOSYNTHESIS</td>
<td>13</td>
</tr>
<tr>
<td>SECTION II: PHOTOSYNTHESIS IN RELATION TO CHLOROPLAST NUMBER AND QUALITY IN SOYBEAN</td>
<td>29</td>
</tr>
<tr>
<td>GENERAL DISCUSSION</td>
<td>53</td>
</tr>
<tr>
<td>ADDITIONAL LITERATURE CITED</td>
<td>56</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>60</td>
</tr>
</tbody>
</table>
DEDICATION

I dedicate this dissertation to four Illinois farmers, who have seen nearly the entire history of American soybean production in their own fields and who are my grandparents.

Merlin Lambert
Lillian Lambert
Romeyn Ford
Bertha Ford
GENERAL INTRODUCTION

The photosynthetic rate per unit leaf area expressed by a leaf is affected by a variety of factors. Exogenous factors include irradiance, temperature, the availability of nutrients and water, and in an experimental setting, the atmospheric concentration of CO$_2$ ($C_a$) and O$_2$. These environmental factors may influence single-leaf apparent photosynthesis per unit leaf area (AP) transiently or persistently depending on the time and duration of their application.

Genotype also influences AP. Among other reasons, AP may differ among species because of differing CO$_2$ fixation pathways: C$_3$, C$_4$, or CAM. Intraspecific differences in AP may be a consequence of the developmental history of both the leaf and the plant to which it is attached, as well as the genotype. The main objective of the experiments herein reported was to determine what might cause genotypic differences in AP in soybean [Glycine max (L.) Merr.]. This section will review those concepts and experimental efforts which have contributed to the understanding of how genotype influences AP intraspecifically. Emphasis will be given to the literature on soybean.

Photosynthesis can be thought of as having three component processes. These are (1) CO$_2$ diffusion from the atmosphere to the site of fixation, (2) photosynthetic carbon fixation and reduction (PCR cycle), and (2) solar energy
transduction via photosynthetic electron transport (PET).

Given two leaves with different AP, the leaf with slower AP can be thought of as being limited. Gaastra (1962) and Björkman (1964) outlined the classic experiment which can determine which component process limits AP. It is based on the hypothesis that CO₂ diffusion, the PCR cycle, and PET depend on C_a, leaf temperature (T₁), and photosynthetic photon flux density (PPFD), respectively. They ignored, or considered insignificant, the dependence of each component process on variables associated with the other two processes. In practice, they measured the dependence of AP on each variable while holding the other two variables constant. AP differences attributable to variation in CO₂ diffusivity presumably would show up in the comparison of each leaf's response to C_a changes, PCR cycle variation in the responses to T₁ changes, and differences in PET from the responses to PPFD.

The dependence of AP on PPFD at low irradiances, where irradiance alone limits AP, varies little among soybean genotypes (Ojima and Kawashima, 1970; Dornhoff and Shibles, 1974). Therefore, differences in the maximum efficiency of PET cannot explain genotypic variation in AP. However, low irradiance studies do not characterize the maximum capacity or activity of PET, only its efficiency. At high, saturating irradiances, both groups of investigators found genotypic
differences in AP (Dornhoff and Shibles, 1970, 1976; Ojima and Kawahima, 1968); a finding corroborated by a host of other researchers (Elmore, 1980).

Only one experiment has been reported that compares the temperature dependence of AP of several soybean genotypes. It showed that genotypes respond similarly to changes in $T_1$. In fact, AP did not respond to $T_1$ between 26 and 35 C for any of the genotypes tested (Dornhoff and Shibles, 1974). Because the temperature dependence of photosynthesis depends primarily on the relative amounts and temperature dependencies of the PCR cycle enzymes, this result suggests that the PCR cycle is qualitatively similar among genotypes. However, it does not rule out the possibility that genotypic variation for total PCR cycle capacity exists.

For soybean, AP is a linear function of $C_a$ below 300 to 400 μL CO$_2$/L air. The slope of the line which describes AP as a function of $C_a$ estimates the inverse of the total resistance to CO$_2$ diffusion into and fixation by the leaf ($\Sigma r'$). The slope depends on (1) the length of the diffusional path­way, (2) the size of the region through which CO$_2$ may diffuse, and (3) the capacity of the leaf for CO$_2$ fixation. The total resistance ($\Sigma r'$) is the sum of the resistances to CO$_2$ diffusion through the leaf boundary layer ($r_a'$), the stomata ($r_s'$), and the mesophyll ($r_m$)--the resistance to CO$_2$ flux from the cell wall to the site of fixation--plus the so-called
carboxylation resistance \( (r_X) \) (Farquhar and Sharkey, 1982). Brun and Cooper (1967) and Dornhoff and Shibles (1970) found that \( \Sigma r' \) varied with AP among genotypes. Using Gaastra's (1959) techniques for quantifying \( r'_a, r'_s, \) and \( r'_m + r'_x \), crop physiologists have set out to determine which impediment to \( \text{CO}_2 \) diffusion or fixation limits AP the most.

All the resistances were more or less negatively correlated with AP (Dornhoff and Shibles, 1970, 1976; Bhagsari et al., 1977; Kaplan and Koller, 1977). But, attention was focused primarily on \( r'_s \) and \( r'_m + r'_x \). The correlations of these resistances with AP and their relative magnitudes (\( r'_s \) is about equal to \( r'_m + r'_x \)) suggest one to be no more limiting to AP than the other. Farquhar and Sharkey (1982) introduced a way to estimate the percent inhibition of AP by \( r'_s \). They did not use the technique to assess the relative contribution of genotypic differences in \( r'_s \) to AP differences, but for most environmentally induced differences, which generally are greater than genotype differences in AP, the limitation imposed by stomata is suggested to be relatively small (Farquhar and Sharkey, 1982).

The residual resistance, or \( r'_m + r'_x \), offers a dilemma: which is the more important of the two resistances? The difficulty is that neither can be directly measured. However, experimental and theoretical efforts have shed some light on this question.
Park S. Nobel and his co-workers have shown that, in some situations, differences in AP are not caused by either $r_m$ or $r_x$, but rather by the size of the region through which CO$_2$ can diffuse into the mesophyll cells. That is, diffusion will proceed more rapidly in leaves with a greater ratio of mesophyll cell surface area to leaf surface area ($A_m/A$). Nobel's group primarily has studied environmentally induced differences in AP (Nobel et al., 1975; Nobel, 1977). Among *Lolium perenne* (L.) genotypes, AP differences are inversely associated with differences in cell size (Wilson and Cooper, 1970). Because leaf dimensions were not altered, Wilson and Cooper concluded that cell number did not vary with cell size; therefore, smaller cells would result in greater $A_m/A$. But, in soybean, Dornhoff and Shibles (1976) observed no consistent association between $A_m/A$ and AP, and found no advantage for genotypes with small cells.

Chartier et al. (1970) and Prioul and Chartier (1977) concluded, on theoretical grounds, that $r_m$ limits AP more than $r_x$. But Raven and Glidewell (1981), using a different theoretical approach, concluded the opposite. Indeed, they held that limitations imposed by the enzymes of the PCR cycle, particularly ribulose 1,5-bisphosphate (RuBP) carboxylase, contributed the most to $r_m + r_x$.

There appears to be good evidence that, under normal conditions and excluding the effect of stomata, AP is co-
limited by the activity of RuBP carboxylase and the leaf's ability to regenerate that enzyme's substrate, RuBP. In brief, the originators of this concept (Farquhar, 1979; Farquhar et al., 1980; von Caemmerer and Farquhar, 1981; Farquhar and Sharkey, 1982) believe that, at lower than ambient \( C_a \), RuBP regeneration is adequate to saturate the enzyme, but at higher than ambient \( C_a \), the photochemical apparatus cannot produce sufficient reducing power to regenerate saturating amounts of RuBP. So at ambient \( C_a \), AP is thought to be co-limited by the capacities for RuBP carboxylation and RuBP regeneration. Each component therefore is used efficiently and von Caemmerer and Farquhar (1981) conclude that AP at ambient \( C_a \) "... can only be increased if the capacities of both components are increased." This concept is consistent with \( r_x \) being the major limitation to AP, because that resistance reflects the rapidity with which \( CO_2 \) can be fixed in the leaf. It is also consistent with the finding that the efficiency of PET and the quality of the PCR cycle do not vary among genotypes. Indeed, the concept implies that these latter factors do not vary, only the amount of photosynthetic apparatus, or total capacity of the leaf, determines AP. Raven and Glidewell (1981) even suggest that this concept is consistent with the finding that variation in \( A_m/A \) explains some or all of the variation in AP. They point out that (1) \( A_m/A \) is probably correlated with
total photosynthetic capacity and (2) a large amount of photosynthetic apparatus requires a high $A_m/A$ to maintain a low $r_m$.

Direct evidence for this concept is not plentiful. That which is available comes from information about AP response to intercellular concentration of CO$_2$ ($C_i$)—this technique removes the effect of $r_a'$ and $r_s'$. Evidence that RuBP levels in the leaf vary in response to increasing $C_i$ in the manner predicted by the concept—greater than saturating at $C_i$ less than that in ambient $C_a$, less than saturating at greater $C_i$—was obtained by Collatz (1978) using Chlamydomonas and spinach (Spinacia oleracea L.) cells, and Mott et al. (1983) using whole Xanthium strumarium (L.) leaves. Others found that soybean RuBP levels do not vary as predicted (Hitz and Stewart, 1980; Creach and Stewart, 1982; Vu et al., 1983), neither did those in wheat (Triticum aestivum L.) leaves (Perchorowicz and Jensen, 1983).

Three studies show that AP at low $C_i$ depends on the capacity of RuBP carboxylation. (1) von Caemmerer and Farquhar (1981) found *in vitro* RuBP carboxylase activity per unit leaf area to be well-correlated with $r_m + r_x$ in Phaseolus vulgaris (L.) leaves, where AP differences were generated by different N-fertility levels, irradiance regimes, or $C_a$ during growth. This supports the idea that $r_x$ is the more important determinant of AP under those conditions. (2) Seeman and Berry (1982) also used different irradiance and
nutrient regimes to induce variation in AP in soybean. By using the kinetic constants of purified RuBP carboxylase and the amount of RuBP carboxylase per unit leaf area, they could predict AP at 100 μL CO₂/L air Cᵢ and 2% O₂. (3) Also with soybean, Laing et al. (1974) found that the kinetics of RuBP carboxylase explained the temperature response of AP in subambient CO₂ environments. Evidence that RuBP regeneration limits AP at high Cᵢ is provided by the work of von Caemmerer and Farquhar (1981). They showed that Cᵢ saturated AP is closely associated with the in vitro rate of electron transport per unit leaf area.

Because rₓ and rₚ are not zero, they contribute to the co-limitation of AP under normal circumstances. Farquhar and Sharkey (1982) conclude that the contribution is small for environmentally induced differences in AP; it remains to be seen if this is true for genotypically induced variation in AP.

The concept that I have described and discussed evidence for suggests that genotypic differences in AP likely result from associated differences in the amount of photosynthetic apparatus per unit leaf area. The results from two experiments are particularly supportive of this notion. First, Hesketh et al. (1981) found that, among 29 soybean genotypes, AP was correlated with a variety of leaf parameters: specific leaf mass (SLM—r=0.49), RuBP carboxylase activity (0.79),
chlorophyll (0.59) and soluble protein (0.57), where the latter three were expressed per unit leaf area. Second, Watanabe (1973a,b) found AP to be correlated with the amount of chlorophyll (0.97), number of chloroplasts (0.88), and PETS activity (0.99)—all expressed per unit leaf area—in the unifoliolate leaves of five soybean genotypes. Furthermore, by measuring AP on trifoliolate leaves exposed to regular flashes of PPFD, Watanabe was able to assess the relative balance of the activities of the light and dark reactions of photosynthesis. He found that the balance was identical for all five genotypes, suggesting that the quality of the photosynthetic apparatus is invariate, but the amount per unit leaf area changes in association with AP.

Other researchers have found AP to be correlated with the per unit leaf area amounts of chlorophyll and/or soluble protein (Buttery and Buzzell, 1977; Secor et al., 1982; Buttery et al., 1981), but the relationships are not universal (Buttery et al., 1981). Similarly, SLM often has been shown related to AP (Dornhoff and Shibles, 1970, 1976; Wiebold et al., 1981; Buttery et al., 1981), but just as often, there seems to have been no relationship (Watanabe and Tabuchi, 1973; Secor et al., 1982) or results were inconsistent (Ojima and Kawashima, 1968; Bhagsari et al., 1977; Kaplan and Koller, 1977). There are several possible reasons for these inconsistencies. (1) AP does not depend on the total amount of
photosynthetic apparatus per unit leaf area after all. (2) The measurements of AP do not reflect the total capacity of the leaf. (3) The measured leaf traits—chlorophyll, soluble protein, SLM—are not related to the total amount of photosynthetic apparatus.

Despite these inconsistencies, the concept developed by Farquhar and his colleagues provides a useful and fresh approach to the study of genotypic differences in AP. It is not likely that AP is universally limited by any single physiologic, anatomic, or biochemical trait; an idea that was foreshadowed by the fact that AP is quantitatively inherited (Izhar and Wallace, 1967; Wiebold et al., 1981). It is more likely that AP depends on the integrated functioning of all the components of the photosynthetic system. In such a system, no component should be produced in excess, so that all components would be used efficiently.

Explanation of the Dissertation Format

This dissertation has been prepared under the alternate format. The following two sections consist of two research papers written in a format suitable for publication in Crop Science. Both papers deal with the question of what causes genotypic differences in AP in soybean. The first is a report of results from an experiment conducted in 1982. That experiment was similar to the classic experiments suggested by
Gaastra (1962) and Björkman (1964). The objective was to determine which component process causes AP differences among experimental lines bred for divergent rates of CO₂ uptake. The second paper is derived from work carried out in 1983. The general aim of it was to confirm and elucidate the role of total photosynthetic capacity (or apparatus—I tend to use these words interchangeably) in explaining AP differences among adapted soybean cultivars. A final section of this dissertation summarizes and discusses both papers. References cited in the General Introduction and General Discussion are listed under Additional Literature Cited; those cited in the two papers are listed in the References sections at the end of each paper.

For both experiments, I conceived the problem to be studied, designed and conducted the experiment, analyzed the data, and wrote the reports. The contributions of my co-author, Dr. Richard Shibles, included advising me at every step and editing the reports which follow.
CAUSES OF DIFFERENCES AMONG SOYBEAN LINES BRED FOR DIVERGENT LEAF PHOTOSYNTHESIS

Duane Merlin Ford and Richard Shibles

Running head: Photosynthesis Differences Among Soybean Lines

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Ames, Iowa 50011


2/ Graduate Research Assistant and Professor, Dept. of Agronomy, Iowa State University, Ames, IA 50011.
SECTION I: CAUSES OF DIFFERENCES AMONG SOYBEAN LINES BRED FOR DIVERGENT LEAF PHOTOSYNTHESIS

Abstract

To facilitate the selection of soybean \( [\text{Glycine max (L.) Merr.}] \) genotypes with improved rates of apparent photosynthesis per unit leaf area (AP), knowledge of the physiologic or anatomical factors that cause genotypic variation in AP would be useful. In previous work, we bred lines with divergent leaf photosynthetic rates. The objective of this experiment was to determine the causes of that divergence. Two rapid and two slow photosynthesizing lines from the original population were grown in the field. About the beginning of rapid seed growth, the dependence of AP on (1) atmospheric \( \text{CO}_2 \) concentration and (2) photosynthetic photon flux density, each at two different leaf temperatures (28 and 18 C), was measured for recently expanded, terminal leaflets. Leaves were harvested for measurement of leaf and cell dimensions.

Evidence indicates that neither stomatal conductance of \( \text{CO}_2 \), nor mesophyll cell size or number, nor the efficiency of the photochemical or enzymatic reactions are causative. This implicates factors internal to the cell. The residual resistance tended to be less for the two rapid photosynthesis lines at 28 C, though the effect was not significant. The evidence in toto implies that amount of photosynthetic material per unit leaf area mediates AP differences among
these soybean lines.

Introduction

This work is a continuation of studies designed to understand and improve leaf photosynthesis in soybean \textit{[Glycine max (L.) Merr.]}]. Dornhoff and Shibles (1970) reported that apparent photosynthesis per unit leaf area (AP) varied among soybean genotypes. This led to an attempt to breed lines with improved leaf AP. Selection for rapid or slow AP among the early generation ($F_2$ and $F_3$) progeny of crosses between Midwest cultivars with rapid and slow AP proved ineffective (Wiebold et al., 1981). But, within a population of 110, $F_6$-derived lines from one of the same crosses ('Amsoy' by 'Ford'), Secor et al. (1982) were able to select two photosynthetically divergent groups based on their rates of $^{14}\text{CO}_2$ uptake. A group of nine rapid photosynthesis lines have consistently outperformed a group of nine slow lines over three years and several locations since the original selection. However, the rate of $\text{CO}_2$ uptake was not correlated with either seed or biomass yield (Ford et al., 1983). The objective of this work was to determine which physiologic or anatomic traits vary with AP, and therefore, might cause divergence for AP within this population.
Materials and Methods

Four, F_7-derived soybean lines from the cross Amsoy by Ford were field grown at ISU's Hinds Research Center near Ames. Two lines selected for rapid ('155' and '175') and two for slow ('47' and '143') rates of photosynthesis per unit leaf area were used. Before planting, 26 kg P/ha and 84 kg K/ha were applied, and alachlor and chloropropham were incorporated. Planting in 9.8 m replicated rows was on 14 June 1982. Irrigation water was applied as needed throughout the season to minimize water stress.

Sampling

On each of 10 days after the beginning of rapid seed growth (R5), 12 recently expanded leaves were sampled, three for each line. On 12, 13, and 19 August, CO_2 and H_2O exchange responses of terminal leaflets to increasing atmospheric CO_2 concentrations (C_a), from 100 to 450 μL CO_2/L air, were measured at 28 C and a photosynthetic photon flux density (PPFD) of 130 nmol photons/s cm^2; the same responses but at 18 C were measured on 9, 18, and 20 August. To estimate efficiency of photochemical reactions, AP responses to low PPFD, 13 and 20 nmol photons/s cm^2, at 28 C and 316 μL CO_2/L air were measured on 14 and 16 August; the responses to PPFD at 18 C were measured on 17 and 21 August.

At the end of each day, the sampled leaves were harvested.
One lateral leaflet was used to prepare leaf clearings for anatomical measurements. Leaf area, measured with a LI-3000 (Li-Cor Inc., Lincoln, NE) area meter, and dry mass of the other lateral leaflet were determined for calculation of specific leaf mass (SLM). Leaf area of the terminal leaflet (LA) was also measured.

Gas exchange and leaf temperature

A Beckman (Beckman Instruments, Inc., Fullerton, CA) Model 865 infra-red gas analyzer was used to measure CO$_2$-exchange in a flow-through leaf chamber system. Relative humidity probes (Weathermeasure Corp., Sacramento, CA), which were inserted in the air stream before and after the leaf chamber, were used to measure H$_2$O-exchange. We measured leaf temperatures with copper-constantan thermocouples linked to a Bat-4 (Bailey Instruments, Inc., Saddle Brook, NJ) thermometer. Air flow rates were monitored with Matheson (Joliet, IL) rotameters.

The CO$_2$ and H$_2$O contents of the air flowing to the leaf chamber were adjusted by (1) dispersal of air in 6 N KOH at -15 C to provide a constant water vapor pressure and remove CO$_2$ and (2) by addition of appropriate amounts of CO$_2$ and H$_2$O back to the air. Processed air was pumped to the leaf chamber and, except for a sample that was withdrawn for gas analysis, exhausted to the atmosphere.

The leaf chamber consisted of a plexiglass box, large
enough to accommodate an entire leaflet, built around a Peltier heat exchanger (Cambridge Thermionic Corp., Cambridge, MA). The leaflet was held between two nylon wire grids, one in the box about 2 cm over the heat exchanger, the other in the lid. A model airplane propellor, mounted on a small, 6 V, electric motor embedded in the heat exchanger, mixed the air within the chamber. A 1000 W metal halide lamp (Westinghouse Electric Corp., Bloomfield, NJ) mounted above the chamber provided light. PPFD was altered by laying filters made of black wire screens on the top of the chamber.

AP and leaf resistance \( (r'_l) \) were calculated from published formulas (Šesták et al., 1971). Intercellular \( \text{CO}_2 \) concentration \( (C_i) \) was calculated as \( C_a - (AP \times r'_l) \). The initial slopes of the AP response to \( C_i \) and PPFD were used to determine residual resistances \( (r'_r) \) and the relative efficiencies of the photochemical reactions, respectively.

**Anatomical measurements**

Dornhoff and Shibles (1976) outline the procedures used to count and measure leaf cells from paradermal leaf clearings. We modified their procedures for calculating the surface area of mesophyll cells per unit leaf area \( (A_m/A) \) to account for the ends of the palisade cells (assuming these ends to be hemispherical).
Results and Discussion

Genotype effects

Figures 1 and 2 show the lines' responses to \( C_i \) at two leaf temperatures. Tables 1 and 2 give line means for the leaf physiologic and anatomic traits, respectively. These means were determined for the same leaves that were used to develop Figures 1 and 2. There were no date by line interactions.

The lines differed with respect to AP at both temperatures at each \( C_i \) level, except the one associated with supra-ambient \( C_a \). As expected, lines 155 and 175 showed more rapid photosynthetic rates than lines 47 and 143. The range at 28 C and ambient \( C_a \) was 22% of the overall mean, similar to the 28 and 20% found by Secor et al. (1982) and Ford et al. (1983) for these same lines. The range at 18 C and ambient \( C_a \) was 23% of the overall mean.

Leaf resistance \( (r_1') \) did not vary significantly among lines at either temperature (Table 1). Therefore, the variation in AP seems not to be caused by differences in stomatal conductance. Farquhar and Sharkey (1982) formulated an equation for calculating, from the AP response to \( C_i \), the percent inhibition of AP by \( r_1' \). According to their equation, \( r_1' \) inhibited AP of our lines by 15% (line 47), 10% (143), 14% (155), and 10% (175) at 28 C. Although these data could not be analyzed statistically, the differences among lines were
Table 1. Line means for leaf and residual resistances

<table>
<thead>
<tr>
<th>Line</th>
<th>47</th>
<th>143</th>
<th>155</th>
<th>175</th>
<th>Sign.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r^\prime$ (s/cm)$^b$</td>
<td>1.31</td>
<td>1.25</td>
<td>1.21</td>
<td>1.37</td>
<td>ns</td>
</tr>
<tr>
<td>$r^\prime$ (s/cm)</td>
<td>3.77</td>
<td>3.24</td>
<td>2.37</td>
<td>2.68</td>
<td>ns</td>
</tr>
</tbody>
</table>

Leaf temperature = 28 (0.3)$^a$

| $r^\prime$ (s/cm)$^c$ | 1.38 | 1.53 | 1.26 | 1.40 | ns |
| $r^\prime$ (s/cm) | 6.99 | 4.50 | 4.52 | 4.47 | ns |

Leaf temperature = 18 (0.2)$^a$

---

$^a$The mean plus or minus the number in parentheses gives the 95% confidence limits. ns = nonsignificant.

$^b$In ambient conditions: $C_a = 322 (4.3) \mu$L CO$_2$/L air; PPFD = 132 (2.1) nmol photons/s cm$^2$; leaf to air vapor pressure deficit = 1.51 (0.11) kPa.

$^c$In ambient conditions; $C_a = 320 (2.9) \mu$L CO$_2$/L air; PPFD = 134 (2.1) nmol photons/s cm$^2$; leaf to air vapor pressure deficit = 0.99 (0.07) kPa.
Table 2. Line means for anatomic traits

<table>
<thead>
<tr>
<th></th>
<th>Line</th>
<th></th>
<th></th>
<th></th>
<th>Sign.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>47</td>
<td>143</td>
<td>155</td>
<td>175</td>
<td></td>
</tr>
<tr>
<td>LA (cm²)</td>
<td>83.2</td>
<td>77.7</td>
<td>72.1</td>
<td>83.6</td>
<td>*</td>
</tr>
<tr>
<td>SLM (mg/cm²)</td>
<td>3.64</td>
<td>4.37</td>
<td>4.03</td>
<td>3.85</td>
<td>**</td>
</tr>
<tr>
<td>LT (µm)</td>
<td>116</td>
<td>129</td>
<td>134</td>
<td>133</td>
<td>*</td>
</tr>
<tr>
<td>Aₘ/A</td>
<td>17.4</td>
<td>18.0</td>
<td>17.8</td>
<td>18.4</td>
<td>ns</td>
</tr>
</tbody>
</table>

*,**Indicate a significant difference among line means at the 5 and 1% probability levels, respectively. ns = nonsignificant.

Small and there was no apparent association with AP. Similar data are not reported for 18 C because negative percent inhibitions resulted (Figure 2). Another argument against a role for r₁' in causing the variation in AP is that Cᵣ did not vary significantly among lines in ambient Cₑ at 28 C. And at 18 C, Cᵣ varied among lines but not with AP, as it must if r₁' causes photosynthetic variation (Farquhar and Sharkey, 1982).

The efficiencies of the photochemical reactions, as judged by the slopes of the AP responses to PPFD at low levels, did not vary among lines at 28 C. This agrees with the work of Ojima and Kawashima (1970) and Dornhoff and Shibles (1974), but Watanabe (1973) found that these slopes and light saturated photosynthetic rates were correlated
Figure 1. The dependence of apparent photosynthesis per unit leaf area (AP) on intercellular CO₂ concentration ($C_i$) at 28 C (leaf temperature) for lines 47 (○), 143 (●), 155 (○), and 175 (●); vertical and horizontal LSD bars are for differences between lines in AP and $C_i$, respectively, at the 5% probability level.
Figure 2. The dependence of apparent photosynthesis per unit leaf area (AP) on intercellular CO$_2$ concentration ($C_i$) at 18°C (leaf temperature) for lines 47 (○), 143 (●), 155 (○), and 175 (■); vertical and horizontal LSD bars are for differences between lines in AP and $C_i$, respectively, at the 5% probability level.
among six soybean cultivars. However, the relationship in Watanabe's data might well have been caused by the inclusion of one chlorophyll deficient genotype which had a very low slope and slow AP. At 18 C, the slopes of the AP responses to low PPFD varied among lines. And the variation was associated with variation in AP at high irradiance ($r^2=0.97$). However, lines did not differ significantly in AP at either 13 or 20 nmol photons/s cm$^2$, the two points upon which the slopes were calculated, and extrapolating the AP versus PPFD curve to the axis of ordinates yielded some positive intercepts. Therefore, there is some doubt whether differences in these slopes at 18 C are real or meaningful.

If the AP response to temperature depends on (1) the temperature dependencies of the photosynthetic enzymes and (2) the amounts of the enzymes relative to one another, then genotypic variation for this response would suggest that the enzymatic reactions are qualitatively different for different genotypes. But the lines responded to temperature similarly: with a 10° decrease in temperature, AP declined by 18, 15, 16, and 11%, while $r_t$ increased by 85, 54, 91, and 67% for lines 47, 143, 155, and 175, respectively. Therefore, qualitative differences in the enzymatic reactions do not appear to explain AP differences.

Among anatomical traits, lines varied with respect to SLM, LA, and leaf thickness (LT) (Table 2). SLM did not account
for very much of the variation in AP: 14% at 28 C. LA did not vary with AP, though the most rapid line (155) had the smallest leaves and the slowest line (47) the largest. The rapid photosynthesis lines tended to have thicker leaves than the slow lines. Hesketh et al. (1981) suggested that small, thick leaves may have an advantage over large, thin leaves after studying 29 soybean cultivars, but we did not find a similar association among six cultivars (Section II). If there is an advantage, it and the differences in line AP were not a consequence of variation in $A_m/A$, which did not differ among lines. Dornhoff and Shibles (1976) also found $A_m/A$ not to be correlated with AP among genotypes.

In fact, they concluded that factors internal to the cell were responsible for the photosynthetic differences they observed. Because we cannot attribute variation in AP to $r_f$ or $A_m/A$ in this study, that conclusion would seem to be appropriate for our lines as well. Though calculated values for residual resistance ($r_r$) do not differ significantly among lines, lines 155 and 175 had numerically smaller $r_r$ than 47 and 143 at 28 C; this trend did not appear at 18 C. Residual resistance depends on the efficiencies of the components of the photosynthetic system as well as the total amount of photosynthetic apparatus present within the cells. Because we have argued that the efficiencies do not vary among lines, it appears that the superior photosynthetic performance
of lines 155 and 175 at 28 C may result from their having more photosynthetic apparatus per unit leaf area than lines 47 and 143.

**Temperature effect**

AP varied significantly between leaf temperatures at ambient $C_a$ and 130 nmol photons/s cm$^2$ (Figures 1 and 2). At 28 C, both $r_{l'}$ and $r_r$ (2.27 and 2.82 s/cm, respectively) were significantly lower than at 18 C (3.33 and 5.35 s/cm). Because $C_i$ at ambient $C_a$ did not vary between leaf temperatures, it does not seem that $r_{l'}$ differences were responsible for the temperature difference in AP. Therefore, we conclude that the major limitation to AP at 18 C relative to 28 C is $r_r$. This agrees with the findings and conclusions of Laing et al. (1974) and Farquhar et al. (1980) who used a biochemical and mechanistic approach, respectively, to show that the response of AP to temperature depends on the response of the photosynthetic enzymes and electron transport system.

**Conclusions**

Though this work allows no specific conclusions about what factors cause genotypic variation for AP, it does suggest that certain factors are not responsible for such differences. Those factors which are not implicated include any external to the mesophyll cells, such as stomatal conductance or mesophyll cell size or number. Of factors
internal to the cells, it seems that the efficiencies of neither the photochemical or the enzymatic reactions vary along with AP. By process of elimination, the evidence implies that amount of photosynthetic material per unit leaf area mediates AP differences among soybean lines.

References


PHOTOSYNTHESIS IN RELATION TO CHLOROPLAST NUMBER AND QUALITY IN SOYBEAN LEAVES

Duane Merlin Ford and Richard Shibles

Running head: Photosynthesis and Chloroplasts in Soybean

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Variation in the photosynthetic rate of leaves may be caused by variation in the capacities of more than one component of the photosynthetic system. The objective of this experiment was to determine (1) whether the amounts or rates of several photosynthetic traits varied together and with the rate of total photosynthesis per unit leaf area (TPs) and (2) whether differences in photosynthetic capacity could be explained by differences in chloroplast number per unit leaf area (CHLP) or in the capacity per chloroplast. Six soybean [Glycine max (L.) Merr.] cultivars were grown in the field. TPs, CHLP, and the per unit leaf area amounts of soluble protein (PRO) and chlorophyll (CHL), and activities of ribulose 1,5-bisphosphate carboxylase (RuBPCase--EC 4.1.1.39) and uncoupled photosynthetic electron transport (PET) were determined for leaves of similar age and nodal position on seven dates.

Among plants within cultivars and among cultivars, TPs was correlated with RuBPCase, PRO, and CHL; significant correlations with PET occurred only late in the leaves' lives among cultivars. The correlations suggested that these traits generally vary together and with TPs. Differences in chloroplast quality explained capacity differences among plants.
within cultivars; the cause among cultivars is unclear. During senescence, the onset and rates of decline in PET, RuBPCase, and TPs were closely coupled. PRO and CHL began to decline 3 weeks later. CHLP did not decline during senescence, so the loss of photosynthetic capacity appeared to be caused by a decline in chloroplast quality rather than number.

Introduction

It has been suggested (Farquhar et al., 1980; von Caemmerer and Farquhar, 1981) that apparent photosynthesis per unit leaf area (AP) at ambient CO₂ concentrations is co-limited by (1) the rate of ribulose 1,5-bisphosphate (RuBP) carboxylation and (2) the rate of RuBP regeneration. The former depends on the activity of RuBP carboxylase (EC 4.1.1.39) (von Caemmerer and Farquhar, 1981; Seeman and Berry, 1982; Laing et al., 1974). RuBP regeneration depends on (1) the activities of photosynthetic carbon reduction cycle enzymes and (2) the activity of the photochemical apparatus, which produces the reducing power required to run the cycle. The co-limitation hypothesis implies that (1) no component of the photosynthetic system has excess capacity and (2) more rapid AP can be attained only by a similar increase in the capacities of all components. If variation in AP among leaves is caused by variation in the capacity of the photosynthetic system, then are differences in the latter a
consequence of differences in the number of chloroplasts per unit leaf area or in the capacity per chloroplast?

With soybean \textit{[Glycine max (L.) Merr.]} (Hesketh et al., 1981) and rice \textit{(Oryza sativa L.)} (Ortani et al., 1979) cultivars, AP is negatively correlated with leaf size (area), but positively related to the per unit leaf area contents of soluble protein (PRO) and chlorophyll (CHL). Hesketh et al. (1981) also found a positive association between AP and RuBP carboxylase activity per unit leaf area (RuBPCase) among the soybean cultivars. They concluded that the photosynthetic apparatus is "diluted" in larger leaves. The question is: What exactly is diluted; does chloroplast number or capacity change with leaf size?

Variation in AP among the five soybean cultivars studied by Watanabe (1973) was mostly explained (96%) by variation in the uncoupled rate of whole-chain electron transport per unit leaf area (PET). This was supported by a highly significant correlation of AP with CHL. The difference in photochemical activity was caused primarily by differences in the number of chloroplasts per unit leaf area (CHLP), rather than by activity per chloroplast. The former explained 77% of the variation in AP; the latter only 13%. Kariya and Tsunoda (1972) also found a positive correlation of AP with CHL and CHLP among \textit{Brassica} genotypes. The decline in AP with leaf senescence seems associated with the degeneration of whole
chloroplasts in soybean (Wittenbach et al., 1980) and wheat (Triticum aestivum L.) (Camp et al., 1982). These results indicate that chloroplast number, as opposed to chloroplast activity, has the major role in determining AP during the active life of a leaf.

This experiment was designed to test the following hypotheses:

1. Leaves of larger area have less photosynthetic capacity per unit leaf area than smaller leaves. Or, photosynthetic capacity is diluted in larger leaves. And the basic unit of dilution is the chloroplast.

2. Cultivar differences in photosynthesis are attributable to differences in the capacity of the photosynthetic system, which in turn, are caused by differences in CHLP.

3. The loss of photosynthetic capability during senescence is associated with a loss of whole chloroplasts.

Materials and Methods

Culture

Six soybean cultivars ('Corsoy', 'Amsoy', 'Harosoy-63', 'Ford', 'Hawkeye', and 'Richland') were field-grown, in 1.5 m replicated rows, near Ames. In previous work, these cultivars expressed a wide range of AP and leaf area (Dornhoff and Shibles, 1970). Before planting, 26 kg P/ha and 84 kg K/ha were applied, and alachlor and chloropropham were incorporated. Planting was on 11 May 1983. Irrigation water was applied as needed to minimize water stress.
Sampling and total photosynthesis

Within each plot, newly unfolding leaves were tagged on 10 plants on 6 July. These leaves, which developed at the same time, were the ones later sampled.

Total photosynthesis per unit leaf area (TPs) was measured on the terminal leaflets of six tagged leaves in each plot by $^{14}$CO$_2$ uptake (Secor et al., 1982) on 11, 18, and 25 July, and 1, 8, 15, and 24 August. Tagged leaves were about two-thirds expanded on 11 July. Two 12.7 mm diameter discs were removed from unexposed portions of each of the same six terminal leaflets and frozen in liquid N for subsequent measurement of the per unit leaf area amounts of chlorophyll (CHL) and soluble protein (PRO), and the activity of RuBP carboxylase (RuBPCase). Additionally, three 6.35 mm diameter discs were taken from each leaflet and preserved in FAA (50:5:10:35, ethanol:glacial acetic acid:37% w/w formaldehyde:water) for chloroplast counting. The lateral leaflets were harvested the following day and taken to the lab on ice for measurement of uncoupled electron transport per unit leaf area (PET).

Leaf areas of the four unsampled leaves were determined using a LI-3000 (Li-Cor Inc., Lincoln, NE) leaf area meter. Specific leaf mass (SLM) is the dry mass of these leaves divided by their area.
Photosynthetic electron transport

Leaflets could be stored on ice for up to 6 h with little loss of PET. Three to six of the twelve lateral leaflets from each plot were pooled and coarsely chopped with a razor blade. The leaf pieces were placed on a brass window screen, which in turn, was over a 100-mesh, brass screen in a 55 mm plastic, "break-apart" Buchner funnel. Nylon cloth netting (20 \(\mu\)m openings—Nitex, Tetko Inc., Elmsford, NY) was clamped between the two halves of the Buchner funnel. With the vacuum off, 20 mL of cold extraction buffer [4 mM Na\(_2\)HPO\(_4\), 16 mM NaH\(_2\)PO\(_4\) (pH=6.5), 0.3 M sucrose, 2% w/v PVP-40, and 12.5 mM K\(_2\)SO\(_4\)] was added and the leaf pieces were vigorously mashed and worked over the screens with a small pestle. A slight vacuum was then applied, and liberated cells were collected on the nylon cloth. The mash was twice washed with 10 mL aliquots of extraction buffer. The Buchner funnel was broken apart and the cells were washed off the netting into a 50 mL centrifuge tube. The extract was centrifuged by accelerating to 1500 \(\times\) g and then decelerating. The supernatant was decanted and the cells resuspended in sufficient PET assay buffer [50 mM Tricine (pH=7.5) 0.2 M sucrose, 2 mM Ca(NO\(_3\))\(_2\), 5 mM KNO\(_3\), 1 mM MgCl\(_2\)] to give about 0.2 to 0.4 mg chlorophyll/ml buffer. This procedure is rapid and yields an abundance of intact cells—judged by their ability to exclude Evan's blue dye (Kanai and Edwards, 1973). Two 0.25
aliquots were removed for chlorophyll determination according to Wintermans and de Mots (1965).

The method for measuring electron transport in whole cells was generously suggested by Dr. Charles Arntzen (personal communication). In an O_2-electrode (Hansatech Ltd., King's Lynn, Norfolk, England) a 0.1 mL aliquot of the cell suspension was combined with 0.9 mL of assay buffer. Aliquots of concentrated NaN_3 and methyl viologen were added, their final concentrations being 0.5 mM. After 60 s, the O_2-electrode was illuminated with a 300 W film strip projector and O_2 consumption was monitored. After 120 to 180 s, uncoupling agents were added—gramicidin (final concentration of 5 µM) in 50% ethanol (0.5% v/v) and NH_4Cl (5 mM)—and the uncoupled rate was determined. Coupled and uncoupled rates were linearly related to chlorophyll up to 50 µg/mL. Photosynthetic electron transport activity per unit leaf area (PET) was calculated as the uncoupled rate of O_2 consumption per unit chlorophyll multiplied by CHL.

**Chlorophyll and soluble protein**

On the second day after TPs sampling, six of the twelve 12.7 mm discs harvested from each plot and frozen in liquid N were homogenized in 100% ethanol using a Polytron (Brinkman, Westbury, NY). The total extract was filtered through No. 4 Whatman filter paper in a 11.5 mm Hirsch funnel. Chlorophyll content of the filtrate was determined spectro-
photometrically according to Wintermans and de Mots (1965).

The filter paper and cell residue was quantitatively transferred from the Hirsch funnel to a 50 mL centrifuge tube. Soluble proteins were extracted in 0.3 N NaOH by overnight incubation at 35 to 40 C. The alkaline protein solution was filtered (No. 1 Whatman). The filtrate was acidified with HCl and assayed for soluble protein by a modified Bradford technique (Bio-Rad Laboratories, Richmond, CA), using bovine serum albumin as the protein standard.

Ribulose 1,5-bisphosphate carboxylase

Also, on the second day after TPs sampling, the remaining discs that had been frozen in liquid N were ground in 5 mL of ice-cold, grinding buffer (Secor et al., 1983) with a small mortar and pestle. The extract was centrifuged at 4200 x g for 20 minutes. Aliquots were removed from the supernatant for determination of soluble protein (by the modified Bradford method) and RuBP carboxylase activity (Secor et al., 1983). RuBP carboxylase activity per unit leaf area (RuBPCase) was calculated as RuBP carboxylase activity per unit soluble protein multiplied by PRO.

Chloroplasts

In the winter of 1983, six of the eighteen 6.35 mm discs that had been preserved in FAA were removed, dehydrated in an ethanol/xylene series, and embedded in paraffin. Sections
2 to 3 \( \mu \text{m} \) thick were cut from these discs with a microtome and stained with Safranin O. Chloroplast number per unit leaf area (CHLP) was estimated as the number of chloroplasts along a 17.5 \( \mu \text{m} \) length of a section, divided by 17.5 \( \mu \text{m} \) and the thickness of the section. A preliminary study indicated that sufficient accuracy could be attained by counting two lengths per section, two sections per leaf disc, and two leaf discs—a total of 8 determinations per replicate. CHLP could not be determined for the 11 July sample because the cells had not reached full expansion and it was difficult to discern individual chloroplasts.

**Results**

Figure 3 shows the average nodal position of the leaves that were sampled on each date. Among cultivars, the range in nodal position above the unifoliolates was only 8.1 to 9.2. All leaves reached full expansion coincidentally at about day 62 (Figure 3). Plant development varied slightly among cultivars: beginning bloom (R1) occurred 42 to 52, beginning of rapid seed growth (R5) 76 to 81, and physiological maturity (R7) 110 to 111 days after plant emergence. Because these ranges are small, it is unlikely that the sampled leaves were affected differently by plant ontogeny. Stem elongation ceased for these indeterminant cultivars slightly after R5 (Figure 3).
Figure 3. Leaf area (LA) and nodal position of the sampled leaves; each point represents the mean of six cultivars and four replications. The LSD bar is for the difference between date means. Nodes above the unifoliolate node (○); nodes below the unfurling leaf (■); R5 is the beginning of rapid seed growth.
Because only leaf area remained constant after full expansion (from day 62), all data are presented on a unit leaf area basis. There were statistically significant differences among cultivars for all measured leaf traits on all dates, except for TPs on day 62. Despite use of cultivars of similar ontogeny and selecting leaves that developed under the same environment, there still were significant cultivar by date interactions for TPs, RuBPCase, PET, PRO, and CHL. These interactions were caused primarily by different rates of leaf senescence for the cultivars. Over the period from 62 to 99 days after emergence, TPs of leaves at node 8 or 9 declined by 64% for the most rapidly senescencing cultivar, but only 39% for the slowest. The rates of decline in TPs, CHL, PRO, PET, and RuBPCase were correlated with LA among cultivars, the larger leaved cultivars showing more rapid senescence of the sampled leaf.

Leaf size effects

Hesketh et al. (1981) hypothesized that photosynthetic capacity is diluted in leaves of large area relative to smaller leaves. To test this hypothesis, it was necessary to make comparisons among cultivars before the onset of rapid senescence, because of the differences in senescence rate among cultivars with different leaf size. However senescence is defined, it surely began before day 76 in this study. Yet, none of the traits were strongly or consistently correlated
with LA on days 62 through 76 (Table 3).

To determine whether or not the dilution hypothesis explains plant-to-plant differences in TPs within a cultivar, we calculated the partial correlations among leaf traits while holding the effects of block, sampling date, and cultivar constant (Table 4). All the leaf traits, except RuBPCase, varied independently of LA, and the correlation with RuBPCase was positive instead of negative as would be expected if dilution were involved. Because photosynthetic capacity did not appear to be diluted in larger versus smaller leaves, either among cultivars or among leaves within a cultivar, our data do not support the dilution hypothesis.

Cultivar effects

Among cultivars, TPs was strongly and consistently correlated with PRO, CHL, SLM, and RuBPCase (Table 5). Correlations with PET were not very strong until the later dates. CHLP was only slightly and inconsistently correlated with TPs among cultivars. There were significant cultivar differences in the ratios TPs/CHLP, CHL/CHLP, RuBPCase/CHLP, and PET/CHLP on a few dates, but in general, chloroplasts were of similar quality among cultivars. These ratios were even less closely correlated with TPs than CHLP (data not shown). Therefore, the data tend to indicate that CHLP may mediate differences in photosynthetic capacity per unit leaf area among cultivars, but this notion is equivocal.
Table 3. Simple correlation coefficients of LA with leaf photosynthetic parameters among cultivars

<table>
<thead>
<tr>
<th></th>
<th>Days after plant emergence</th>
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<tbody>
<tr>
<td></td>
<td>62</td>
<td>69</td>
<td>76</td>
<td>83</td>
<td>90</td>
<td>99</td>
</tr>
<tr>
<td>TPs</td>
<td>0.03</td>
<td>-0.61</td>
<td>-0.50</td>
<td>-0.65</td>
<td>-0.84</td>
<td>-0.98</td>
</tr>
<tr>
<td>CHLP</td>
<td>-0.19</td>
<td>-0.71</td>
<td>-0.81</td>
<td>-0.92</td>
<td>-0.97</td>
<td>-0.70</td>
</tr>
<tr>
<td>RuBPCase</td>
<td>0.15</td>
<td>-0.10</td>
<td>-0.33</td>
<td>-0.66</td>
<td>-0.76</td>
<td>-0.91</td>
</tr>
<tr>
<td>PET</td>
<td>0.49</td>
<td>-0.08</td>
<td>-0.36</td>
<td>-0.88</td>
<td>-0.82</td>
<td>-0.80</td>
</tr>
<tr>
<td>PRO</td>
<td>-0.20</td>
<td>-0.67</td>
<td>-0.51</td>
<td>-0.77</td>
<td>-0.79</td>
<td>-0.97</td>
</tr>
<tr>
<td>CHL</td>
<td>-0.47</td>
<td>-0.51</td>
<td>-0.51</td>
<td>-0.80</td>
<td>-0.72</td>
<td>-0.81</td>
</tr>
<tr>
<td>SLM</td>
<td>-0.71</td>
<td>-0.72</td>
<td>-0.54</td>
<td>-0.89</td>
<td>-0.68</td>
<td>-0.99</td>
</tr>
</tbody>
</table>

^r > 0.72 and r > 0.81 differ significantly from zero at the 10 and 5% probability levels, respectively (n=6).
Table 4. The partial correlation coefficients among leaf parameters holding the effects of block, sampling date, and cultivar constant; from data collected after full leaf expansion.

<table>
<thead>
<tr>
<th></th>
<th>CHLP</th>
<th>RuBPCase</th>
<th>PET</th>
<th>PRO</th>
<th>CHL</th>
<th>LA</th>
<th>SLM</th>
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</thead>
<tbody>
<tr>
<td>TPs</td>
<td>-0.11</td>
<td>0.34**</td>
<td>0.08</td>
<td>0.44**</td>
<td>0.35**</td>
<td>0.14</td>
<td>0.15</td>
</tr>
<tr>
<td>CHLP</td>
<td>0.05</td>
<td>0.10</td>
<td>0.11</td>
<td>0.03</td>
<td>0.11</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>RuBPCase</td>
<td>0.17</td>
<td>0.67**</td>
<td>0.51**</td>
<td>0.21*</td>
<td>-0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PET</td>
<td>0.23**</td>
<td>0.32**</td>
<td>0.10</td>
<td></td>
<td></td>
<td>-0.01</td>
<td></td>
</tr>
<tr>
<td>PRO</td>
<td>0.67**</td>
<td>0.11</td>
<td></td>
<td>-0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHL</td>
<td></td>
<td>0.13</td>
<td></td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.25*</td>
<td></td>
</tr>
</tbody>
</table>

*,**Indicate that r differs significantly from zero at the 5 and 1% probability levels, respectively.
Table 5. The simple correlation coefficients of TPs with leaf photosynthetic parameters among cultivars

<table>
<thead>
<tr>
<th>Days after plant emergence</th>
<th>62</th>
<th>69</th>
<th>76</th>
<th>83</th>
<th>90</th>
<th>99</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHLP</td>
<td>0.62</td>
<td>0.02</td>
<td>0.85</td>
<td>0.66</td>
<td>0.77</td>
<td>0.78</td>
</tr>
<tr>
<td>RuBPCase</td>
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<td>0.79</td>
<td>0.90</td>
<td>0.82</td>
<td>0.91</td>
<td>0.89</td>
</tr>
<tr>
<td>PET</td>
<td>0.03</td>
<td>0.52</td>
<td>0.82</td>
<td>0.85</td>
<td>0.96</td>
<td>0.87</td>
</tr>
<tr>
<td>PRO</td>
<td>0.80</td>
<td>0.96</td>
<td>0.94</td>
<td>0.87</td>
<td>0.96</td>
<td>0.93</td>
</tr>
<tr>
<td>CHL</td>
<td>0.76</td>
<td>0.95</td>
<td>0.95</td>
<td>0.76</td>
<td>0.97</td>
<td>0.78</td>
</tr>
<tr>
<td>SLM</td>
<td>0.59</td>
<td>0.91</td>
<td>0.96</td>
<td>0.91</td>
<td>0.74</td>
<td>0.97</td>
</tr>
</tbody>
</table>

\(^a r \geq 0.72 \text{ and } r \geq 0.81 \text{ differ significantly from zero at the 10 and 5\% probability levels, respectively (n=6).}\)
Within cultivars, TPs was significantly correlated with PRO, CHL, and RuBPCase (Table 4). However, the partial correlations between TPs and PET or SLM were not close. All traits varied independently of CHLP. TPs was significantly correlated with CHL/CHLP ($r=0.23$) and RuBPCase/CHLP (0.37). The relation with PET/CHLP was not significant (0.19). Therefore, differences among leaves within a cultivar seem to be primarily attributable to capacity per chloroplast rather than to differences in CHLP, though the correlation coefficients on which this conclusion is based are small.

**Senescence effects**

Figures 4 through 6 show the ontogenetic trends averaged for all cultivars for six of the leaf traits. Because the cultivar by date interactions were caused primarily by differences in the slopes of the curves for each cultivar, not by the shapes of the curves or the dates when the curves reached maximum, a single curve for each trait appropriately illustrates the trend for all cultivars.

TPs, RuBPCase, and PET all attained maximum on day 62, when leaves had just attained full expansion. Subsequently, these activities declined nearly linearly at rates dependent on cultivar, CHL and PRO both peaked later and for a longer period before declining. Thus, the decline in TPs was most closely coupled to the loss of RuBPCase and PET. We could detect no decline in CHLP, but RuBPCase/CHLP, PET/CHLP,
Figure 4. Ontogenetic trends of mean total photosynthesis and chloroplast number per unit leaf area (TPs and CHLP, respectively); each point represents the mean of six cultivars and four replications; the LSD bars are for differences between date means.
Figure 5. Ontogenetic trends of mean in vitro RuBP carboxylase activity and uncoupled electron transport per unit leaf area (RuBPrCase and PET, respectively); each point represents the mean of six cultivars and four replications; the LSD bars are for differences between date means.
Figure 6. Ontogenetic trends of mean soluble protein and chlorophyll per unit leaf area (PRO and CHL, respectively); each point represents the mean of six cultivars and four replications; the LSD bars are for differences between date means.
TPs/CHLP, and CHL/CHLP all declined with time in patterns similar to the declines for the numerators. Thus, our data do not support the hypothesis that the loss of photosynthetic capability during senescence is associated with a loss of whole chloroplasts.

Discussion

Our data support the conclusions of von Caemmerer and Farquhar (1981) that photosynthesis is co-limited by more than one component of the photosynthetic system, and that to achieve an increased rate of photosynthesis requires a proportional increase in all the components of the system. The various photosynthetic traits were strongly interrelated and generally correlated with TPs among cultivars and among leaves within cultivars (Tables 4 and 5). The relationships of PRO, CHL, and RuBPCase with TPs were particularly close. However, the ratios—PET/RuBPCase, PET/CHL, PET/PRO, RuBPCase/CHL, RuBPCase/PRO, and CHL/PRO—all varied significantly among cultivars. It appears that most of these traits vary together and generally with TPs among cultivars or leaves within cultivars, but the proportions are not identical for all cultivars.

Significant correlations have been reported among soybean cultivars between photosynthetic rate and CHL (Watanabe, 1973; Buttery and Buzzell, 1977; Hesketh et al., 1981) or
PRO (Hesketh et al., 1981); but others have found them small (Secor et al., 1982) or inconsistent (Buttery et al., 1981). Hesketh et al. (1981) showed that AP was positively correlated with RuBPCase among 29 soybean cultivars. In our study, PET was less uniformly correlated with TPs than were the other traits, but the association was closer later in the leaves' lives. Watanabe (1973) showed that among the means of five cultivars AP was positively related to PET.

During the senescence phase, the decline in TPs was closely coupled with declines in RuBPCase and PET, but less closely related to losses of PRO or CHL. Others have also found that PRO (Mondal et al., 1978; Secor et al., 1983) or CHL (Wittenbach, 1983) may decline later in the senescence process than photosynthesis. However, losses of RuBPCase usually coincide with decline in photosynthesis (Mondal et al., 1978; Wittenbach, 1983; Secor et al., 1983).

We counted about $10^6$ chloroplasts/mm$^2$ or about twice as many as Watanabe (1973) found in soybean unifoliolates and Kariya and Tsunoda (1972) counted in Brassica. This discrepancy might be caused by different growing conditions, nodal positions, or species. Furthermore, the coefficient of variability was 24% for CHLP versus 19, 18, or 9% for RuBPCase, TPs, or CHL, respectively. But even with this large error, differences of 14 and 9% of the mean could be statistically detected among cultivar and date means, respectively.
If photosynthesis depends on the capacity of the photosynthetic system per unit leaf area, what causes the variation in photosynthetic capacity—CHLP or chloroplast quality? Our data did not provide a clear-cut answer to this question for differences among cultivars, but the differences among leaves within cultivars and the decline in photosynthetic capacity during senescence appeared to be caused by differences in chloroplast quality.

The finding that chloroplast quality, not CHLP, declines during senescence disagrees with the conclusions of others. Wittenbach et al. (1980) reported that chloroplasts declined in number, and the membranes of the envelop and stroma lamellae of the remaining chloroplasts appeared to degenerate, as soybean leaves aged. Their conclusions were based on qualitative observations from electron micrographs. Camp et al. (1982), working with wheat, did not determine CHLP directly but suggested that it declined during senescence because (1) PET declined earlier than PET/CHL (we found they declined coincidentally), (2) CHL/CHLP did not vary over time (we found it followed a similar course as CHL—Figure 6), and (3) light scattering by the thylakoids remained constant over time. They did observe that the activities of two stromal enzymes, NADP-triose-P dehydrogenase and RuBP carboxylase, declined at a faster rate than the loss of whole chloroplasts, which is in agreement with our data.
References


GENERAL DISCUSSION

Differences in the amount of photosynthetic apparatus per unit leaf area appear to cause genotypic variation in the rate of photosynthesis (Ps) of soybean leaves. In this section, I will discuss why I believe this statement to be true.

First, by process of elimination, variation for Ps is caused by factors internal to the cells (Dornhoff and Shibles, 1976; Section I). It is true that Ps is often negatively correlated with $r_s^*$ or $r_s^* + r_a^*$ among genotypes (Dornhoff and Shibles, 1970; Bhagsari et al., 1977; Kaplan and Koller, 1977), but Hesketh et al. (1981) found no significant relationship. And I calculate from the data of these four groups of researchers that $C_i$ did not vary with Ps as it must if the variation in Ps is caused by variation in $r_a^*$ and/or $r_s^*$ (Farquhar and Sharkey, 1982). The ratio of mesophyll cell surface area to leaf area ($A_m/A$), which accounts for both cell size and number, is not related to Ps in soybean (Dornhoff and Shibles, 1970; Section I), so leaf anatomy seemingly does not regulate Ps. Because differences in neither the resistances to CO$_2$ diffusion external to the cells nor the size of number of leaf cells can be convincingly related to the genotypic differences in Ps in soybean, factors internal to the cells must cause the variation.
Within leaf cells, the efficiencies and/or the capacities of the components of photosynthesis may vary among genotypes. However, the efficiency of neither PET nor the PCR cycle appears to vary much among genotypes because the AP responses to PPFD at low irradiances (Ojima and Kawashima, 1970; Dornhoff and Shibles, 1974; Section I) and to $T_1$ (Dornhoff and Shibles, 1974; Section I) do not differ among genotypes. The efficiency with which CO$_2$ is transferred from the cell wall to the site of fixation ($r_m$) may explain some of the variation. But most evidence suggests carboxylation resistance ($r_X$) to be of greater importance (Laing et al., 1974; von Caemmerer and Farquhar, 1981; Seeman and Berry, 1982), though these researchers were trying to explain environmentally induced variation in $P_s$ rather than genotypic differences. Carboxylation resistance reflects both the capacity and the efficiency of PET and/or the PCR cycle, but if the efficiencies of each of these components are similar for all genotypes, as previously suggested, then the capacity of one or both of these components likely determines $P_s$. This notion is supported by the recent modeling of photosynthesis done by Farquhar and his colleagues (Farquhar, 1979; Farquhar et al., 1980; von Caemmerer and Farquhar, 1981; Farquhar and Sharkey, 1982).

If variation for the total capacity, or the total amount of photosynthetic apparatus per unit leaf area, causes the
variation in Ps among genotypes, then various measurements of the amount of photosynthetic apparatus should be correlated with Ps. Indeed, PRO, CHL, RuBPCase, PET, and SLM have often been found to be correlated with Ps (Dornhoff and Shibles, 1970, 1976; Watanabe, 1973a,b; Buttery and Buzzell, 1977; Hesketh et al., 1981; Wiebold et al., 1981), but the relationships are not universal (Watanabe and Tabuchi, 1973), or they are inconsistent (Ojima and Kawashima, 1968; Bhagsari et al., 1977; Kaplan and Koller, 1977; Buttery et al., 1981; Secor et al., 1982) (see General Introduction).

In Section II, I reported that TPs was positively correlated with CHL, PRO, RuBPCase, and on the later dates (leaves senescing) with PET among cultivars. Because no other factors can convincingly account for genotypic variation in Ps (Section I), and because there is evidence that the amount of photosynthetic apparatus per unit leaf area does account for the variation (Section II), I conclude that it is the cause.
ADDITIONAL LITERATURE CITED


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