Soybean Adaptation to Water Stress at Selected Stages of Growth¹

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ABSTRACT
Soybean (Glycine max [L.] Merr. cv Braxton) plants were grown in sandy soil with only natural rainfall (N) or with supplemental irrigation (I). Water-stressed plants grew more extensive root systems, whereas irrigated plants developed larger shoots and smaller root systems. Maximum stomatal apertures were observed at the beginning of each photoperiod. Partial stomatal closure occurred each afternoon, but stomata of I plants remained open longer than those of N plants. Significant reductions in net carbon fixation rate generally accompanied decreases in stomatal aperture, which coincided with periods of high temperature, low relative humidity, maximum solar radiation, and water stress. Leaf water potential decreased from morning to afternoon, with a greater decrease observed for N plants. Midafternoon stomatal closure did not occur in N plants with very large root systems following a heavy rain which saturated the soil profile. With smaller root systems and greater evaporative demand from larger shoots, the I plants continued to show midafternoon stress following the heavy rain. The large root systems of the N plants absorbed sufficient water to meet shoot evaporative demand for several days following the rain. Root soil system resistance apparently contributed to the afternoon water stress in the I plants.

Inasmuch as photosynthetic rate is a function of temperature and PPFD³, the highest CO₂ fixation rates should be measured during midafternoon when light intensity and temperature reach their daily maximum, provided all other factors are optimum and temperatures are not detrimental. Solar radiation and air temperature also affect leaf water status (15, 18). Seed yield and short term photosynthetic rates are reduced when soybean leaf water potentials fall below −11 bars (2, 3), a water potential often observed on warm bright afternoons even under conditions of adequate soil moisture (3, 17, 23). Leaf water potentials below −11 bars may affect photosynthesis through stomatal closure. Thus, leaf water status, particularly turgor, can affect photosynthetic carbon fixation rate.

For maximum photosynthetic productivity, leaves must maintain turgor by obtaining sufficient water from the xylem to replace evaporative loss (9). The xylem, in turn, obtains water by root absorption from soil reserves. If the water supply is not adequate, leaves will initiate stomatal closure, thus limiting photosynthesis. Ishihara et al. (14) noted that leaf water stress induced stomatal closure even in rice plants growing in flooded paddies. The time of stomatal closure coincided with periods of high temperature and maximum solar radiation, conditions which would otherwise facilitate maximum photosynthetic rates (13). Because the rice roots grew in standing water, any reduction in leaf water potential under conditions of high evaporative demand implies that water movement from soil to and through roots and the xylem transport system was too slow.

Maintenance of an active root system requires continued growth into new regions of soil to replace older roots which become suberized or die. Thus, continued growth of both shoot and root systems must be coordinated and regulated as the plant adapts to its environment during the growing season (22). The regulatory mechanisms are poorly understood at present (5); this study was initiated to determine effects of diurnal and long term water stress on soybean shoot and root growth.

MATERIALS AND METHODS
Soybean (Glycine max [L.] Merr. cv Braxton) plants were grown in rows 1 m apart in a uniformly packed surface soil material from a Dothan loamy sand (fine loamy, siliceous, thermic Plinthic Paludeults) at Auburn, AL. The soil was installed in six adjacent compartments of the rhizotron at Auburn (12), and fertilized by liming and surface application of a commercial 0-20-20 fertilizer according to standard recommendations from the Auburn University Soil Testing Laboratory for soybeans. One vertical wall of each compartment, extending to a depth of 2 m below the surface, was fitted with glass viewing panels to permit daily observations of root growth.

In early June, rhizobium-inoculated soybean seeds were planted 1 to 2 cm apart in rows perpendicular to the windows. A single row of soybeans was planted across the center of each 50 × 100-cm compartment; each row extended 2 m beyond the edge of the observation compartment to eliminate border effects. Following emergence, the foliage was periodically sprayed with insecticide. When plants had reached the unifoliate stage (V-1) (7), they were thinned to 15 to 20 plants per 50-cm row.

Three compartments, selected at random, received only naturally
by tracing the outline of all visible roots onto the glass viewing panels with china-marking pencils at intervals of 3 to 7 d. New root growth (roots not covered by a mark from previous observations) was recorded, and then the marks were extended with different color so that further new root growth could be distinguished when the next root growth measurements were made 3 or 4 d later.

Soil and air temperature, rainfall, and incident solar radiation were automatically recorded (4). PPFD was measured using a Li-Cor 190 SB transducer and was recorded manually to ensure correspondence between the time and place of these measurements and the determinations of photosynthetic rate, leaf water potential, and degree of stomatal opening described below.

\( \psi_r \) was estimated with a Scholander pressure chamber. A selected leaflet was first enclosed within a polyethylene bag just before cutting the petiole. Within 30 to 45 s after cutting, the leaflet was transferred to the pressure chamber which was lined with moist paper towels to ensure vapor saturation of the chamber gas. After recording the pressure at which water exuded from the petiole, completed 3 to 5 min after cutting, leaflets were transferred to stainless steel vials that were sealed with rubber stoppers. These vials were initially held at 4°C, and subsequently frozen at -20°C. After thawing, cell sap was expressed in a modified sodium press. Osmotic potential of the expressed sap was estimated with a Wescor model 2400 osmometer.4

An improved infiltration method for measuring stomatal apertures of rice plants (13, 16) was adapted for use on soybean leaves by extending the range of infiltration scores to include 6 and 7. These correspond to 60 and 70% ethylene glycol, respectively, in the series of test solutions, and were necessary to indicate fully open pores of the larger stomata of soybean leaves. Unpublished studies of the authors, as well as those of Schorn (20) have shown good correlations between infiltration scores and other methods of measuring stomatal apertures. Test solutions were applied to one of the lateral leaflets of a trifoliate leaf immediately following measurements of CO2 exchange rate on the center leaflet.

Single leaf carbon fixation rates were measured as the reduction in CO2 concentration of an air stream after it passed through a cuvette. The cuvette design of Tsunoda (25), originally developed for rice leaves, was modified as shown in Figure 1 to accommodate soybean leaflet. A steady state CO2 level was established by pumping air at the rate of 7 L min\(^{-1}\) from a 2-m\(^3\) chamber held at 25°C by an air-conditioning system. Because

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4 Mention of a trade name is for the convenience of the reader and does not imply endorsement by the U. S. Department of Agriculture, Auburn University, or the Japanese Society for the Promotion of Science.
Fig. 3. Diurnal course of events on calendar day 209 (July 28), during late anthesis (see Fig. 2). A, Stomatal aperture changes. Vertical bars indicate mean standard error (n = 3) for each data point. B, Mean CO₂ exchange rates for young (second or third node below terminal mainstem node) and old (fifth or sixth node) leaflets growing on irrigated plants. Horizontally measured PPFD is compared with PPFD measured by Li-Cor 190SB sensor held perpendicular to incoming solar radiation (the position of leaf cuvette at time of measuring CO₂ exchange rates). C, Individual leaflet CO₂ exchange rates as a percentage of the maximum rate observed for that leaflet at any time during the day of July 28. Maximum rates were 27.4 mg dm⁻² h⁻¹ for young leaflets and 22.5 mg dm⁻² h⁻¹ for older leaflets on I plants, and 23.5 mg dm⁻² h⁻¹ and 23.0 mg dm⁻² h⁻¹ for young and older leaves, respectively, on N plants.

Fig. 4. Normalized CO₂ exchange rates just prior to anthesis (calendar day 197 or July 16). Maximum rates (100% values) for leaflets on I plants were 19.8 mg dm⁻² h⁻¹ for young (second node below terminal meristem) and 23.5 mg dm⁻² h⁻¹ for old (fifth node) leaves and 20.2 mg dm⁻² h⁻¹ and 23.3 mg dm⁻² h⁻¹ for young and old leaves, respectively, on N plants. PPFD measurements are comparable to those shown in Fig. 3B.

the sampling system removed such a small volume of the recirculating air, baseline drift was slow and could be corrected. A second pump, with an in-line flowmeter, removed air from the leaf cuvette through 10 m of flexible tubing at the rate of 4 L min⁻¹, 1.0 L min⁻¹ of which was pumped into a Beckman model 864 IR gas analyzer calibrated against reference standards from Linde Air Products Corporation. Excess air escaped through the narrow leaflet insertion slit, eliminating the requirement for a gas-tight seal around the petiole.

Kinetic tests showed the flow cuvette to be relatively insensitive to CO₂ perturbations such as those resulting from wind gusts or the operator’s breath. Steady state equilibrium at the gas analyzer was generally achieved within 15 to 20 s after leaflet insertion. Changes in light intensity at the leaf surface induced by clouds or intentional shading produced measurable changes within seconds, the response time being a function of pumping rate and dimensions of the system. The very rapid response to changing light intensity implied that sufficient air circulation occurred around the soybean lamina. The cuvette design facilitated rapid changes from one leaflet to the next; in practice, measurements could be repeated every 60 s with an initial 15 to 20 s for equilibration, 10 to 15 s for reading the output of the analyzer, and 25 to 35 s for moving the cuvette to the next leaf.

All CO₂ exchange measurements were made with the cuvette in full sun and perpendicular to incident radiation. PPFD was reduced by 20% at the leaf surface due to absorption by the cuvette. Apparent net photosynthetic rates were computed by multiplying the gas sampling rate (4 L min⁻¹) times the difference in CO₂ concentration between air entering the leaf cuvette and that returned to the analyzer. In most cases, CO₂ measurements were repeated every 2 to 3 h, using the center leaflet of a particular leaf on each plant. At the end of each day, leaf areas were measured with a Li-Cor leaf area meter.

RESULTS AND DISCUSSION

There were four extended periods of warm, bright weather during the 1981 growing season when evaporotranspiration greatly exceeded rainfall, and soil water potential fell below −0.4 bars in the N treatment (Fig. 2). The first of these coincided with the beginning of anthesis (R1), whereas the other three occurred during the later stages of pod development (R5, R6, and R8, respectively). In these experiments with ‘Braxton’ soybeans, a determinate cultivar, the influence of plant water status upon
because of the strong light dependence of CO₂ fixation. After anthesis, younger leaflets were more active in CO₂ fixation than old leaves (Fig. 3B), in general agreement with other studies (18, 26). The lower rates of older leaves may result from their adaptation to partial shading within the canopy (8).

The influence of soil water regime upon the diurnal course of CO₂ fixation rates can be seen more clearly in Figure 3C, where normalized rates are expressed as a percentage of the maximum observed for each leaflet that day. After anthesis, leaflets on irrigated plants continued CO₂ fixation at near-maximum rates well into the afternoon, while water-stressed plants showed a significant reduction from maximum photosynthetic rate by noon. The reduction in CO₂ fixation rate was more severe with older leaves than with younger leaves.

After anthesis was initiated (days 200–202, see time line of Fig. 2), the determinate Braxton soybeans used in these experiments produced no more mainstem nodes; thus, when leaves from the terminal mainstem node completed expansion about day 210 to 214, no further change in leaf length was observed. The ‘young’ leaves at the second or third mainstem node below the last fully expanded leaf on day 209 were probably at about the same stage of physiological development as ‘old’ leaves at the fifth node on day 197, shown in Figure 4, when the terminal shoot was still elongating and new leaf initiation was not yet completed. Absolute CO₂ fixation rates were greater for young leaves on the postanthesis plants, but greater for old leaves before anthesis.

While the number of main stem nodes on I and N plants was comparable, I plants were taller because their internodes were
lochrone, or rate of appearance of new leaves along the main stem axis, was comparable in both N and I plants: one new leaf appeared each 2 to 3 d (Fig. 2). Root growth continued throughout the life of the plant, although it was much slower after anthesis. Extremely rapid growth of new roots under nonirrigated conditions characterized the dry period from days 190 to 210.

New roots forming during the dry period from days 190 to 210 showed a 3- to 5-fold increase in length for N plants when compared to I plants. When the rainfall of days 211 and 212 rewet the soil, new root growth for N plants subsided to a rate comparable to that observed for the I plants. Frequent trickle irrigation (1-h intervals) maintained a uniformly wet profile, and roots grew slowly. N plants survived the dry period from days 197 to 210 because of rapid root penetration or extension (Fig. 6) into deeper and moister soil layers (Fig. 7).

A computer simulation model (11) describing the interactions of photosynthesis, transpiration, and soil hydraulics using catenary equations (6, 9) offers an explanation for these growth responses. Carbohydrates used to support the increased root proliferation under severe water stress must be diverted from growth of new shoot tissue (19). Irrigated plants used more of their photosynthetic production for shoot growth, reflected as consistently greater length of newly formed leaflets and a slower, steady rate of new root growth during the periods when N plants were under water stress (Fig. 5B).

The potential loss of dry matter production from midafternoon stomatal closure can be very large in rice (13). In these experiments with Braxton soybeans, we observed the same phenomenon previously described for rice grown under flooded paddy conditions (14). Even though soil moisture was maintained at high levels throughout the growing season in this experiment, CO₂ fixation rate was less than maximum for a substantial fraction of the day. These reductions were accompanied by stomatal closure and relatively low leaf water potential.

One possible explanation for the observed decline in photosynthetic rate with increasing light intensity and temperature (conditions which should have favored higher CO₂ fixation rates) is that root-soil system resistance to water flow may have limited the water supplied to rapidly transpiring leaves on warm, bright afternoons (28).

To test the hypothesis that it was root-soil system resistance which limited water flow, causing midafternoon reduction in both stomatal apertures and CO₂ fixation rates, we compared

Table I. Stomatal Aperture Scores, PPFD, CO₂ Fixation Rates, and Leaf Water Potential Values for Soybean Plants Grown Under Rainfall only or Supplemental Irrigation

<table>
<thead>
<tr>
<th>Calendar Day</th>
<th>n</th>
<th>Time</th>
<th>Stomata</th>
<th>PPFD</th>
<th>CO₂ Fixation</th>
<th>Leaf Water Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>score</td>
<td>μE m⁻² s⁻¹</td>
<td>mg dm⁻² h⁻¹</td>
<td>ψₑ</td>
</tr>
<tr>
<td>N only</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>209</td>
<td>2</td>
<td>8 AM</td>
<td>6.8 ± 0.1</td>
<td>727</td>
<td>15.9 ± 2.4</td>
<td>-3.5 ± 0.3</td>
</tr>
<tr>
<td>209</td>
<td>2</td>
<td>6 PM</td>
<td>3.0 ± 0.2</td>
<td>1380</td>
<td>12.5 ± 1.6</td>
<td>-11.9 ± 0.6</td>
</tr>
<tr>
<td>215</td>
<td>2</td>
<td>3 PM</td>
<td>5.7 ± 0.2</td>
<td>1470</td>
<td>17.0 ± 0.4</td>
<td>-7.6 ± 0.4</td>
</tr>
<tr>
<td>238</td>
<td>2</td>
<td>3 PM</td>
<td>2.1 ± 1.2</td>
<td>1195</td>
<td>11.8 ± 1.4</td>
<td>-8.3 ± 0.3</td>
</tr>
<tr>
<td>I</td>
<td></td>
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</tr>
<tr>
<td>209</td>
<td>2</td>
<td>8 AM</td>
<td>6.8 ± 0.1</td>
<td>727</td>
<td>18.5 ± 2.2</td>
<td>-2.5 ± 0.2</td>
</tr>
<tr>
<td>209</td>
<td>2</td>
<td>6 PM</td>
<td>4.3 ± 0.2</td>
<td>1380</td>
<td>14.5 ± 1.7</td>
<td>-6.7 ± 0.4</td>
</tr>
<tr>
<td>215</td>
<td>2</td>
<td>3 PM</td>
<td>5.9 ± 0.3</td>
<td>1470</td>
<td>19.4 ± 1.1</td>
<td>-6.8 ± 0.4</td>
</tr>
<tr>
<td>238</td>
<td>2</td>
<td>3 PM</td>
<td>3.4 ± 0.6</td>
<td>1195</td>
<td>16.6 ± 2.2</td>
<td>-4.3 ± 0.6</td>
</tr>
</tbody>
</table>

* Number of individual observations.

* Turgor computed by subtracting osmotic potential φ from leaf water potential ψₑ for each replicate. Mean turgor computed by averaging sums of individual differences.

* kPa × 10⁻².
our results from the 3 PM measurements made on day 209 with comparable midafternoon measurements made on day 215. On day 209, N plants were under severe water stress, whereas on day 215, water stress in these plants had been alleviated by the heavy rains of days 212 and 213. The early season water stress had induced large differences in root/shoot ratios between the I and N plants (21). The heavy rains on days 212 to 213 quickly rewet the soil around the large root systems of the N plants. However, soil water potential around the smaller root systems of the I plants was relatively unaffected by the rains because irrigation had maintained a wet soil profile throughout the growing season.

On day 215, I plants were transpiring rapidly, with slight turgor loss in midafternoon (Table I). Shoots of the N plants, however, were supplied with water from a much larger root system, which removed water from soil with a water content comparable to that for the I plants. Under these conditions, no loss of turgor was observed in the N plants, even in midafternoon. For the first day after the rains, stomata of all plants remained open in midafternoon. Canopy growth of N plants proceeded very rapidly after the rain, while new root formation returned to a rate comparable to that measured under irrigation (Fig. 5). Table I shows that the midafternoon carbon fixation rate (3 PM) for N plants on day 215 was comparable to the maximum rate measured at 8 AM 1 week earlier, and was nearly as large as the CO₂ fixation rate of the I plants which had been adapted to high photosynthetic rates all along.

On day 215, ψᵣ of the N plants with large root systems in wet soil (−7.6 ± 0.4 bars) was similar to that of the I plants (−6.8 ± 0.4 bars), suggesting that the much larger root systems of the N plants were absorbing sufficient water to meet transpirational requirements. Under conditions of maximum radiation in mi-
dafonoon on day 215, evaporative demand of the larger shoot systems of the I plants, however, was so large that some wilting occurred; the N plants, with their extensive root systems now occupying wet soil, exhibited no signs of wilting for several days immediately following the rains.

Three weeks after the rains, by day 238 when the weather had again been dry for some time and soil moisture for N plants was again very low, the stomatal responses and CO₂ fixation rates of both groups of plants were similar to those recorded prior to day 215 (Table 1). Although shoots of the N plants grew very rapidly immediately after the rain, these plants quickly reverted to their earlier CO₂ fixation rates as the soil dried (Fig. 5). Irrigated plants, however, grew steadily throughout the season.

The increase in available soil water following the rain on days 212 and 213 illustrates a special situation introduced by rapid changes in microenvironment. Under irrigation, plants were always near equilibrium with their environment. Long term stress induced a shift in shoot/root ratio (1) in the N plants. Rapid and significant changes in the relative growth rates of root and shoot systems following the rains on days 211 to 212 illustrate how quickly the soybean plant's homeostasis mecha-
nism responded to alleviation of water stress. Following a marked change in soil water status, a much lower root soil resistance to water flow was observed. The plants responded almost immedi-
ately by growing and adapting to the new environment.

Thus, root soil system resistance due to limited root surface area may be responsible for midafternoon water stress, stomatal closure, and reduced carbon fixation of rapidly transpiring leaves on well watered plants. While supplemental irrigation may limit long term water stress of field-grown soybean plants, the short term diurnal water stress resulting from total root system resist-

ance plays an important role in limiting maximum photosyn-
thetic productivity, even for plants having adequate soil moisture.

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LITERATURE CITED

2. BOYER JS 1970 Differing sensitivity of photosynthesis to low leaf water poten-
6. BURCH GJ, RCC SMITH, WK MASON 1978 Agronomic and physiological responses of soybean and sorghum crops to water deficits. II. Crop evapora-
7. FEHR WR, CE CAVINESS, DT BURNOOD, JS PENNINGTON 1971 Stage of develop-
ment descriptions for soybeans, Glycine max (L.) Merrill. Crop Sci 11: 929–931
15. ISHIHARA K, T NISHIHARA, T OGURA 1971 The relationship between environ-
mental factors and behavior of stomata in the rice plant. I. On the measure-
18. LUGG DG, TR SINCLAIR 1981 Seasonal changes in photosynthesis of field-
19. PATE JS, DF HERRIDGE 1978 Partitioning and utilization of net photosyn-
20. SCHORN M 1929 Untersuchungen über die Verwendbarkeit der Alkohol fixi-
erung und der Infiltrations-Methode zur Messung von Spaltöffnungswinden. Jahrb Wiss Bot 71: 783–840
23. STANLEY CD, TC KASPAR, HM TAYLOR 1981 Modeling soybean leaf water
24. TAYLOR HM, MG HUCK, B KLEPPER, ZF LUND 1970 Measurements of soil-
grown roots in a rhizostron. Agron J 62: 807–809
26. TURNER NC, JE BEGG, HM RAWSON, SD ENGLISH, AB HEARN 1978 Agronomic and physiological responses of soybean and sorghum crops to water de-
ficits. III. Components of leaf water potential, leaf conductance, ¹⁴CO₂