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Light Acclimation During and After Leaf Expansion in Soybean[1]

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ABSTRACT

Soybean plants (*Glycine max* var. Ransom) were grown at light intensities of 850 and 250 μeinsteins m^{-2} sec^{-1} of photosynthetically active radiation. A group of plants was shifted from each environment into the other environment 24 hours before the beginning of the experiment. Net photosynthetic rates and stomatal conductances were measured at 2,000 and 100 μeinsteins m^{-2} sec^{-1} photosynthetically active radiation on the 1st, 2nd, and 5th days of the experiment to determine the time course of photosynthetic light adaptation. The following factors were also measured: dark respiration, leaf water potential, leaf thickness, internal surface area per external surface area, chlorophyll content, photosynthetic unit size and number, specific leaf weight, and activities of malate dehydrogenase, and glycolate oxidase. Comparisons were made with plants maintained in either 850 or 250 μeinsteins m^{-2} sec^{-1} environments. Changes in photosynthesis, stomatal conductance, leaf anatomy, leaf water potential, photosynthetic unit size, and glycolate oxidase activity occurred upon altering the light environment, and were complete within 1 day, whereas chlorophyll content, numbers of photosynthetic units, specific leaf weight, and malate dehydrogenase activity showed slower changes. Differences in photosynthetic rates at high light were largely accounted for by internal surface area differences with low environmental light associated with low internal area and low photosynthetic rate. An exception to this was the fact that plants grown at 250 μeinsteins m^{-2} sec^{-1} then switched to 850 μeinsteins m^{-2} sec^{-1} showed lower photosynthesis at high light than any other treatment. This was associated with higher glycolate oxidase and malate dehydrogenase activity. Photosynthesis at low light was higher in plants kept at or switched to the lower light environment. This increased rate was associated with larger photosynthetic unit size, and lower dark respiration and malate dehydrogenase activity. Both anatomical and physiological changes with environmental light occurred even after leaf expansion was complete and both were important in determining photosynthetic response to light.

The light intensity under which leaves develop has been shown to affect both leaf anatomy and physiology (5, 13). Anatomical changes typically observed are in leaf thickness and mesophyll surface area. Among the physiological and biochemical changes known to occur are changes in leaf Chl content, photosynthetic unit size, density of photosynthetic units, total protein, carboxylase activity, dark respiration, and specific leaf weight (6-8, 10, 11, 18). The question remains how do these anatomical and physiological factors influence photosynthetic activity during light acclimation. It has been suggested (16, 17, 19) that the changes in photosynthetic rates at high light intensities associated with differences in growth light intensity can be accounted for largely by changes in leaf anatomy. It is not known the extent to which light acclimation can occur without a change in leaf anatomy. Furthermore, little is known about the time course of the physiological and anatomical changes which occur during light acclimation. Therefore, our study was designed to compare changes in photosynthesis and anatomy in response to light intensity treatments imposed during and after leaf expansion. In addition, we examined a number of physiological factors in order to determine which ones were of most importance in explaining the photosynthetic characteristics which could not be accounted for by anatomical changes.

MATERIALS AND METHODS

Light Treatments. Soybeans (*Glycine max* var. Ransom) were grown from seed in a controlled environment room of the Duke University unit of SEPEL (14). Plants were grown in a 1:1 vermiculite-gravel mixture and watered three times/day with modified half-strength Hoagland solution. Growth conditons were 12-hr thermoperiod with day and night temperatures of 26/20 C, and a daytime relative humidity of 80%. Full fluorescent and incandescent light was provided 12 hr/day. One hr of incandescent lights (50 μeinsteins m^{-2} sec^{-1}) of photosynthetically active radiation was provided at the beginning and end of the full lighting period, and also in the middle of the night to delay flowering. Plants were grown at a light intensity of 750 μeinsteins m^{-2} sec^{-1} until the first trifoliolate leaf was fully expanded, then 19 plants were exposed to 850 μeinsteins m^{-2} sec^{-1}, and 18 plants exposed to 250 μeinsteins m^{-2} sec^{-1}. These light intensities were achieved by raising plant height, and by shading with plastic screens, respectively. Leaf temperatures of plants in the high light environment were about 1 C higher than in the lower light environment as measured with thermocouples. After 10 days (during which time the second through the fourth trifoliolate leaves had fully expanded) nine plants from each of the two light environments were placed in the opposite light environment. This created four light treatments of nine plants each: plants grown and maintained at 850 μeinsteins m^{-2} sec^{-1} (designated hereafter as HH); plants grown and maintained at 250 μeinsteins m^{-2} sec^{-1} (LL); grown at 250 then switched to and maintained at 850 μeinsteins m^{-2} sec^{-1} (LH); and plants grown at 850 then switched to and maintained at 250 μeinsteins m^{-2} sec^{-1} (HL). Plants were switched to the light environments predawn on June 26. Physiological and anatomical measurements were made on three plants from each treatment on June

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27 and 28, and July 1. All measurements were made on the fourth trifoliolate leaf, which was fully expanded before June 25.

Physiological Measurements. The net photosynthetic rate of the terminal leaflet was determined at 100 and 2,000 μeinsteins m^{-2} sec^{-1} and 340 μl l^{-1} CO_2 using a Beckman 865 model IR analyzer as a differential instrument in an open system. The cuvette was a clamp-on type with a coated polypropylene film window (18). The airstream was split and passed over both leaf surfaces and was mixed before returning to the analyzer. Light was provided by an incandescent lamp and was filtered through water. Leaf temperature ranged from 25 to 27 C. Following determination of net photosynthesis, the stomatal resistance was determined with a Lambda diffusion porometer for each leaf surface at the same two light intensities and CO_2 concentration. On June 30 dark respiration was measured at 25 C for three plants from each light treatment.

Residual or mesophyll resistance to CO_2 uptake was calculated from net photosynthesis and stomatal resistance using zero as the internal CO_2 concentration, so that all nonstomatal limitations were included. The boundary layer resistance to CO_2 was estimated by filter paper models to be 1.8 sec cm^{-1}.

Leaf water potentials were determined for two plants from each light treatment under the treatment growth conditions using an *in situ* hygrometer (Wescor model L-51).

After photosynthesis and stomatal resistance measurements, leaf discs were removed for determination of Chl photosynthetic unit size, photosynthetic unit number, malate dehydrogenase activity, glycolate oxidase activity, and specific leaf weight. Chl was determined by the method of Arnon (4). Chloroplast lamella were prepared from leaf tissue by the methods of Alberte *et al.* (3), then solubilized in Triton X-111 by the procedure of Shiozawa *et al.* (20). Light-induced oxidation of P700 was measured in Triton extracts (20), and photosynthetic unit size was calculated from the ratio of total Chl to P700 (1). A crude leaf extract was prepared as described by Treharne and Eagles (21), and malate dehydrogenase and glycolate oxidase activities assayed spectrophotometrically (9, 12).

Anatomical Measurements. A disc was removed from each plant near the center of the terminal leaflet. Cross-sections were made using an Oxford model G vibratome, and leaf thickness, thickness of palisade, spongy mesophyll, and epidermal layers, lengths and diameters of palisade cells, average diameter of spongy mesophyll cells, and spacing of spongy mesophyll cells were determined. Paradermal sections made by hand sectioning were used to determine the spacing of palisade cells. Mesophyll surface area was estimated by assuming palisade cells to be cylinders with hemispherical ends, and spongy mesophyll cells to be spheres.

RESULTS AND DISCUSSION

Gas Exchange. Net photosynthesis measured at 2,000 μeinsteins m^{-2} sec^{-1} averaged over the 3 measurement days was highest in the HL treatment, and progressively less in the HH, LL, and LH treatments (Table I). Net photosynthesis measured at 100 μeinsteins m^{-2} sec^{-1} averaged over the 3 days was higher in the low maintenance light (LL and HL) treatments than in the high maintenance light treatments (HH and LH) (Table I). The fact that net photosynthetic rates differed in the four light treatments indicates that substantial adjustment to light intensity did occur both during (HH and LL plants) and after (HL and LH plants) leaf expansion. These photosynthetic alterations were apparently complete 24 hr after exposure to the new light conditions since photosynthetic rates did not change significantly (at the 95% level) with time in any light treatment. Averaged over all treatments and measurement times standard deviations were 9.3% and 20% of the mean photosynthetic rates at 2,000 and 100 μeinsteins m^{-2} sec^{-1}, respectively. Adjustment during leaf

Table I. Photosynthetic rates, stomatal and residual resistances.
Average net photosynthetic rates (Pn) at 2000 and 100 μE m^{-2} sec^{-1} are either uncorrected or corrected to stomatal resistances of 0.91 and 2.2 sec cm^{-1} to water vapor, respectively. Residual (r_r) and stomatal (r_s) resistances to CO_2 uptake are at 2000 and 100 μE m^{-2} sec^{-1} and are per external area.

	Pn^{2000}	Pn^{100}	Pn^{2000}	Pn^{100}	r_r^{2000}	r_r^{100}	r_s^{2000}	r_s^{100}
	mg dm^{-2} hr^{-1}		corrected		sec cm^{-1}		sec cm^{-1}	
LL	21.0a*	5.5a	20.7a	5.5a	7.0a	34.8a	1.4a	3.4a
HL	26.0b	5.9a	26.4b	4.9a	4.8b	40.2a	1.6a	3.5a
LH	16.4c	3.3b	17.9c	3.4b	8.9c	62.9b	2.4b	6.2b
HH	22.7d	3.5b	25.2b	3.6b	5.1b	55.1b	2.5b	6.9b

* Values in columns followed by the same letter are not statistically different at the 95% level.

expansion, represented by the LL and HH light treatments, occurred in an anticipated manner, in that leaves which developed at higher light intensities had higher rates of net photosynthesis at high light and lower rates at low light. Leaves which were expanded under low light, and then were placed under high light showed reduced net photosynthesis at both high and low light intensities compared with leaves maintained in low light. These findings are in agreement with those of Gauhl (11). Leaves developed under high light, then placed under low light showed increased net photosynthesis at low light compared to leaves maintained in high light.

Although all plants were watered three times daily, the leaf water potentials of the plants maintained under high light (HH, LH) were more negative, averaging −7.1 bars, than those maintained under low light (LL, HL) which averaged −4.4 bars. The lower water potentials were associated with higher stomatal resistances, as indicated by a significant (95% level) regression of stomatal resistance on leaf water potential. The light acclimation response of photosynthesis in soybean was influenced by changes in leaf water potential and stomatal resistance, factors which may be overlooked in light acclimation experiments. Removing the effect of different stomatal resistances by correcting photosynthetic rates at 2,000 μeinsteins m^{-2} sec^{-1} to the mean stomatal resistance to water vapor (.91 sec cm^{-1}) of the plants maintained under low light (HL, LL) did not alter the ranking of the treatments (Table I) except that the HH and HL treatments were no longer statistically different at the 95% level and the difference between the HH and LL treatments was accentuated. Stomatal resistances do not account for all differences between treatments although they may have accounted for differences between the HH and HL treatments.

Corresponding to the differences in photosynthetic rate, residual resistance to CO_2 uptake at 2,000 μeinsteins m^{-2} sec^{-1} was least in the plants grown initially in high light (HH and HL treatments), and progressively greater in the plants grown initially in low light (LL and LH treatments) (Table I). At 100 μeinsteins m^{-2} sec^{-1} the residual or mesophyll resistance was on the average 7.8 (±2.4) times higher than at 2,000 μeinsteins m^{-2} sec^{-1}, and was higher in the treatments maintained at high light (HH and LH) than in the treatments maintained under low light (LL and HL) (Table I). Stomatal resistance at 100 μeinsteins m^{-2} sec^{-1} was on the average 2.49 (±0.24) times higher than at 2,000 μeinsteins m^{-2} sec^{-1}. Changes in total resistance to CO_2 uptake at 100 compared to 2,000 μeinsteins m^{-2} sec^{-1} were thus dominated by residual rather than stomatal resistance.

Leaf Anatomy. Leaves were thickest in the HH light treatment, and progressively thinner in the HL, LH, and LL treatments (Table II). Therefore, leaf anatomy was not only affected by the light environment during expansion, but was also modified by light environment after expansion was complete. Changes after expansion were not as great as those during expansion. Since the anatomical characteristics did not change (at the 95% level) between June 27 (day 2) and July 1 (day 6) in the light-shifted groups (HL and LH), the changes observed must have been completed within 24 hr of initial exposure. All leaves had two layers of palisade cells and changes in leaf thickness were accompanied by altered thickness of both the

palisade and spongy mesophyll tissue. The ratios of internal mesophyll surface area to external area were greater in the HH and HL treatments than in the LL and LH treatments (Table II). The changes in thickness which occurred after leaf expansion were complete (HL and LH), occurred without a significant change in internal surface area.

The effects of anatomical changes on net photosynthesis rates at 2,000 μeinsteins m^{-2} sec^{-1} are best evaluated by a consideration of the residual resistances/unit internal area (Table II) because expressing net photosynthesis on an internal area basis would introduce differences between light treatments in stomatal and boundary layer resistances. Residual resistances to CO_2 uptake/internal surface area were very similar except for the LH treatment (Table II). Physiological explanations must be sought to explain the higher residual resistance/internal area in the LH treatment. Activities of the respiratory and photorespiratory enzymes expressed on an internal area basis were highest in these plants (see Table IV). Nobel *et al.* (16, 17) also found the residual resistances much more similar per internal than external area. We can conclude that anatomical changes which affect internal surface area have an important effect on leaf photosynthetic activity.

Chlorophyll Content, Photosynthetic Unit Size and Density. The leaf Chl content/unit external area increased by an average of 10% in all light treatments from June to July 1 (Table III), a phenomenon commonly observed in maturing leaves (15). Analysis of the organization of leaf Chl into photosynthetic units revealed that the photosynthetic unit sizes of the plants kept in constant light environments (HH and LL groups) remained constant with time (Table III). The high light groups had smaller photosynthetic units than the low light group, in agreement with earlier reports (2, 8). Plants which were shifted from low to high light showed photosynthetic unit sizes characteristic of the new environment within 24 hr (Table III). A similar rapid response was observed in the plants shifted from high to low light. Large photosynthetic unit size provides more efficient capture of the available light energy (2, 8) and may be partially responsible for higher photosynthesis at low light (Table I). In all light treatments except HL there was an increase in the number of photosynthetic units/area with time (Table III). In these cases the increased number of photosynthetic units was correlated with an increase in the leaf Chl content (on an area basis, Table III). We concluded that changes in photosynthetic unit density/dm^2 primarily reflect changes in Chl content, whereas changes in photosynthetic unit size were independent of leaf Chl content and directly dependent on light intensity.

Enzyme Activities and Specific Leaf Weight. Averaged over the three sampling times, malate dehydrogenase activity was lower in the treatments maintained under low light (LL and HL) than in the high light-maintained treatments (LH and HH) Table IV), as was also found in dry beans (10). These activities were correlated with the respiration rates (measured only on June 30) (Table IV). Unlike the other enzymes, malate dehydrogenase activity showed significant changes with time (Table V) with activity decreasing in plants put into low light and increasing in plants put into high light. This may reflect an adaptation of respiration to changing light intensity.

Glycolate oxidase activity was highest in the LH treatment (Table IV), suggesting that high photorespiration in these plants may have accounted for their low photosynthetic rates.

Specific leaf weight was highest in the HH treatment (Table IV), and progressively less in the LH, HL, and LL treatments. Changes in specific leaf weight between treatments were partially attributable to differences in mesophyll thickness, but were also caused by differences in cell components since HL leaves were thicker than LH leaves, but had lower specific leaf weight. Specific leaf weight increased with time (Table V) in the plants maintained in high light (HH and LH). Since leaf thickness did not increase during this time, this increase must have been a result of synthesis of starch and other cell components.

Table II. Anatomical characteristics, and residual resistance per unit internal area. Residual resistances to CO_2 uptake are at 2000 μE m^{-2} sec^{-1}.

	Total Thickness	Mesophyll Thickness	Mesophyll/External Surface Area	Residual Resistance
	mm		ratio	sec cm^{-1}
LL	0.146a*	0.116a	22.2a	156a
HL	0.202b	0.172b	31.5b	152a
LH	0.170c	0.141c	21.9a	195b
HH	0.214d	0.184d	32.0b	164a

* Values in columns followed by the same letter are not statistically different at the 95% level.

Table III. Chlorophyll content, photosynthetic unit size and density. Changes in chlorophyll content, number of photosynthetic units (PSU) per unit external leaf area, and photosynthetic unit size from June 27 to July 1.

	chlorophyll (mg/dm^2)		PSU/dm^2 (nmol P700/dm^2)		PSU size (chlorophyll/P700)		
	6/27-	7/1	6/27-	7/1	6/27-	6/28-	7/1
LL	4.0-	4.4	14.5-	15.4	320-	320-	320
HL	3.8-	3.9	14.4-	13.6	315-	320-	320
LH	3.9-	4.3	14.2-	16.5	285-	290-	290
HH	3.7-	4.4	14.4-	17.3	285-	290-	285

CONCLUSIONS

The differences in photosynthetic rates at low light seem to be related to the rates of dark respiration with a difference in respiration between the LL and HL treatments and the HH and LH treatments of about 1.3 mg CO_2 dm^{-2} hr^{-1} compared to the difference in photosynthesis of about 1.9. Respiration was measured only on June 30, so the time course of change is not known, although the malate dehydrogenase activity, which may

Table V. Malate dehydrogenase activity and specific leaf weight on July 1 minus activity (or specific leaf weight) on June 27.

	malate dehydrogenase (μmol min^{-1} cm^{-2})		specific leaf weight (g dm^{-2})	
LL	0.22	ns*	0.02	ns
LH	1.33	s	0.13	s
HL	-0.72	s	-0.05	ns
HH	0.31	ns	0.14	s

* Values followed by ns are not significant at the 95% level; values followed by s are significant at the 95% level.

Table IV. Dark respiration, malate dehydrogenase activity, glycolate oxidase activity, and specific leaf weight.

	dark respiration	malate dehydrogenase	malate dehydrogenase	glycolate oxidase	glycolate oxidase	specific leaf weight
	(mg dm^2 hr^{-1})	(μmol min^{-1} dm^{-2})	(per dm^2 internal)	(nmol O_2 min^{-1} dm^{-2})	(per dm^2 internal)	(g dm^{-2})
LL	0.96a*	130a	5.86	120a	5.45	0.19a
HL	0.89a	150a	4.76	130a	4.13	0.28b
LH	2.26b	190b	8.69	210b	9.59	0.34c
HH	2.31b	200b	6.25	150a	4.69	0.43d

* Values in columns followed by the same letter are not statistically different at the 95% level.

reflect dark respiration in these experiments, showed a more gradual change than did photosynthetic rates. Differences in photosynthetic unit size may also have contributed to differences in photosynthesis at low light.

Except for the LH treatment, differences in photosynthetic rate at high light are accounted for by changes in internal to external surface area ratios, since residual resistances/internal area are very similar. The high residual resistance/internal area of the LH treatment may be due to increased photorespiration and dark respiration as suggested by the elevated activity of glycolate oxidase and malate dehydrogenase.

Our results indicate that light acclimation is a complex phenomenon which involves both physiological and anatomical changes. Many physiological parameters, *e.g.* enzyme activities, photosynthetic unit size, and CO_2 exchange rate, change within 24 hr after plants are shifted to different light intensities. The amount and nature of photosynthetic adjustment to light are determined by the developmental stage at which the leaf was exposed to altered light conditions. Differences in photosynthetic rates measured at low light intensities appear to be primarily caused by physiological changes and are fully reversible, while differences in photosynthetic rates measured at high light intensities appear to be primarily caused by anatomical changes and are not completely reversible. Finally, it is clear from our results that anatomical as well as physiological leaf characteristics can change in response to a new light regime even after leaves are fully expanded.

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