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Limiting Factors in Photosynthesis

I. USE OF IRON STRESS TO CONTROL PHOTOCHEMICAL CAPACITY *IN VIVO*[1]

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ABSTRACT

The possibility of using Fe stress as an experimental tool in the study of limiting factors was explored. Results show that Fe stress decreased the chlorophyll (Chl) *a*, Chl *b*, carotene, and xanthophyll content of leaves of sugar beets (*Beta vulgaris* L.) and that the maximum rate of photosynthetic CO_2 uptake (P_{max}) per unit area was linearly related to Chl (*a* + *b*) per unit area. Measurements of noncyclic ATP formation by isolated chloroplasts at light saturation indicate that photosynthetic electron transport capacity decreased concomitantly with pigment content under Fe stress.

Iron stress decreased Chl per chloroplast but had no effect on the number of leaf cells per unit area, average leaf cell volume, number of chloroplasts per unit area, or leaf soluble protein per unit area. Average chloroplast volume, protein N per chloroplast, and ribulose bisphosphate carboxylase activity were diminished by Fe stress but to a lesser extent than Chl per chloroplast. The reduction in pigment concentration with Fe stress led to a relatively small decrease in light absorption, the fraction of incident light absorbed remaining high (49%) even at very low leaf Chl contents. There was no apparent change in the quantum yield of attached leaves at low irradiances, but at high irradiances, the capacity to convert absorbed light to chemical energy was greatly diminished in Fe-stressed leaves.

The results suggest: (*a*) that P_{max} per unit area are decreased linearly with Chl per unit area because of a decrease in photochemical capacity rather than a change in light absorption; and (*b*) that the effect of Fe stress may be sufficiently specific for it to be used as an experimental tool for the control and study of photochemical capacity *in vivo*.

Elucidation of rate-limiting steps in photosynthesis has largely been approached by the use of chemical agents, such as the electron transport inhibitor DCMU, and by the use of photosynthetic mutants (12). Relatively few investigators (6, 28) have used mineral nutrient stress as a means of studying limiting factors in photosynthesis. In our laboratory we are currently exploring the possibility that the capacity of parts of the photosynthetic apparatus may be varied independently of other factors by withholding specific mineral elements from the growing plant. It is eventually hoped to use mineral stress (in combination with leaf gas exchange techniques to measure photosynthesis and control leaf environment) to gain useful information on the quantitative effects of specific internal factors on the rate of photosynthesis.

Here, we report an investigation into the possibility of using Fe stress to control photochemical capacity, *i.e.* the capacity of a leaf to convert absorbed light into chemical energy. The approach employed was to induce Fe stress in sugar beet plants by withholding Fe from the culture solution and then follow the changes in various leaf attributes which might potentially affect photosynthetic performance. The attributes measured included Chl and carotenoid contents, maximum rates of photosynthesis, leaf absorptance and quantum yield, noncyclic ATP formation by isolated chloroplasts, RuBP[2] carboxylase activity, leaf and chloroplast protein, and numbers and volumes of leaf cells and chloroplasts.

[1] This work was supported by the Beet Sugar Development Foundation.

MATERIALS AND METHODS

Plant Culture. Sugar beets (*Beta vulgaris* L. cv. F58-554H1) were cultured hydroponically at 25 C, and illuminated at 35,000 lux over a 16-h day. The plants were cultured for 2 weeks following planting in Vermiculite with half-Hoagland solution. They were then transplanted into a culture solution containing (in mM): 2.5 $Ca(NO_3)_2$, 1.0 KH_2PO_4, 2.5 KNO_3, 1.0 $MgSO_4$, and 0.5 NaCl, and (in μM) 23.1 B, 4.6 Mn, 0.38 Zn, 0.16 Cu, 0.052 Mo, and 8.95 Fe (as ferric-sodium EDTA complex). After 2 weeks, the plants were transferred to solutions with the same composition as above except for Fe, which was withheld; 2 M NaOH and solid $CaCO_3$ were added to raise the pH to 8.5.

Most of the measurements described below were carried out on young, rapidly expanding leaves which were about 150 to 200 cm^2 in area.

Chl and Carotenoids. Total leaf Chl content was determined as described previously (25). The leaf carotenoid content was determined as follows (see ref. 7 for the original method). Fresh leaf samples (4 g) were blended in 50 ml of a mixture of acetone and hexane (2:3, v/v) with 0.1 g of sodium ascorbate and 0.1 g $MgCO_3$. The extract was filtered, washed with water, 9 ml acetone added, and made up to 100 ml with hexane. The pigments were separated on a chromatographic column packed with equal parts by weight of activated magnesia and diatomacious earth with anhydrous Na_2SO_4 above the column. The separation of the carotenes was completed by washing through with 50 ml of 1:9 (v/v) acetone-hexane mixture. The xanthophylls were washed through with 50 to 100 ml of acetone-hexane mixture (1:4, v/v). Each eluate was made up to 100 ml and *A*-determined at 436 nm for the carotenes ($\epsilon_{436} = 1.05 \times 10^5$ l/mol·cm), or 445 nm for the xanthophylls ($\epsilon_{445} = 1.42 \times 10^5$ l/mol·cm).

The eluates were separated further by TLC. The major constituent of the carotene fraction was β-carotene. The xanthophyll fraction appeared to be a mixture of several xanthophylls which were not identified.

Cell Numbers, Cell Volume. Twenty-four leaf discs (each 0.504 cm^2) were selected at random from three or four leaves; 10 discs

[2] Abbreviations: RuBP: ribulose 1,5-bisphosphate; I_i, I_a, I_r, I_t: spectral quantum flux densities for monochromatic light of wavelength λ (incident, absorbed, reflected, and transmitted, respectively); A_λ: absorptance at wavelength λ ($A_\lambda = I_a/I_i$); Q_i, Q_a: total quantum flux densities for PAR over the range 400–700 nm (incident and absorbed, respectively); P: rate of photosynthetic CO_2 uptake; P_{max}: rate of photosynthetic CO_2 uptake measured at 30 C, 1000 μl CO_2 l^{-1} air and at optimum irradiance.

were used for determination of fresh and dry weight, 10 for Chl, and the remaining four leaf discs were placed in 3 ml of chromic acid (5% w/v) and kept at 4 C for 2 days. The four discs were broken up by mixing on a Vortex mixer for 60 s, made up to 10 ml, and an aliquot of cells counted using a hemacytometer. The number of cells per leaf and the average leaf cell volume were determined as described previously (22).

Chloroplast Numbers. After the sampling for cell numbers, the remaining leaf material was cut into 1-cm^2 squares and placed in 3% glutaraldehyde solution for 48 h at 4 C. The fixed leaf was blended for 15 s in a solution of 4% glutaraldehyde and 0.33 M NaCl, filtered through six layers of fine nylon mesh, and centrifuged at 200*g* for 60 s at 0 C. The pellet was discarded and the supernatant centrifuged at 800*g* for 10 min. The pellet, which from microscopic examination appeared to be predominately composed of chloroplasts, was resuspended in 5 ml of 0.3 M NaCl. One aliquot (1 ml) of the suspension was made up to 5 ml with 100% acetone, centrifuged at 10,000*g* for 10 min to remove particles, and the Chl content determined. A second aliquot (0.1–1 ml depending on the number of chloroplasts present) was diluted to 10 ml with 0.3 M NaCl solution, mixed, and the number of chloroplasts counted using a hemacytometer. The number of chloroplasts per unit leaf area was obtained from the number of chloroplasts per unit volume of suspension, and from the Chl contents per unit volume of chloroplast suspension and per unit area of leaf.

Chloroplast Volume. The diameters of chloroplasts isolated by the glutaraldehyde-NaCl procedure were measured by micrometer. The volume of each chloroplast was calculated from the formula for the volume of a prolate spheroid (an ellipse rotated about its major axis), *i.e.* 4/3 πab^2, where a and b are each half of the lengths of the major and minor axes of the chloroplast, respectively. Twenty chloroplasts were measured per leaf sample.

Chloroplast Dry Weight and Protein N. Chloroplasts were isolated nonaqueously and the dry weight and protein N content per chloroplast determined. Three leaves of uniform Chl content were cut into 1-cm^2 squares and mixed. Half of the squares was used for nonaqueous chloroplast isolation and the other half for measurement of the number of chloroplasts using the glutaraldehyde-hemacytometer procedure.

The procedure used to isolate chloroplasts nonaqueously was modified from that of Stocking (20) and only essential differences are presented here. After lyophilizing, the leaf sample was ground in a carbon tetrachloride-hexane medium (density 1.44 g/ml), filtered through glass wool, and centrifuged at 12,000*g* for 5 min. The chloroplasts were removed from the surface of the medium and resuspended in a second carbon tetrachloride-hexane medium with a density of 1.31 g/ml. After determination of Chl content, the suspension was centrifuged at 3,000*g* for 5 min; the supernatant was removed, and its Chl content determined. The Chl content of the pellet was calculated by difference; the pellet was dried at 70 C and weighed. The chloroplast dry weight/Chl ratio (w/w) was determined.

Soluble nitrogen compounds in the chloroplast pellet were extracted with 10% (w/v) trichloroacetic acid. After 10 min the extract and chloroplasts were diluted with distilled H_2O and centrifuged at 3,000*g* for 5 min. The pellet was digested in a solution of equal volumes of concentrated H_2SO_4 and saturated potassium hydrogen sulfate. The protein N content was determined by liberating N as ammonia from the digest with 40% (w/v) KOH, and titrating with 0.02 N HCl (5).

The dry weight per chloroplast was calculated from the ratios of chloroplast dry weight/Chl (w/w) and chloroplast numbers/Chl.

RuBP Carboxylase and Soluble Protein. The RuBP carboxylase extract was prepared from a chopped leaf sample and its activity measured according to the method described previously (25) except that the enzyme was activated in the presence of the assay medium for 5 min prior to the addition of RuBP. Soluble proteins in the enzyme extract were precipitated by 10% (w/v) trichloroacetic acid for 10 min, and centrifuged for 5 min at 10,000*g*. The protein was dissolved in 2 ml of 2 M NaOH and measured by the Lowry method (8) using standard solutions of BSA.

Absorptance and Quantum Flux Density. Leaf absorptance was measured in an integrating sphere (50-cm diameter) by the method described by McSwain and Arnon (11). The interior of the integrating sphere was painted with Eastman 6080 white reflectance coating. The monochromatic light entering the sphere had a 10 nm bandwidth, which was obtained by Balzers interference filters, and its intensity was measured by a photomultiplier/volt meter unit. The intensity of light entering the sphere, equivalent to I_i, was set to the arbitrary value of 100. A small portion of an individual leaf of measured Chl content was then placed in the sphere. The light remaining in the sphere after absorption was equal to $(I_r + I_t)/I_i$, and absorptance was calculated as $A_\lambda = 1-(I_r + I_t)/I_i$. In some instances I_r/I_i was determined by placing a black card directly underneath the leaf portion to absorb transmitted light.

The total incident quantum flux density for PAR, Q_i, is given by $400^{\int 700}(I_i)d\lambda$. It was measured using a Lambda Instruments LI-190S quantum sensor.

Leaf Gas Exchange. The rate of photosynthetic CO_2 uptake per unit area was determined on individual attached leaves in the manner described by Terry (23). The leaf was inserted in the leaf chamber and irradiated at a quantum flux density of 100 μE m^{-2} s^{-1} for 1 h. Measurements were made of CO_2 uptake, water vapor efflux, and leaf temperature. The leaf was then successively irradiated at 500, 1,000, 1,500, 2,000, and 3,000 μE m^{-2} s^{-1} for 1 h at each irradiance, unless the leaf was chlorotic, in which case it was irradiated at lower irradiances ranging from 100 to 1,500 μE m^{-2} s^{-1}. The ambient CO_2 concentration was maintained at 1,000 ± 50 μl CO_2 l^{-1} air, and leaf temperature at 30 ± 0.5 C.

Quantum Yield. Quantum yield was estimated from measurements of the rate of photosynthetic CO_2 uptake by an attached leaf, Chl/area and Q_i as described below. Leaf CO_2 efflux was measured for 1 h in darkness; then, after illuminating the leaf with red light (see Fig. 7B for emission spectrum), CO_2 uptake was measured for 2 h at 25 μE m^{-2} s^{-1} and for 1 h at 50, 75, and 100 μE m^{-2} s^{-1}, respectively. Leaf temperature was 30 ± 0.2 C. Ambient CO_2 concentration was maintained at 300 ± 1 μl CO_2 l^{-1}, both in air during the first half of the measuring interval, and in nitrogen for the second half. Quantum yield was calculated as the slope of the linear plot of P/area with $Q_a \cdot Q_a$, which is given by $400^{\int 700}(I_a)d\lambda$, was estimated from Q_i using the relationship of Q_a/Q_i to Chl/area shown in Figure 8A.

RESULTS

Pigment Composition and Photosynthesis. In control plants the concentration of Fe (see ref. 26 for method of measurement) in the leaf blade usually ranged from about 2.0 to 3.5 μmol g^{-1} dry weight, while Chl contents were from 40 to 60 μg cm^{-2}. Values below 40 μg cm^{-2} were obtained by withholding Fe hydroponically for periods of 3 to 10 days. Young leaves began to develop a chlorotic appearance within 3 to 4 days after Fe was withheld, and by 6 or 7 days most of the young leaves were completely yellow. The quantitative relation between Chl content and leaf Fe concentration is shown in Figure 1. Chl/area decreased sharply with leaf Fe concentration below 2 μmol g^{-1}. The ratio of Chl *a*/Chl *b* did not change during Fe stress and remained at about 3.2 (w/w).

Carotene (mainly β-carotene) and xanthophyll contents decreased linearly with Chl content during Fe stress (Fig. 2). In control leaves (40–60 μg Chl cm^{-2}) the average molar ratio was about 0.091 for carotene/Chl and 0.11 for xanthophyll/Chl; as leaves became severely Fe-stressed these ratios increased to 0.15

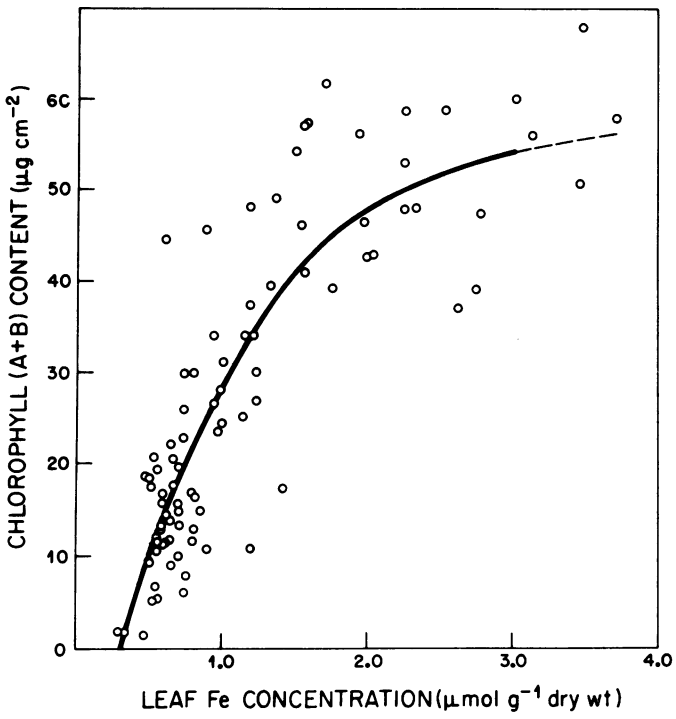

FIG. 1. Relation between Chl content and leaf blade Fe concentration ($y = 2.29\ x^3 - 20.1\ x^2 + 63.4\ x - 16.7$).

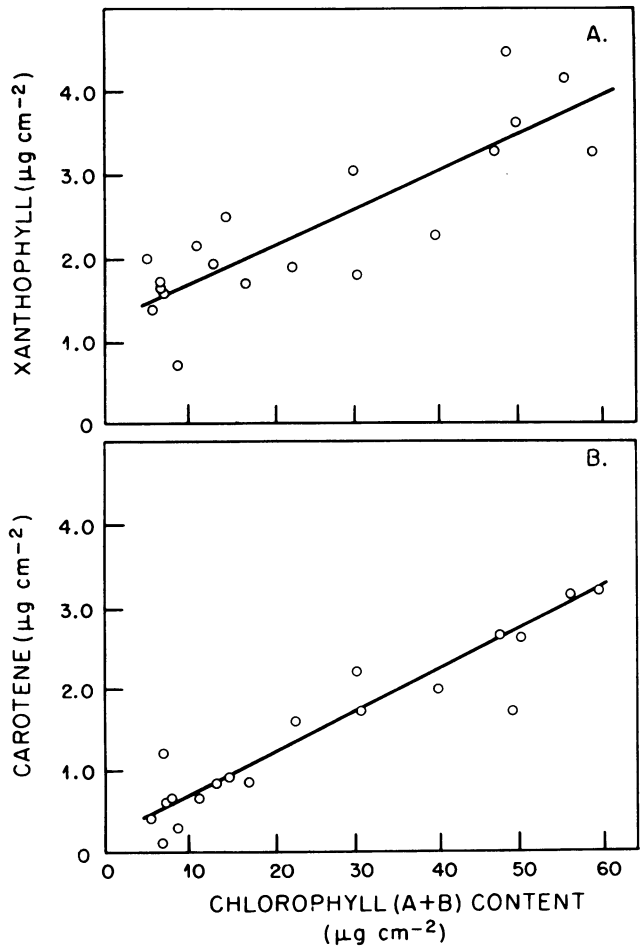

FIG. 2. Relationship between carotenoid pigment content and Chl content under conditions of Fe stress. A: xanthophyll ($y = 1.27 + 0.0436\ x$; $r = 0.85$); B: carotene ($y = 0.182 + 0.0508\ x$; $r = 0.94$).

and 0.47, respectively. These data show that there was a concomitant decrease in the per unit area amounts of all photosynthetic pigments (Chl *a*, Chl *b*, carotene, and xanthophyll) with Fe stress, but that xanthophyll decreased relatively less than the other pigments (the proportion of xanthophyll increasing from 6 to 22% by weight).

The rate of photosynthetic CO_2 uptake per unit leaf area was measured at 30 C under optimal conditions of irradiance, CO_2 supply, nutrition, and leaf age; it is referred to as P_{max} and was the maximum rate obtainable under these specified experimental conditions. The level of irradiance at which P_{max} was attained varied with the Chl content of the leaf: leaves with smaller Chl contents tolerated less irradiance than those with higher Chl contents (Fig. 3). P_{max} data were, therefore, obtained at irradiances appropriate to the Chl content of each leaf. The high ambient CO_2 concentrations used in the photosynthesis measurements (1,000 $\mu l\ CO_2\ l^{-1}$ air) ensured that rates of photorespiration were minimal.

The data show that P_{max}/area is linearly related to Chl/area (Fig. 4A). The linear regression of the data is significant to $P = 0.001$ with a correlation coefficient of 0.95. For ease of discussion the relationship between P_{max}/area and Chl/area is referred to as "linear" but it should be pointed out that it is almost certainly slightly curvilinear. Statistically, the fit to the data was improved significantly by the addition of a quadratic term to the regression. Furthermore, the quadratic regression ($y = -4.93 + 2.57\ x - 0.014\ x^2$ yielded a negative rather than a positive intercept; a negative intercept is more appropriate since P would attain a negative value (*i.e.* respiration) when leaves reached very low Chl contents (<2 $\mu g\ cm^{-2}$).

Although Fe stress decreased the rate of photosynthesis per unit area, it did not decrease the rate of photosynthesis per unit Chl (Fig. 4B). The rate of photosynthetic CO_2 fixation remained high at 437 ± 98 $\mu mol\ CO_2\ mg\ Chl^{-1}\ h^{-1}$ throughout the range of leaf Chl contents. These data suggest that the photosynthetic apparatus associated with Chl functioned efficiently even at the very low leaf Chl contents associated with severe Fe stress.

Leaf and Chloroplast Attributes. There are several possible ways in which P_{max}/area might be causally related to Chl/area. First, Fe stress might reduce the number or size of cells resulting in less leaf tissue per unit area, thereby decreasing both Chl and photosynthetic capacity per unit leaf area. Second, Fe stress might reduce the number or amount of chloroplasts per unit volume leaf tissue. A third possibility is that Fe stress might diminish the amount of light-harvesting and electron transport apparatus (*i.e.*

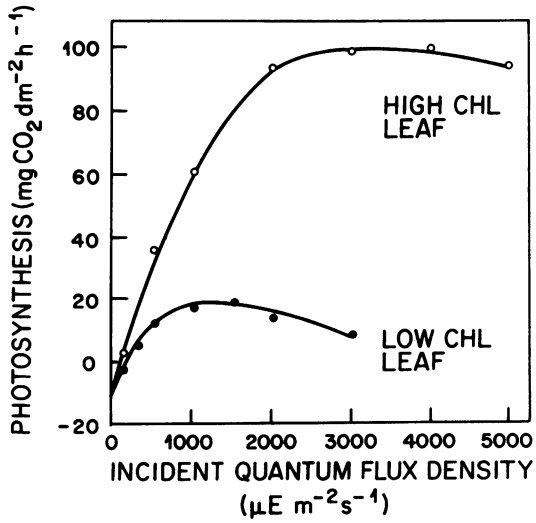

FIG. 3. Relation between the rate of photosynthesis and irradiance for a high Chl (50 $\mu g\ cm^{-2}$) control leaf and for a low Chl (6.4 $\mu g\ cm^{-2}$) Fe-stressed leaf. Photosynthesis was measured as leaf CO_2 uptake at 30 C at saturating levels of CO_2 supply (1,000 $\mu l\ CO_2\ l^{-1}$ air).

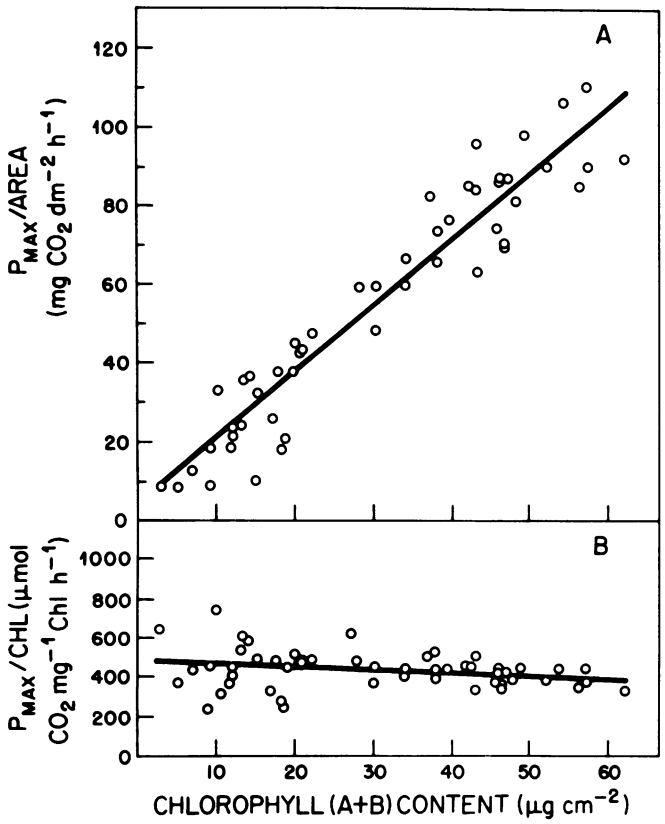

FIG. 4. Relationship between maximum photosynthesis and Chl content under conditions of Fe stress. A: rate expressed per unit leaf area ($y = 4.97 + 1.69\ x$; $r = 0.95$); B: rate expressed per unit Chl ($y = 482 - 1.53\ x$; $r = -0.26$).

membranes, pigments, reaction centers and electron carriers, etc.) per chloroplast. The latter possibility might result in a reduction in light absorption, or, photochemical capacity. These possibilities were explored experimentally.

The data show that Fe stress did not affect the number of cells/leaf area or average leaf cell volume, nor did it change the number of chloroplasts/area or per cell (Table I). In an earlier experiment it was observed that for sugar beet leaves of comparable size and development, Fe stress had no effect on fresh weight/area, leaf thickness, or leaf tissue volume, *i.e.* volume exclusive of leaf intercellular air space (24). Collectively, these data imply that Fe stress did not diminish Chl/area and photosynthetic capacity via an effect on the amount of leaf tissue, or the number of chloroplasts per unit area.

There was some evidence that Fe stress reduced the size of chloroplasts. Direct measurement of the radii of the individual chloroplasts yielded an estimated volume which was smaller for severely Fe-stressed than for mildly stressed or control leaves (Table I). In order to obtain an alternative measure of chloroplast size, chloroplasts were isolated nonaqueously and the dry weight per chloroplast determined; these data show a progressive decrease in chloroplast weight with Fe stress (Table I). However, chloroplasts isolated in this manner may have variable amounts of starch, which complicates interpretation of the data. In an attempt to overcome this problem, the amount of protein N per chloroplast was measured to provide a measure of chloroplast size, which was independent of fluctuations in starch content; these data show that Fe stress had less effect on chloroplast protein N content (34% decrease) than on chloroplast dry weight (42% decrease).

The reduction in Chl/area was due to a decrease in Chl/chloroplast (Fig. 5A). Thus, the relation of P_{max}/area with Chl/area is more specifically a relation between P_{max}/area and Chl/

Table I. *Effect of Fe Stress on Some Leaf and Chloroplast Attributes*

	Control Leaves >40 μg Chl cm^{-2}	Fe-stressed Leaves 20–40 μg Chl cm^{-2}	Severely Fe-stressed <20 μg Chl cm^{-2}
	N = 14	N = 12	N = 8
No. of cells/leaf area (10^6 cm^{-2})	1.30 ± 0.30	1.21 ± 0.19	1.35 ± 0.28
Mean leaf cell volume (10^{-8} cm^3)	2.64 ± 0.75	2.78 ± 0.61	2.75 ± 0.90
No. of chloroplasts/leaf area (10^8 cm^{-2})	1.02 ± 0.19	0.91 ± 0.21	0.89 ± 0.18
No. of chloroplasts/cell	72 ± 32	77 ± 9	83 ± 24
Average chloroplast volume (μ^3)	42 ± 8.3	37 ± 12	21 ± 7.3
Soluble protein/leaf area (mg cm^{-2})	0.57 ± 0.08	0.56 ± 0.07	0.53 ± 0.07
	N = 6	N = 6	N = 7
Nonaqueously isolated:			
Dry wt/chloroplast (pg)	30.1 ± 5.2	23.0 ± 4.8	17.4 ± 8.1
Protein N/chloroplast (pg)	1.88 ± 0.37	1.34 ± 0.25	1.24 ± 0.46

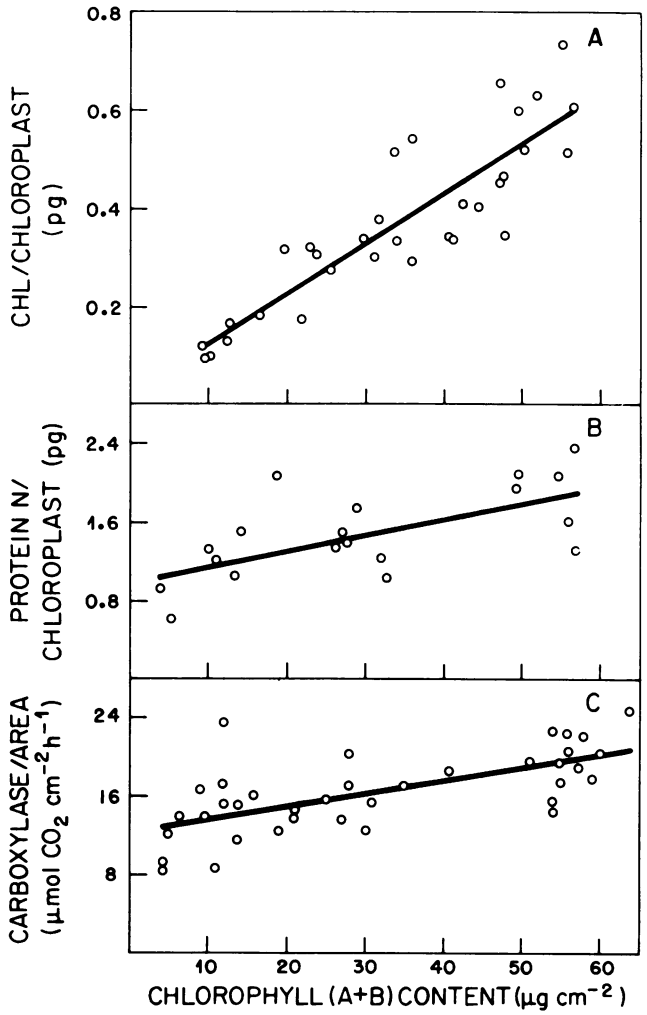

FIG. 5. Changes in three chloroplast attributes with leaf Chl content under conditions of Fe stress. A: amount of Chl per chloroplast ($y = 0.025 + 0.01\ x$; $r = 0.88$); B: amount of protein N per chloroplast ($y = 0.99 + 0.016\ x$; $r = 0.65$); C: per unit leaf area rate of CO_2 fixation by extracted RuBP carboxylase ($y = 12.5 + 0.13\ x$; $r = 0.65$).

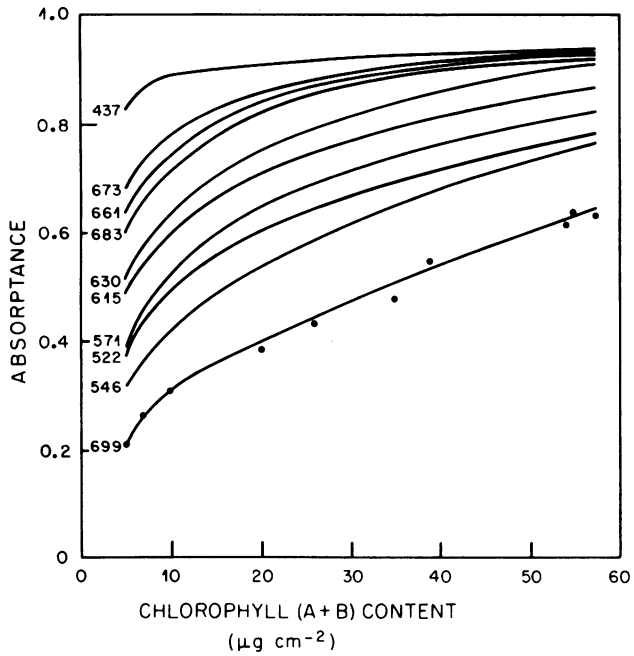

FIG. 6. Relation between leaf absorptance and Chl content at different wavelengths of monochromatic light. Except at 699 nm, data points are excluded for clarity.

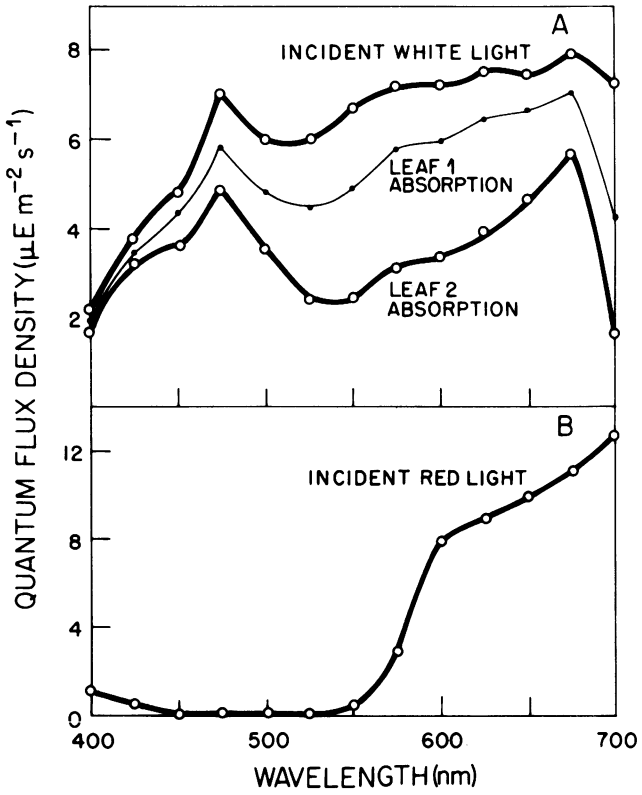

FIG. 7. Spectral distribution of quantum flux density. A: white light source; B: red light source used in determining quantum yields. The estimated spectral distribution of absorbed quantum flux density, Ia, is shown for two leaves irradiated with white light. Leaf 1 was a control leaf containing 54 μg Chl cm^{-2} while leaf 2 was an Fe-stressed leaf of similar size and development containing 6.8 μg Chl cm^{-2}.

chloroplast. Both of these latter two attributes decreased by about 80% as Fe stress became severe. The decreases in chloroplast protein and RuBP carboxylase activity were less, about 30% (Fig. 5, B and C), while leaf-soluble protein, up to 45% of which may be RuBP carboxylase (16), exhibited no decrease (Table I). These data imply that the decrease in P_{max}/area was more likely to have been related to an effect of Fe stress on the light-harvesting and electron transport apparatus than to an effect on the capacity of the stromal enzymes for CO_2 fixation and reduction.

Light Absorption and Photochemical Capacity. The apparent correlation between P_{max}/area and Chl/chloroplast under conditions of Fe stress suggests that photosynthetic capacity may have been causally related to light-harvesting pigment content in two possible ways. First, the reduction in pigment content could have diminished photosynthesis by decreasing light absorption. The second possibility is that Fe stress reduced the amount of the entire light-harvesting and electron transport apparatus, including reaction centers and electron carriers, and that photosynthesis was decreased because of a decrease in photochemical capacity. Thus, Fe-stressed leaves with low pigment content may have absorbed light energy at a fairly high rate but perhaps were less able to convert the absorbed light to chemical energy.

The effect of pigment content on light absorption was investigated by measuring absorptances of leaves with different Chl contents. Absorptance of monochromatic light at all wavelengths decreased with decrease in the content of Chl and carotenoids, the extent of the reduction depending on the wavelength of incident light (Fig. 6). The absorption of green light (*e.g.* 546 nm) and the longer wavelengths of red light (*e.g.* 699 nm) was strongly diminished with decrease in pigment content while the absorption of blue light at 437 nm and red light at 661, 673, and 683 nm was far less affected.

It is perhaps surprising that absorptance decreased so little, especially at red wavelengths, with the large reduction in Chl content (Fig. 6). However, although the Beer-Lambert Law for light absorption by a homogeneous pigment solution indicates that *absorbance* increases proportionately with pigment concentration, absorptance is not proportional[3]. Furthermore, leaves are more efficient absorbers of light energy than are pigment solutions of comparable concentration. This is because of the increase in the effective path length which results from the multiple reflections and scattering of light as it passes through the leaf (1). The results of the present work are consistent with those obtained by Björkman (1) who obtained absorptances (at 673 nm) of 0.89 and 0.85 at Chl contents of 49 and 25 μg cm^{-2}, respectively. In the present investigation, comparable absorptances of 0.93 and 0.88 at corresponding Chl contents were obtained (Fig. 6).

P_{max} was measured using a xenon arc illumination system which provided a source of white light. The emission spectrum of the white light source (and the red light source in the case of quantum yield) was obtained using an ISCO spectroradiometer (Fig. 7). The absorption spectrum for a leaf of a given Chl content was then estimated using the A_λ values. Absorption spectra determined in this way are shown for two different leaves in Figure 7A. By integrating the spectral quantum flux density (from the area under each curve) over 400–700 nm wavelength it was possible to obtain a value for Q_a/Q_i, the proportion of the total incident quantum flux density which was absorbed.

The ratio, Q_a/Q_i, was calculated for leaves of different Chl contents (Fig. 8A). The absorption of white light was substantial even in leaves with very low Chl contents (Fig. 8A), *e.g.* an Fe-stressed leaf with 5 μg Chl cm^{-2} absorbed 49% of the incident quantum flux compared to 81% for a control leaf with 10 times the Chl content. These data suggest that it is unlikely that the

[3] The Beer-Lambert Law for a homogeneous pigment solution states that absorbance ($\log_{10} I_i/I_t$) is equal to ϵcb, where ϵ = molar absorption coefficient, c = pigment concentration, and b = path length of light. However, absorptance for such a solution (*i.e.* $[I_i - I_t]/I_i$) is equal to $1 - 10^{-\epsilon cb}$ and is therefore not directly proportional to c.

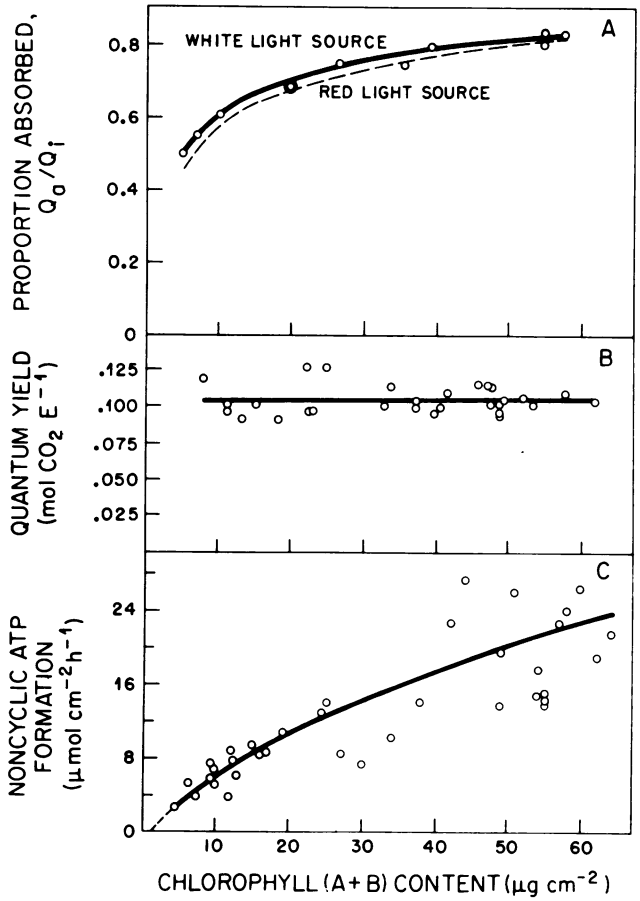

FIG. 8. Changes in light absorption, quantum yield, and photosynthetic electron transport with leaf Chl content under conditions of Fe stress. A: proportion of incident total quantum flux density absorbed by leaves of different Chl contents (the relation of Q_a/Q_i with Chl/area for red light, broken line, was used to estimate quantum yield); B: quantum yield of attached leaves irradiated with red light in the absence of O_2; C: per unit leaf area rate of light-saturated noncyclic ATP formation by isolated chloroplasts (potassium ferricyanide as electron acceptor [25]).

decrease in light absorption which accompanied the 10-fold decrease in Chl/area would be large enough to account for the observed reduction in P_{max}/area under conditions of Fe stress.

The effect of Fe stress on the photochemical conversion of absorbed light was explored by determining the quantum yield of attached leaves irradiated with red light (Fig. 8B). The data show that quantum yield, which was about 0.10 mol CO_2/E, was not altered by Fe stress even though Chl/area decreased to less than 20% of the control. Thus, at low values of Q_i severely Fe-stressed leaves were able to absorb a fairly high proportion of the incident quantum flux and photochemically process it as efficiently as control leaves.

As Q_i increased, however, Fe-stressed leaves, while still absorbing a high proportion of Q_i, were less able to utilize photosynthetically the incoming quanta. This may be illustrated by considering the photosynthesis data for the high and low Chl leaves shown in Figure 3. At a Q_i of 1,000 μE m^{-2} s^{-1}, the high Chl leaf fixed CO_2 at a rate of 38.2 μmol CO_2 m^{-2} s^{-1} while the rate for the low Chl leaf was 10.6 μmol CO_2 m^{-2} s^{-1}. Using the relation between Q_a/Q_i and Chl/area for white light (Fig. 8A), one can estimate that the high Chl leaf probably absorbed about 810 μE m^{-2} s^{-1} and the low Chl leaf 530 μE m^{-2} s^{-1}. From calculations based on these data, one can show that the high Chl leaf absorbed 1.45 quanta per molecule Chl per second and fixed 0.047 CO_2 molecules per absorbed quantum. The low Chl leaf absorbed 7.35 quanta molecule^{-1} s^{-1} and fixed only 0.020 molecules CO_2/absorbed quantum. Thus, at a fairly high irradiance of 1,000 μE m^{-2} s^{-1} (about one half of full sunlight), the Fe-stressed low Chl leaf absorbed quanta faster per Chl than the high Chl leaf, but was much less efficient in utilizing the absorbed quanta in photosynthesis.

The rate of noncyclic ATP formation by isolated chloroplasts at light saturation was not diminished by Fe stress, and there was no uncoupling of photophosphorylation from electron transport. The rate expressed per unit leaf area, however, decreased curvilinearly with Chl/area (Fig. 8C). This suggests that as Fe stress decreased Chl/area, there was a concomitant decrease in the per unit leaf area capacity for photosynthetic electron transport. This may have been responsible for the decrease in P_{max}/area with Chl/area.

DISCUSSION

Iron stress has a pronounced effect on the formation of the light-harvesting and electron transport apparatus of the chloroplast. Iron stress diminished the per chloroplast amounts of all photosynthetic pigments, there being correlative decreases in Chl *a*, Chl *b*, carotene, and xanthophyll. Similar correlative decreases in Chl and carotenoid pigments with Fe stress were observed in potato (3) and barley (10). The reduction in light-harvesting pigment content was accompanied by an apparent reduction in photosynthetic electron transport, suggesting that Fe stress may have decreased the content of electron carriers along with pigments; subsequently, P_{700} and Cyt *f* were shown to decrease proportionately with Chl/area under Fe stress (19). Electron microscope studies show that Fe stress markedly reduces the number of granal and stromal lamellae in chloroplasts of corn (21), tomato, spinach, and maize (27) and *Xanthium* (2), as well as sugar beets (19), while Machold (9) showed that Fe chlorosis was associated with a substantial reduction in Chl-protein complexes. Thus, Fe stress may affect the entire light-harvesting and electron transport apparatus, including lamellae, pigment complexes, reaction centers, and electron carriers.

The Fe stress effect on the light-harvesting and electron transport apparatus appeared to be remarkably specific, as Fe stress had no effect on several attributes of leaf growth. The number of cells per unit leaf area, average leaf cell volume, soluble leaf protein, and numbers of chloroplasts per unit leaf area were not affected even by severe Fe stress. Iron stress did decrease chloroplast volume, chloroplast protein content, and RuBP carboxylase activity to some extent, but to a much smaller degree than it decreased pigment content or photosynthetic electron transport. Iron stress effects may be more severe than those reported here. Shetty and Miller (17) and Perur *et al.* (14) showed that Fe stress considerably reduced the protein content of chloroplasts (but not cytoplasmic protein), while Stocking (21) found that RuBP carboxylase activity was much reduced in corn. Öquist (13) showed that Fe stress impaired several photosynthetic components of *Anacystis nidulans*. These apparently wide ranging effects of Fe stress could be attributed to differences between species in their response to Fe stress, or to the severity of Fe stress imposed. On the other hand, Bottrill *et al.* (4) and Spencer and Possingham (18) found that Fe stress did not depress photosynthetic activity per unit Chl in spinach and tomato, respectively.

Price and Carell (15) showed that although the rate of Chl synthesis in *Euglena gracilis* was rapidly affected after Fe was withheld, the rate of growth and the activities of several enzymes proceeded normally. A similar study with sugar beets showed that Chl production by young leaves ceased almost immediately when Fe was withheld but that the leaves continued to expand at a constant rate; when Fe was resupplied the leaves regreened within 48 h (24). These two studies illustrate both the specificity of the Fe stress effect, and that it is reversible.

Since P_{max}/area was determined under essentially nonlimiting environmental conditions, the rate of photosynthesis was limited

by the capacity of the photosynthetic apparatus. The data presented above support the view that the linear decrease of P_{max}/area with Chl/area was associated with a reduction in photochemical capacity. Although the reduction in Chl/area was also accompanied by decreases in light absorption and RuBP carboxylase activity, these decreases did not appear to be large enough to have been responsible for the observed reduction in P_{max}/area. The absence of an effect of Fe stress on P_{max}/Chl suggests that Fe stress did not affect photosynthetic unit size, and that Fe stress may have caused chlorosis and reduction in photochemical capacity by decreasing the number of photosynthetic units per unit leaf area. Further evidence to this effect is presented in the following paper (19).

The data presented here and elsewhere (19) support the view that Fe stress may be sufficiently specific for it to be used as an experimental tool for the control and study of photochemical capacity *in vivo*.

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