Plant Physiol. (1970) 46, 1-5

The Photosynthetic Action Spectrum of the Bean Plant[1]

Received for publication August 21, 1969

S. E. BALEGH[2] AND O. BIDDULPH
Department of Botany, Washington State University, Pullman, Washington 99163

ABSTRACT

The photosynthetic action spectrum of the bean plant leaf, *Phaseolus vulgaris* L. (variety Red Kidney), has been determined with a diffraction grating illuminated by a 6500-watt xenon arc. An infrared CO_2 analyzer was used to determine the gross photosynthetic rate of the terminal leaflet of the first trifoliate leaf. The rate was measured as a function of the light intensity at steps of 12.5 nanometers which approximates the length of the leaflet used. Twenty-five curves between 400 and 700 nanometers were used to establish the action spectrum. All light curves were some linear function of the incident intensity, and all were extrapolated to zero. The action spectrum shows the following features. (*a*) There are two peaks (*i.e.*, at about 670 and 630 nanometers) and a shoulder between 600 and 612 nanometers in the red region where the highest rate of photosynthesis is found. Lower peaks in descending order are found in the blue (at about 437 nanometers) and the green (at about 500 nanometers) regions. (*b*) There are two small minima at about 650 nanometers and between 470 and 480 nanometers, and a broad minimum is found between 540 and 530 nanometers. (*c*) The photosynthetic rate declines rapidly above 680 nanometers, reaching the lowest value at 700 nanometers. (*d*) At wave lengths below the blue maximum, the rate decreases progressively to 400 nanometers.

Despite the large number of papers published on the action spectra of algae, we have not been able to find one complete reliable action spectrum of a higher plant that has been determined at many different narrow wave length bands. The recent work of Egnéus (5) and Lundegårdh (13) is of qualitative nature and covers only limited portions of the visible spectrum. Most of the early work was done by using filters that isolate a rather wide band of light. The work of Hoover (11) with such a system has been widely published and furnishes the basis for our present understanding of action spectra for higher plants. It is the purpose of this study to establish the photosynthetic action spectrum for the bean plant leaf, *Phaseolus vulgaris* L. (variety Red Kidney), with a diffraction grating spectrograph.

MATERIALS AND METHODS

Experimental material consisted of Red Kidney bean plants grown in areated half-strength Hoagland solution with micronutrients, under the following environmental conditions: temperature 23 ± 1 C; fluorescent light of spectral distribution shown in Figure 1, of an intensity of 1200 to 1750 ft-c on a 12-hr photoperiod, and a relative humidity of 60 ± 5%. The age of the plants used varied between 18 and 21 days from the seed. Only the intact terminal leaflet of the first trifoliate leaf was used.

Photosynthesis was measured by means of an infrared CO_2 analyzer (Beckman, L/B, 15 A) in a closed system. It consisted of a Plexiglass leaf chamber, a pump (Randolph Pump, model 500 with a Zero-Max gearhead Q1-Qw55-M52) and a flowmeter. Air was circulated at a constant flow rate of 1.1 liters/min. The air temperature of the chamber was controlled by controlling the ambient temperature. Apparent photosynthesis was determined from the average CO_2 uptake during two consecutive 4- to 5-min light exposures at each intensity and wave length. Each CO_2 uptake period was followed by an 8- to 10-min dark period, wherein CO_2 evolution was measured. The system was flushed with a 500 μl/liter CO_2 stream for 1 min between the two sequential determinations. Flushing with standard CO_2 whenever the wave length or intensity was changed obviated the use of a drying agent in the closed system and assured against CO_2 depletion.

The rate of gross photosynthesis was calculated as the absolute sum of the apparent photosynthetic rate and light respiration. The latter was assumed to equal the average dark respiration before and after a light exposure. Two observations have established confidence in this assumption. First, when the gross photosynthetic rate, computed this way, was plotted against the light intensity, it was found to be a straight line that invariably extrapolated to zero. Second, the rate of dark respiration was almost identical before and after light exposure. Several workers (2, 6) in agreement with our results, have found evidence that with alternating 10-min periods of light and darkness, the dark reading ordinarily led to a close approximation of the rate of respiration during the light exposures. Although the work of Ozbun *et al.* (17) indicates that the rate of CO_2 release decreases sharply upon illumination of bean leaves, their results are not comparable to ours. This is because, apart from using excised leaves, the rate of CO_2 evolution was measured during a 3-hr period of darkness.

In our work the photosynthetic rate at each wave length, in steps of 12.5 nm, was determined as a function of the light intensity. The light intensities used were selected to fall in the upper half of the linear range of the light curves in order that the respiration correction would be small in comparison with CO_2 uptake and the percentage of error of the measurements would also be small. The data were used to establish 25 light curves, one for each wave length employed, and these in turn were used to determine the photosynthetic action spectrum.

THE LIGHT SOURCE

A large spectrograph capable of producing a 16-ft long continuous spectrum was used. It has a nearly constant linear dispersion of 0.115 nm/mm. Exact values are shown in Table I. The primary light source was an Osram XBO 6500-w high pressure xenon arc. Light intensity of the diffracted beam was con-

[1] This investigation was supported in part by Project (AT-45-1)-1380 by the United States Atomic Energy Commission. Their support is gratefully acknowledged.

[2] Present address: Biophysics Program, Washington State University, Pullman, Washington 99163.

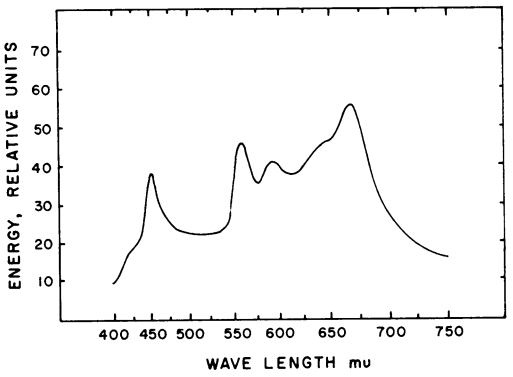

FIG. 1. The spectral energy distribution of the light in which the plants were grown.

trolled by the power input to the light source and by an adjustable entry slit which opens to a maximum of 25 mm with a micrometer control to 10 nm. The diffracted beam was produced by a 1200 lines/mm plane grating (B and L Optical Co., Buffalo, N.Y.) with a ruled face of 200 × 308 mm. A spectral span covering 550 nm between 225 and 775 nm was attained without rotating the grating. The energy range available over the useful spectrum was in excess of 592.2 μw cm^{-2} (*i.e.*, 19.8 × 10^{-10} Einsteins cm^{-2} sec^{-1} at 400 nm). The spectral band was 8 inches high at the focal curve, thus permitting a specimen 4 × 8 inches to be irradiated with full diffracted beam intensity within a range of 10 nm. The intensity of the contaminating light at its peak wave length, at any position on the focal curve has been found to be no more than 10^{-3} to 10^{-4} of the focal curve intensity. The instrument could be used as a monochromator by holding the sample stationary and rotating the grating on its calibrated mounting.

DETERMINATION OF THE SPECTRAL ENERGY DISTRIBUTION OF THE LIGHT SOURCE

An Epply Thermopile (No. 6581) was used. The xenon arc was operated at 110 amp (operating range 60–160 amp), and the slit was fully open, *i.e.*, 25 mm. The thermopile readings were recorded at each step of 2.5 nm between 375 and 750 nm. The radiant flux density per unit area (cm^2) was determined, and a summary of the data (in steps of 12.5 mμ) is plotted in Figure 2.

DETERMINATION OF THE RADIATION INCIDENT ON THE LEAF

Quantum Flux. Levels of energy were first determined at 400 nm, the energy minimum for the lamp at the wave lengths used, and the energies at other wave lengths were adjusted to this quantum flux. The conversion from radiant flux density units to photons cm^{-2} sec^{-1} and vice versa was done through the following relationship (19):

$$\text{Energy flux of 1 photon} - \text{sec}^{-1} = \frac{1987}{\lambda(\text{A})} \times 10^{-12}\mu\text{w}$$

Details of the calculations are shown by Balegh (1).

Wave Length Range and Resolution. The wave length limits of the flux incident on a leaf 125 mm long will be $\lambda + (D \times l/2)$ and $\lambda - (D \times l/2)$ where λ = wave length at the center of the leaf, D = dispersion (Table I) and l = leaf length. For λ = 500 nm one edge is at 507.1 nm and the other at 492.9 nm. With a slit width of 25 mm, a magnification of 2.32, and a dispersion of 0.114 nm/mm, the range of the wave length falling at each point on the leaf will be 25.0 mm × 2.32 × 0.114 nm/mm = 6.6 nm (15). Then the extreme range of wave lengths falling between the two edges of the leaf will be 507.1 + 3.3 nm and 492.9 − 33. nm, or 510.4 nm and 489.6 nm. This is a range of 20.8 nm.

The average wave length range for leaves in all positions along the spectrograph is 21.23 nm: it varies between 22.03 nm

Table I. *Optical Properties of the Spectrograph and the Spectral Energy Distribution of the Light Source, at 110 amp with a Slit of 25 mm*

Wave Length	Dispersion (D)	Magnification	Spectral Energy Distribution
nm	*nm/mm*		*μw cm^{-2}*
700.0	0.1220	2.179	321.2
687.5	0.1213	2.192	395.4
675.0	0.1206	2.205	363.2
662.5	0.1199	2.217	345.4
650.0	0.1194	2.229	353.5
637.5	0.1186	2.239	317.9
625.0	0.1180	2.249	329.3
612.5	0.1175	2.259	347.0
600.0	0.1170	2.269	350.0
587.5	0.1166	2.277	390.6
575.0	0.1162	2.285	361.5
562.5	0.1158	2.292	388.9
550.0	0.1154	2.299	418.0
537.5	0.1150	2.305	424.5
525.0	0.1147	2.311	429.3
512.5	0.1144	2.316	432.6
500.0	0.1142	2.319	435.8
487.5	0.1140	2.324	451.9
475.0	0.1138	2.327	500.3
462.5	0.1136	2.329	508.4
450.0	0.1134	2.331	442.2
437.5	0.1133	2.332	388.9
425.0	0.1132	2.333	374.4
412.5	0.1133	2.333	356.7
400.0	0.1134	2.333	345.4

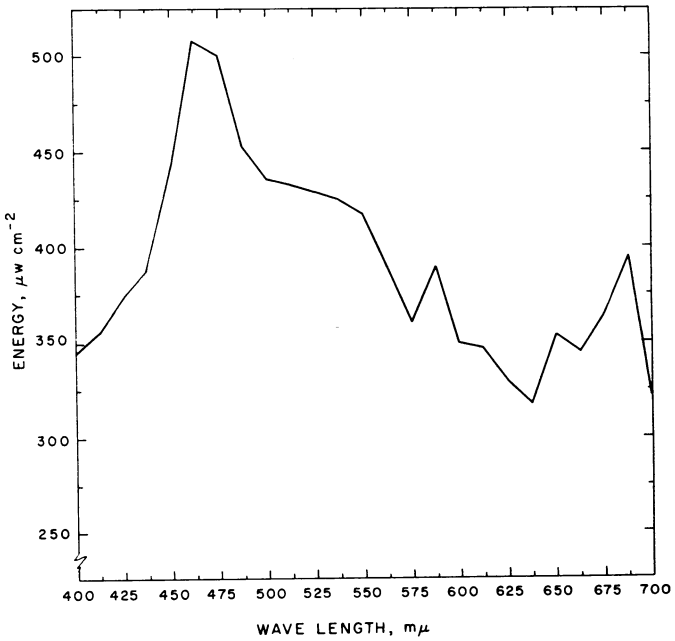

FIG. 2. Spectral energy distribution of the light source.

at 700 nm and 20.72 nm at 400 nm. Thus, it should be possible to distinguish photosynthetic peaks that are separated by 25 nm or more. To improve resolution it would be necessary to use a narrower slit width, which would mean lower light intensities, shorter and less reliable photosynthetic cruves, and ncreased errors from the respiration measurement.

RESULTS

The results of at least four and mostly six determinations were used to calculate the average photosynthetic rate at each wave length used. The light curves plotted from these data are shown in Figures 3 to 6. The action spectrum derived from the slope of these curves by reading the photosynthetic values for each wave length at the light intensity of 11.9×10^{14} photons cm^{-2} sec^{-1} is shown in Table II. These values were then converted to molecules of CO_2 per 1000 photons and plotted as a function of the wave length (Fig. 7). The horizontal lines at data points represent the average length of the leaf. Precision was lowest at 675 nm, where the average photosynthetic rate (at light intensity = 14.31×10^{14} photons cm^{-2} sec^{-1}) was 0.419 ± 0.024 μl of CO_2 cm^{-2} min^{-1}. At 500 nm (at light intensity = 11.9×10^{14} photons cm^{-2} sec^{-1}) the precision was the highest. The average photosynthetic rate was 0.261 ± 0.007 μl of CO_2 cm^{-2} min^{-1}.

The action spectrum shows the following features. (*a*) There are two peaks and a shoulder in the red region where the highest rate of photosynthesis is found. Lower peaks in descending order are found in the blue and green regions. (*b*) The two red peaks are found at about 670 and 630 nm and are separated by a small minimum at about 650 nm. Both red maxima are rather broad, and the one at 670 nm is a little higher. The shoulder is located between 600 and 612 nm. (*c*) There is a green peak at about 500 nm and a blue peak at about 437 nm. The blue maximum is relatively broad. (*d*) A broad minimum is found between 540 and 530 nm and a lesser one between 480 and 470 nm. (*e*) The photosynthetic rate declines rapidly at wave lengths longer than 680 nm, reaching the lowest rate at 700 nm. (*f*) At wave lengths below the blue maximum, the rate decreases progressively to 400 nm.

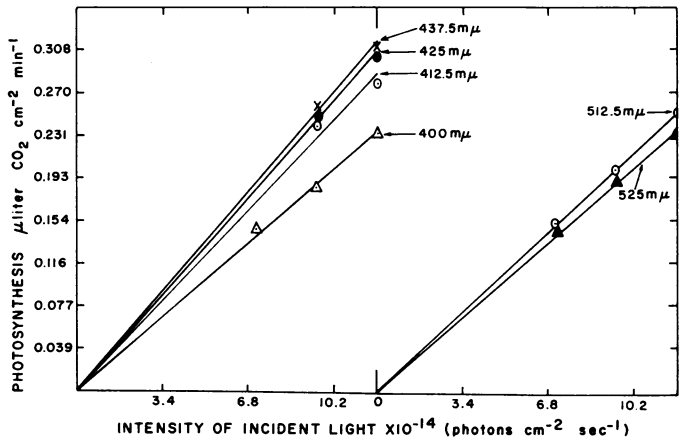

FIG. 3. Light intensity curves, 400 to 437.5 nm, 512.5 nm, 525 nm.

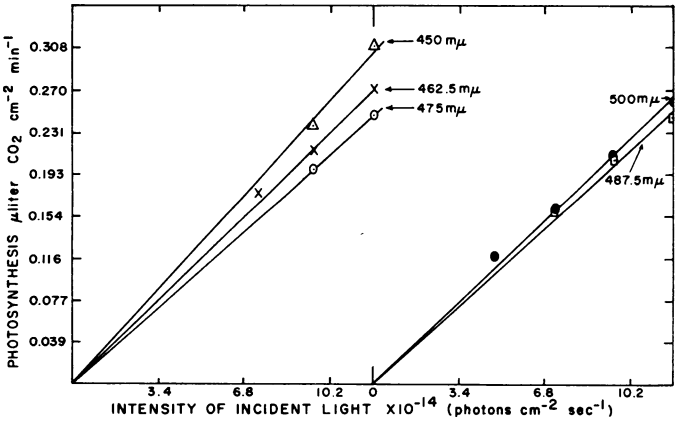

FIG. 4. Light intensity curves, 450 to 500 nm.

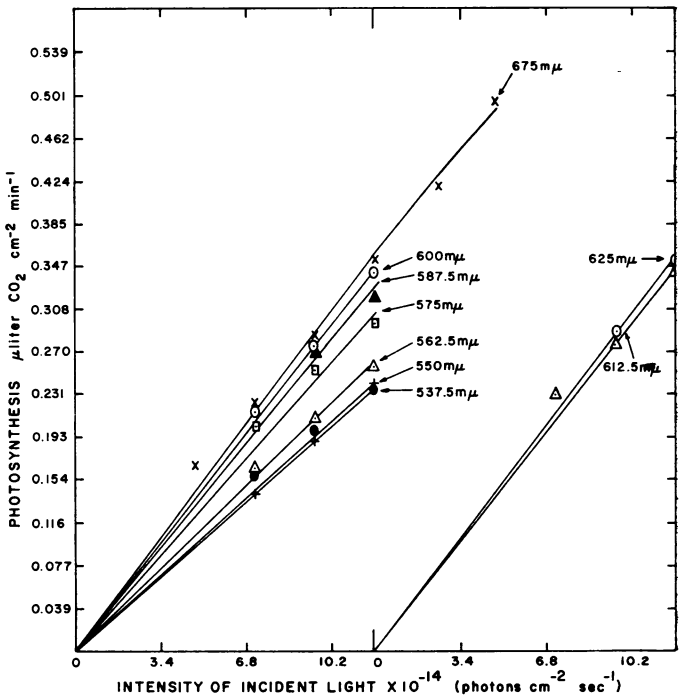

FIG. 5. Light intensity curves, 537.5 to 625 nm, 675 nm.

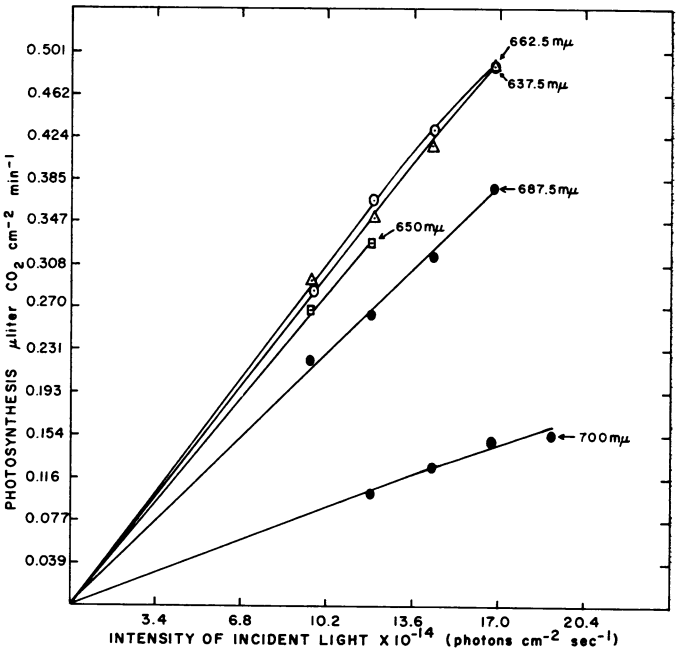

FIG. 6. Light intensity curves, 637.5 to 650 nm, 687.5 nm, 700 nm

Table II. *Derived Values of the Action Spectrum*

Wave Length	$\frac{\mu liter\ CO_2}{cm^2 min}$[1]	$\frac{Photons\ (Incident)}{CO_2\ (Reduced)}$
nm		
400.0	0.232	13.8
412.5	0.285	11.2
425.0	0.302	10.6
437.5	0.316	10.1
450.0	0.304	10.5
462.5	0.270	11.8
475.0	0.246	12.9
487.5	0.250	12.8
500.0	0.264	12.1
512.5	0.250	12.8
525.0	0.233	13.7
537.5	0.233	13.7
550.0	0.241	13.2
562.5	0.260	12.3
575.0	0.302	10.6
587.5	0.325	9.81
600.0	0.343	9.30
612.5	0.343	9.30
625.0	0.351	9.08
637.5	0.352	9.08
650.0	0.331	9.66
662.5	0.362	8.80
675.0	0.358	8.92
687.5	0.270	11.8
700.0	0.109	29.3

[1] At light intensity of 19.8×10^{-10} Einsteins $cm^{-2}sec^{-1}$ (*i.e.*, 11.9×10^{14} photons $cm^{-2}sec^{-1}$).

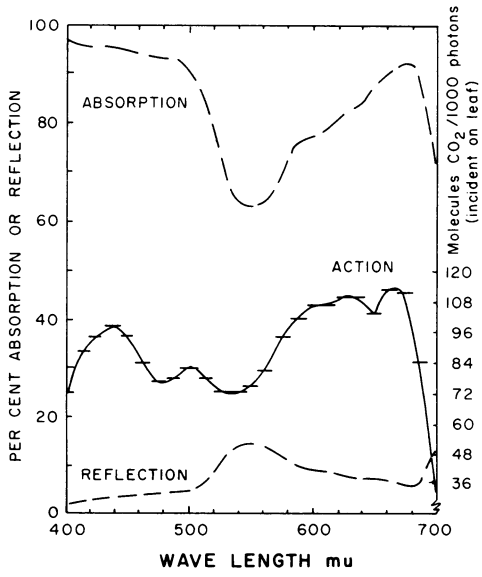

FIG. 7. The detailed photosynthetic action spectrum of the bean leaf, presented with the absorption and reflection spectra as determined by Moss and Loomis (16).

DISCUSSION

The action spectrum presented was determined on an optically thick tissue, *i.e.*, the bean leaf. It follows that analysis of the spectrum in terms of the absorption peaks of the aetivc pigments must be considered only as an approximation.

The broad nature of the two peaks between 662 and 675 nm and between 625 and 637 nm in the action spectrum may be due to the superposition of two or more absorption bands with their peaks close enough to merge into a single band when a 12.5-nm interval is used. The relatively well sustained photosynthetic rate at 687 nm with the rapid decline to 700 nm may be an indication of a Chl band at about 683 nm. All plants appear to have a form of chlorophyll absorbing at about 670 to 673 nm, referred to as $C_a673 \pm 2$, and another form, $C_a683 \pm 2$ (20). Krasnovsky and Kosobutskaya (cited by Rabinowitch, Ref. 18) suggested that two forms of chlorophyll are present in bean leaves: an active monomeric form with a peak at 665 to 670 nm and a more abundant aggregated form with a peak at 683 nm. Butler (4), in 1965, reported the presence of two forms of Chl *a* in the bean leaf with absorption peaks at approximately 673 and 683 nm.

The photosynthetic maximum at 625 to 637 nm probably corresponds to the Chl *a* secondary absorption maximum reported to occur between 620 and 626 nm in various species (8). This absorption band is barely detectable in the absorption spectra of higher plants; however, plants lacking Chl *b* show this band clearly, *e.g.*, diatoms and brown algae. The observed photosynthetic depression at 650 nm (where Chl *b* is known to absorb significantly) in the bean action spectrum may have accentuated the appearance of this secondary photosynthetic maximum. The presence of this photosynthetic peak is in agreement with the curve analysis of the Chl *a* red band in *Chlorella* by French (9), which indicates that a large fraction of the light is absorbed by the Chl *a* secondary peak at 625 nm. He also suggested that the peak may have a doublet structure with broad components at about 620 and 630 nm. The photosynthetic action spectrum of the wheat leaf as determined by Egnéus (5) indicates the broad nature of this maximum and confirms the major peak at 675 nm.

The minor peak at 500 nm indicates the participation of the carotenoids. Emerson and Lewis (6) estimated that in *Chlorella* the carotenoids absorb significantly from 530 nm downward. They estimated that 75% of the total absorption at 500 nm and about 55% at 460 nm are due to these pigments. A recent action spectrum for *Chlorella* by Lundegårdh (13) shows a similar maximum at about 500 nm. Lundegårdh (14) presents an action spectrum for the reduction of NADP-ferrodoxin by spinach chloroplasts with a band at 500 nm.

The identification of the active pigments in the blue-green region is more difficult because of the overlapping of the chlorophyll and carotenoid bands. Nevertheless, the peak at 437 nm agrees with the Chl *a* absorption band reported to occur in different plants between 435 and 438 nm (8). The broad nature of this peak probably indicates the participation of carotenoids and Chl *b*. There is a suggestion of an absorption band at about 412 nm as evidenced by the sustained level of photosynthesis to 412 nm and the sudden drop at 400 nm. It may correspond to the minor blue band of Chl *a* found between 416 and 418 nm in various plants (8).

The drop in photosynthetic activity by the bean leaf at 650 nm may be due to a possible partial inactivity of Chl *b*. The derivative spectrum for Ulva by French (7) shows that the red Chl *b* band may have two components, one at about 647 nm and the other at about 641 nm. Thus, if there were two forms of Chl *b* in the bean plant, the long wave length form (647 nm) would be inactive. However, overlapping of two active bands could also account for the observed dip. The data of Emerson and Lewis (6) on the quantum yield of *Chlorella* show a small but presumably significant minimum at 650 to 660 nm.

The depression at about 480 nm again implies reduced efficiency of Chl *b*. Chl *b* has an absorption band reported between 470 and 490 nm in different plants (8). If Chl *b* is the cause for

he depression at 650 nm, it must be also contributing to that at 480 nm.

A comparison between the absorption (16) and the action spectra (as shown in Fig. 7) indicates that there are general similarities, especially at wave lengths longer than 500 nm. There seems to be much wasted absorption on either side of the action spectrum peak at 437 nm. We have confirmed the reported observation that bean leaves contain large amounts of water-soluble nonplastid yellow pigment (18). A similar pigment has also been found in conifer needles by Burns (3), who attributed the photosynthetic inefficiency of blue-green light to the presence of this pigment. The loss of light to this nonplastid pigment depends on the length of the light path. This in turn depends on the photosynthetic pigment abosrption capacity and the degree of light scattering (21). With low absorption capacity or high light scattering, or both, the light transverses much more cellular material before it is absorbed and hence a greater loss to the nonactive pigments. This could explain the low efficiency of photosynthesis in the blue region since light scattering is inversely proportional to the fourth power of the wave length.

Quantum Number. Since the absorption of blue and red light is almost complete (Fig. 7), the incident energy can be taken as approximating the absorbed energy. Quantum numbers calculated in this way (Table II) were found in the range of 9 to 13 $h\nu/CO_2$, values that are close to those for unicellular organisms. Quantum numbers determined for leaves of higher plants are in good agreement with our results. Briggs (as cited by Kok, Ref. 12), using gas analysis to measure the oxygen exchange of *Phaseolus*, *Sambucus*, and elm leaves, observed values approaching 11 $h\nu/O_2$. Gabrielsen (as cited by Gaffron, Ref. 10), using gas analysis to measure CO_2 exchange with leaves of several higher plants, found quantum numbers between 10 and 12 $h\nu/CO_2$. Wassink (as also cited by Kok, Ref. 12), using manometric techniques to determine quantum numbers for several horticultural plants, reported values between 11 and 13 $h\nu/O_2$. Our detailed results are in satisfactory agreement with generally accepted values.

LITERATRUE CITED

1. Balegh, S. E. 1969. Photosynthetic studies on the bean plant. Ph.D. thesis. Washington State University, Pullman.
2. Bassham, J. A., K. Shibata, and M. Calvin. 1955. Quantum requirement in photosynthesis related to respiration. Biochim. Biophys. Acta 17: 332–340.
3. Burns, G. R. 1942. Photosynthesis and absorption in blue radiation. Amer. J. Bot. 29: 381–387.
4. Butler, W. L. 1965. Development of photosynthetic systems 1 and 2 in a greening leaf. Biochim. Biophys. Acta 102: 1–8.
5. Egnéus, H. 1968. Action spectrum for the transient and the normal photosynthetic oxygen evolution in wheat leaves. Physiol. Plant. 21: 602–614.
6. Emerson, R. and C. M. Lewis. 1943. The dependence of the quantum yield of *Chlorella* photosynthesis on wavelength of light. Amer. J. Bot. 30: 165–178.
7. French, C. S. 1958. Various forms of chlorophyll *a* in plants. Brookhaven Symp. Biol. 2: 65–73.
8. French, C. S. 1959. The chlorophylls *in vivo* and *in vitro*. *In:* W. Ruhland, ed.. Encyclopedia of Plant Physiology, Vol. 5, No. 1. Springer-Verlag, Berlin, pp. 232–297.
9. French, C. S. 1968. The absorption spectra of chlorophyll *a* in algae. Carnegie Inst. Wash. Year B. 66: 177–186.
10. Gaffron, H. 1960. Energy storage: Photosynthesis. *In:* F. C. Steward, ed., Plant Physiology—A Treatise, Vol. 1B. Academic Press, New York and London. pp. 116–119.
11. Hoover, W. H. 1937. The dependence of carbon dioxide assimilation in a higher plant on wavelength of radiation. Smithson. Misc. Collect. 95: 1–13.
12. Kok, B. 1959. Efficiency of photosynthesis. *In:* W. Ruhland, ed., Encyclopedia of Plant Physiology, Vol. 5, No. 1 Springer-Verlag, Berlin. p. 168.
13. Lundegårdh, H. 1966. Action spectrum and the role of carotenoids in photosynthesis. Physiol. Plant. 19: 754-769.
14. Lundegårdh, H. 1967. Role of carotenoids in photosynthesis of green plants. Nature 216: 981–985.
15. Monk, G. S. and C. F. Ehret. 1956. Design and performance of a biological spectrograph. Radiat. Res. 5: 88–106.
16. Moss, R. A. and W. E Loomis. 1952. Absorption spectra of leaves. I. The visible spectrum. Plant Physiol. 27: 370–391.
17. Ozbun, J. F., R. J. Volk, and W. A. Jackson. 1964. Effects of light and darkness on gaseous exchange of bean leaves. Plant Physiol. 39: 523–527.
18. Rabinowitch, E. I. 1951. Photosynthesis and Related Processes, Vol. 2, Parts 1 and 2. Interscience. New York.
19. Seliger, H. H. and W. D. McElroy. 1965. Light: Physical and Biological Action. Amer. Inst. Biol. Sci. and E. A. C. Academic Press, New York and London. pp. 16–21.
20. Smith, I. H. C. and C. S. French. 1963. The major and accessory pigments in photosynthesis. Ann. Rev. Plant Physiol. 14: 181–224.
21. Strain, H. H. 1950. Cellular opacity and the activity of chloroplast pigments in photosynthesis. Science 112: 161–164.