ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XXX — Nr 3—4
1961

The influence of chloroplast displacements on the optical properties of leaves

by

J. ZURZYCKI

INTRODUCTION

Changes in the arrangement of chloroplasts in leaf cells alter the optical properties of leaves, e.g. the profile arrangement assumed by chloroplasts under the influence of strong light leads to a drop in the amount of absorbed light energy. As early as 1880 S t a h l making direct prints of leaves on photographic paper demonstrated that the parts of leaves previously exposed to strong light transmitted more of the photochemically active light rays and gave darker prints than the parts not exposed to light. B a b u s k i n (1955), using the same method for determinating the action spectrum of phototaxis, assumed that the density of sensitized paper after exposure was a criterion of chloroplast arrangement. Recently B i e b l (1954, 1955) applied a photovoltaic cells placed directly under a leaf exposed to light for measurements of light transmission. In many instances the light transmission of leaves changed according to expectation in view of the changes of the chloroplast arrangements. However, in the leaves of some species there was no clear dependence between chloroplast arragements and transmission or the dependence was directly opposite to what was expected. E.g. the light transmission of *Sambucus nigra* leaves was lower after exposure to strong light when measured with the photovoltaic cell, though with S t a h l ' s method of direct prints on photographic paper a higher transmission of these leaves was observed (B i e b l 1955). Is this result interpreted as caused by the difference in the spectral sensitivity of the two detectors?

Only a few of the investigations just mentioned were connected with the microscopical control of the chloroplast arrangements (B i e b l 1954), and thus as was rightly stressed by S e y b o l d they usually could not precisely define what was the influence of the chloroplast arrangements on the transmission of leaves. Similarly little is known of the actual changes in the absorption of light energy that take place after the chloroplasts are regrouped. Theoretical consideration lead to the conclusion that changes of this kind must take place, but their magnitude is not very great (Zurzycki 1953, Seybold 1955). However some simplifications and not always verifiable assumptions have to be of nesessity introduced into the theoretical calculations and accurate data on the actual changes in the amount of absorbed energy may only be obtained from experimental spectrophotometric measurements.

Some information on this problem is contained in the reports of Detlefson (1888) as well as of Schanderl and Kaempfert (1933). Detlefson measured with a thermocouple the transmission of illuminated leaves of *Humulus lupulus* and found that transmission increased by 12 per cent during 37 minutes. He considered the displacement of chloroplasts to be a possible cause of this change. Working with leaves of *Tradescantia viridis* Schanderl and Kaempfert found that after exposure to sun light transmission increased from 6 to 12 per cent in the yellow-green part of the spectrum and from 3 to 10 per cent in the blue-violet range. Seybold, who doubts the accuracy of these results (1955), has been the first to study the full characteristic of changes in the optical properties of leaves (1956). Using a Hardy spectrophotometer he determined the changes of spectral reflection, transmission and absorption of leaves of *Begonia multiflora*. He found that absorption was distinctly altered, but not more than 5 per cent, after the leaves were exposed to strong light. It seems that in the thin and more translucent leaves of water plants, which are usually used for experiments on the displacement of chloroplasts, the changes of absorption should be somewhat greater. The investigations here reported were carried out on the leaves of water plants and were intended to supply an answer to the following questions:

1. Did a simple measurement with a photovoltaic cell of light transmission of leaves having a single or a few layers of cells correlate with the chloroplast arrangement and could it be regarded as indicative of the chloroplasts arrangements?
2. How did the actual optical conditions of leaves change for various chloroplasts arrrangements?

METHOD

The measurements were carried out on fronds of *Lemma trisulca* and on leaves of *Funaria hygrometrica, Elodea canadensis* and *Fontinalis antypiretica*. In the leaves of the last of these species there are no chloroplast movement and the species was used for obtaining comparative data.

The optical properties of all these species could be determined on a small surface ranging 0,5 to 2 mm. in diameter. The simple method of measuring light transmission consisted in measurements of light intensity with a photovoltaic cell in the plane of the microscope image. The microscope was of the kind designed for microphotography with a side eyepiece. The photovoltaic cell was placed in the plane of the photographic plate and the arrangement of chloroplasts was observed simultaneously through the side eyepiece. The microscope used for these measurements .was equiped with a 10 × objective and a 7 × eyepiece. The light was white or blue (filter BG 23—2 mm). The diameter of the lighted field and at the same time the field of vision was 0,92 mm.

Being translucent but not transparent leaves reflect and transmit light differently than solution. The spectrometric techniques for that kind of biological objects was developed by Shibata (1958, 1959) and

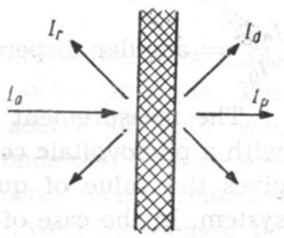

Fig. 1. Path of light in translucent materials (leaves). I_0 — incident light, I_p — parallel transmitted light, I_d — diffuse transmitted light, I_r — reflected light

his method was applied in the present work. Shibata's terminology was supplemented by the concept of transluctance; the logarithm of the reciprocal of transluctance corresponds to Shibata's attenuance. For the sake of precision the various values determined in the experiments are listed and defined below, where I_0 is the intensity of incident light, I_r the intensity of reflected light, I_p the intensity of parallel transmitted light and I_d the intensity of diffuse transmitted light (Fig. 1).

$$\frac{I_p}{I_0} = \text{rectilinear transluctance}$$

$$\frac{I_p + fI_d}{I_0} = \text{quasi transluctance}$$

$$\frac{I_p + I_d}{I_0} = \text{semi-integral transluctance}$$

$$\frac{I_p + I_d + I_r}{I_0} = \text{integral transluctance}$$

$$\frac{I_r}{I_0} = \text{reflectance}$$

$$1 - \frac{I_p + I_d + I_r}{I_0} = \text{absorptance}$$

$$\lg \frac{I_0}{I_p} = \text{rectilinear attenuance}$$

$$\lg \frac{I_0}{I_p + fI_d} = \text{quasi attenuance}$$

$$\lg \frac{I_0}{I_p + I_d} = \text{semi-integral attenuance}$$

$$\lg \frac{I_0}{I_p + I_d + I_r} = \text{integral attenuance}$$

$$\lg \frac{I_0}{I_r} = \text{reflex attenuance}$$

$$\frac{f_a I_r}{I_0} = \text{angular reflection}$$

$$\frac{f_a I_d}{I_0} = \text{angular dispersion}$$

The measurement of the intensity of light transmitted by an object with a photovoltaic cell fitted to a microscope in the way described above gives the value of quasi transluctance, where f depends on the optical system. In the case of the 10× (N.A. 0,24) objective used in the experiments the light detector was reached by rays dispersed by not more than 14 degrees.

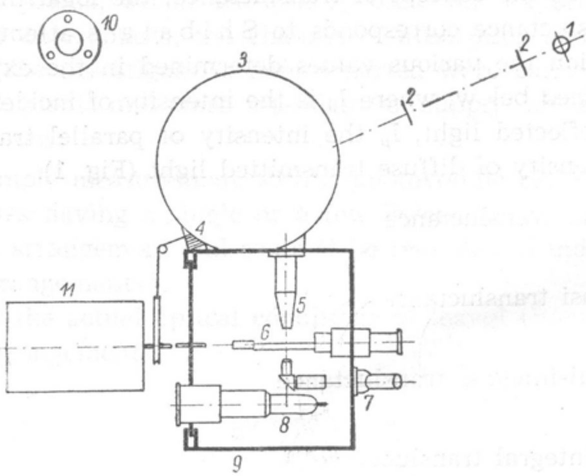

Fig. 2. Schematic diagram of apparatus. *1* — 250 W projection lamp; *2* — light focusing optical system; *3* — mirror monochromator; *4* — monochromator drum; *5* — optical system focusing monochromatic light; *6* — stage for holding the object; *7* — control microscope; *8* — photomultiplier; *9* — chamber in wchich observations are made; *10* — galvanometer; *11* — optical kymograph

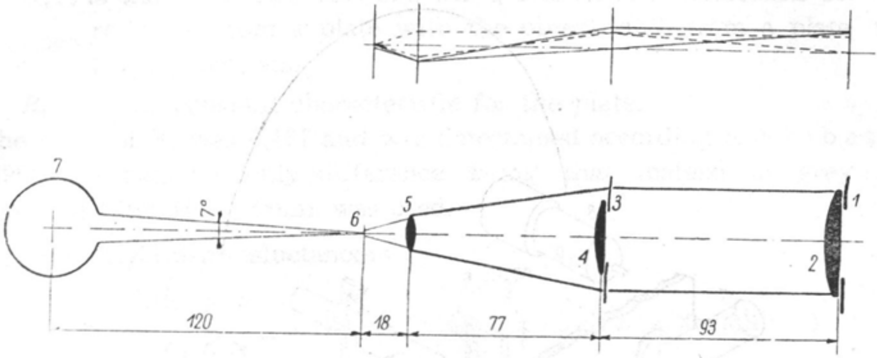

Fig. 3. Optical system focusing monochromatic light. *1* — aperture of monochromator; *2* — lens $f = 7{,}15$ cm.; *3* — iris diaphragm, *4* — lens $f = 4{,}55$ cm.; *5* — lens $f = 1{,}2$ cm.; *6* — light spot; *7* -- photomultiplier. Dimensions in the diagram in milimeters

The absolute measurements of the other optical properties were made in a special apparatus utilizing S h i b a t a's opal plate principle. The design of this apparatus is shown in fig. 2. The source of light was a 250 W lamp supplied from a stabilizer; the light condensed by a system of lenses reached the entrance slit of a Z e i s s mirror monochromator; the light rays coming out from the monochromator were condensed by an optical system shown in fig. 3 and gave an uniformly illuminated light spot 2.5 mm. in diameter. The size of the light spot could be freely reduced by the iris diaphragm incorporated in the optical system. The aperture of the lighting system was about 0.25. The object i.e. the microscopic sample on an opal slide held on a special stage, was placed in the plane of the light spot. The stage with the slide held on it could be moved and fixed in three positions for making zero and blanck measurements. A system of levers working on the principle of a cross stage made possible the placing of any part of a leaf in the light beam. An auxiliary microscope with a low magnification objective was used for the control of the part of the sample exposed to light. The details of the arrangement are shown in fig. 4. The optical system directing the light from the monochromator, the microscopic stage and the objective of the control microscope were placed in a lightproof box in which the preparations were irradiated. The light detector, i.e. a Z e i s s M 12 S photomultiplier, also placed in the box could be adjusted at various angles ranging from 0 to 150 degrees with regard to the beam of light. This equipment allowed the measurement of the following optical properties of leaves:

1. Rectilinear transluctance — the leaf was mounted on a transparent slide and the detector was set at 0 degrees, i.e. the axis of the light beam (the angle of the light beam that reached the photomultiplier being

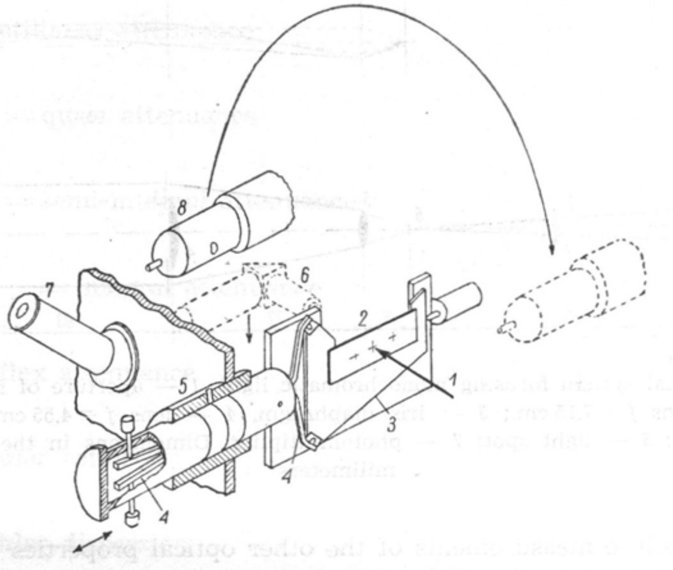

Fig. 4. Stage for holding object and the control microscope. *1* — path of the light beam; *2* — slide; *3* — movable stage adjusted with levers *4*, *5* — tube with spring catch; *6* — prism and microscope objective (can be shifted downwards); *7* — tube of microscope; *8* — photomultiplier

7 degrees the light rays may be regarded as almost parallel). The reference light intensity was the light transmitted by the slide with no leaf.

2. Angular dispersion and reflection — sample on transparent slide, detector adjusted at various angles ranging 0 to 150 degrees the reference light intensity same as in point 1.

3. Semi integral transluctance without correction for multiple reflection — sample on opal slide, detector in the 0 degrees position, the reference light intensity same as in point 1.

4. Reflectance — preparation behind the opal slide, detector in the 150 position, the reference light intensities were black paper on slide and an additional layer of opal glass (S h i b a t a 1958).

The values of reflectance and semi integral transluctance have been calculated from the following formulae:

Reflectance:

$$R_s = \frac{1}{R_1 + \frac{D_2}{D_{1+s}} \cdot \frac{1}{R_1} + R_1} \quad \text{(Shibata 1958, formula 14)}$$

where D_2 is the difference between the galvanometer deflections for light reflected from two opal plate and from one plate with black paper,

D_{1+s} is the difference between the galvanometer deflections for light reflected from a plate with the object and from a plate with black paper, and

R_1 is the constant characteristic for the plate.

The value of R_1 was 0,467 and was determined according to Shibata's (1958) method, the only difference being that instead of grey film a neutral filter NG 5.2 mm. was used.

Semi integral transluctance:

$$T_s = \frac{1 - R_1 R_s}{K_1 / K_{1+s}}$$ (deduced from Shibata's formula 7)

where T_s is semi integral transluctance corrected for multiple reflection:

$$\frac{I_p + I_d}{I_0},$$

K_1 is the deflection of the galvanometer for light passing through opal glass,

K_{1+s} is the deflection of the galvanometer for light passing through opal glass and the sample.

Absorptance:

$$A_s = 1 - (R_s + T_s).$$

The output current of the photomultiplier was measured with a rapid response galvanometer of inner resitance 680 Ω, oscillation period 1.5 sec. and sensitivity $4 \cdot 10^{-10}$ A/mm. m. The deflections of the galvanometer were recorded on an optical kymograph. The axis of the kymograph could be coupled either with the angular gear of the photocell — the graph that illustrated the angular dispersion — or with the drum of the monochromator and then the graph represented the galvanometer deflection with regard to the wave length. An additional lamp lit by suitable connections on the monochromator drum marked light lines on the kymograph drum and these lines were the datum points for the scale of wave lengths. From the graphs thus obtained the values of R_s, T_s and A_s were calculated in the manner already described at intervals of 5 or 10 mμ for the scale of wave lengths and at intervals of 5 degrees for the angular scale. The recording of the leaf spectrum could be complete during a relatively short time, i.e. 8 to 10 minutes, which was important in so far as the chloroplast arrangement obtained in the initial exposure to light was not stable and changed in the dark chamber in which the measurements were made. However, during 10 minutes the changes of the chloroplast arrangement were on the whole insignificant.

RESULTS

I

In the part of the fronds of *Lemna trisluca* with a single mesophyll layer as well as in the leaves of the other species used in the experiments the value of quasi transluctance for the flat arrangement of chloroplasts was 30 to 60 per cent. Under stabilized light conditions — in weak light — the value of quasi transluctance changed but little, the range of the changes not exceeding 2 per cent. After the chloroplasts had been rearranged into the profile position (strong light) quasi transluctance increased by 8 to 20 per cent. The use of the blue filter increased the recorded differences up to 30 per cent.

Table 1

Quasi transluctance of leaves for the flat and profile arrangements of chloroplasts

Object	Light	Quasi transluctance in epistrophe %	Quasi transluctance in parastrophe %	Increment %	Relative increase in relation to epistrophe = = 100%
Lemma trisulca	white	45.9	51.8	5.9	12.86
Lemna trisulca	white	44.4	52.2	7.8	17.56
Lemna trisulca	blue	36.8	46.8	10.0	27.38
Lemna trisulca	blue	47.5	56.9	9.4	19.77
Lemna trisulca	blue	35.5	43.9	8.4	23.66
Funaria hygrometrica	blue	58.3	65.0	6.7	11.50
Funaria hygrometrica	blue	62.0	67.3	5.3	8.55
Elodea canadensis	blue	30.2	40.1	9.9	32.75

Some values of quasi transluctance obtained in various experiments and for leaves of various species are assembled in table 1. The differences of the values of quasi transluctance and of the changes in this value when the chloroplasts passed into the profile arrangement were rather high even within one species. These differences were comprehensible in view of the considerable individual differences in the anatomic structure of leaves and chloroplasts, the concentration of pigments in the plastids etc.

In order to check whether the changes of quasi transluctance conformed with the course of the chloroplast rearrangement measurements were carried out for recording simultaneously in the course of a phototactic reaction the percentage of epistrophe — established by counting the number of chloroplasts in the flat arrangement — and the changes of quasi transluctance (Figs. 5—9). The course of a typical reaction of this kind is shown in fig. 5. A change in the number of chloroplasts in the flat arrangement corresponded to the increase of quasi transluctance. When all the chloroplasts assumed the profile arrangement quasi transluctance increased from the initial value of 48.8 to 56.7 per cent (i.e. by 16.18 per cent) in white light. In blue light the increase was from 41 to

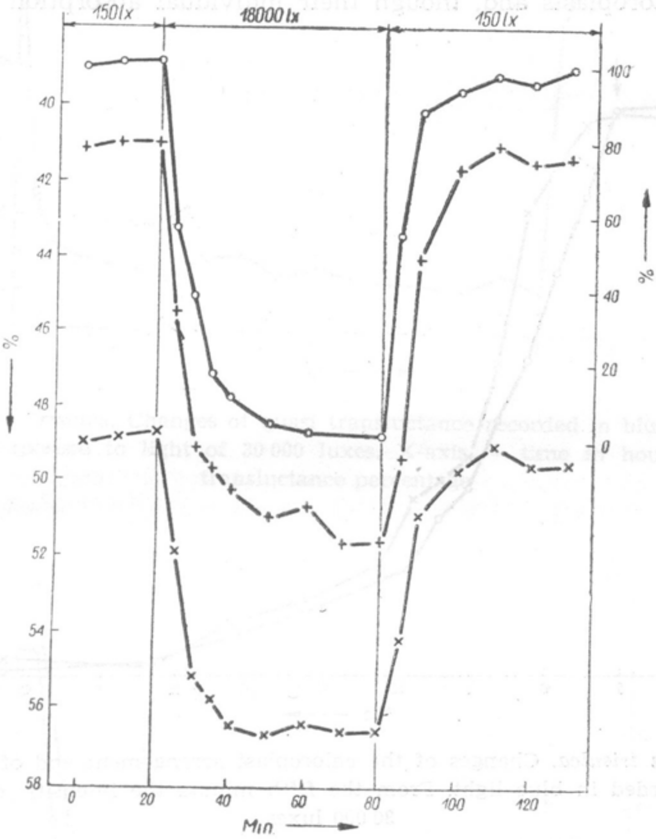

Fig 5. *Lemna trisulca.* Changes in the chloroplast arrangement (right scale) and of quasi transluctance (left scale) —o— percentage of epistrophe; —×— quasi transluctance in white light;—+— quasi transluctance in blue light. X-axis — time in minutes. The figures above indicate the intensity of light

51.7 per cent, i.e. by 26.21 per cent. The return of chloroplasts to the flat arrangement was accompanied by a drop of quasi transluctance to approximately the original value. The changes in the value of quasi transluctance and in the arrangement of chloroplasts as determined numerically are distinctly parallel. This conformity was only disturbed in the first stage of the phototactic reaction the increase of transluctance at first being slower than the decrease of the number of chloroplasts in the flat arrangement. The lack of conformity was better brought out by a series of measurements carried out at shorter intervals (Fig. 6). The certain disagreement between the two methods of measurement is easy to explain. In the initial stage of reaction the part of chloroplasts which had moved to the side cell walls merkedly decreased the percentage of epistrophe, but in the cell they occupied spaces where previously there were no chloroplasts and, though their individual absorption diminished

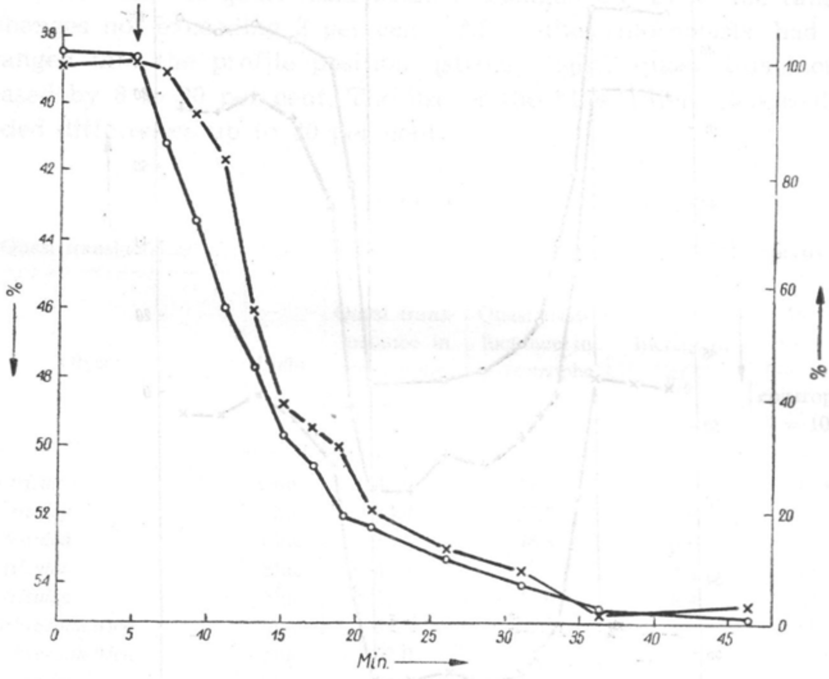

Fig. 6. ***Lemna trisulca.*** **Changes of the chloroplast arrangement and of quasi transluctance recorded in blue light. From the fifth minute the intensity of light was 30 000 luxes**

owing to their different orientation with regard to the direction of light (Zurzycki 1953), the change was relatively small and only slightly influenced the overall absorption of the leaf. It was not till the later stages of the reaction when the chloroplasts were arranged in two or more layers by the side cell walls that the change of absorption was more distinct.

Investigations on the changes of light transmission of terrestial plants in very strong light have shown that after the initial increase transmission sometimes decreased (Schanderl and Kaempert 1934, Biebl 1954). This effect was usually regarded as being caused either by the accumulation of assimilating starch or by changes in the water content of leaves. In water plants the latter of these possibilities seems out of the question. To check whether also in water plants transmission would

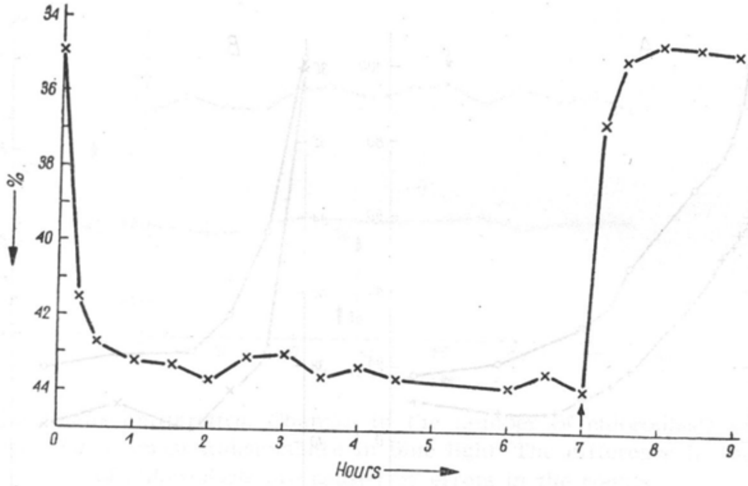

Fig. 7. *Lemna trisulca*. Changes of quasi transluctance recorded in blue light during 7 hours of exposure to light of 30 000 luxes. X-axis — time in hours; Y-axis — transluctance percentage

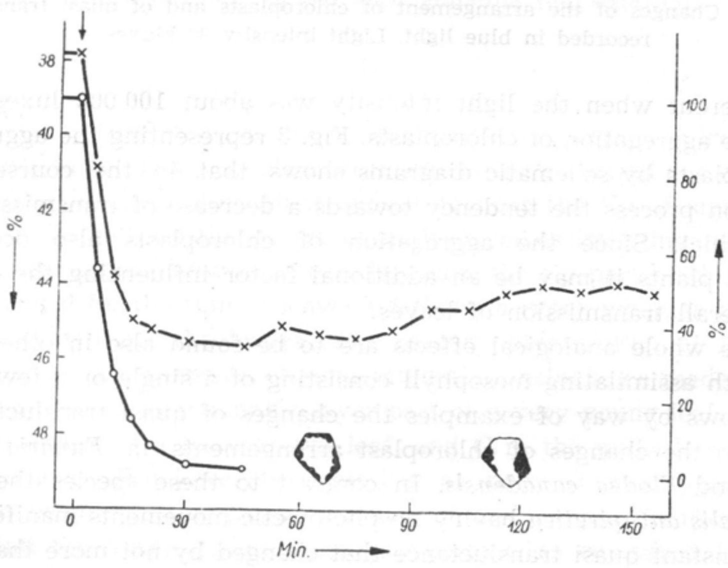

Fig. 8. *Lemna trisulca*. Changes of chloroplast arrangements and of quasi transluctance recorded in blue light. Light intensity 100 kluxes. The degree of the aggregation of chloroplasts is marked schematically

decrease after long exposure to strong light experiments were carried out with *Lemna trisulca* in light of about 30 000 luxes (Fig. 7). During 7 hours of irradiation only small directionless changes of quasi transluctance not exceeding 2 per cent were observed. However, the results

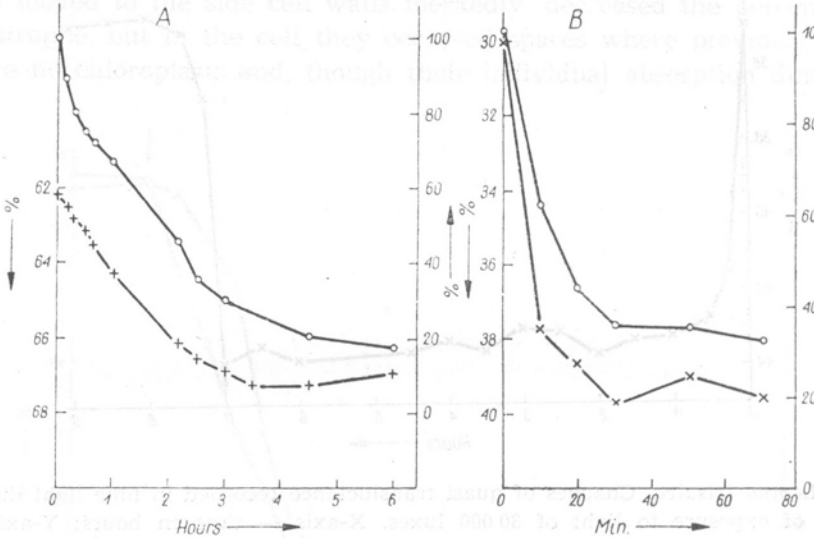

Fig. 9. *A — Funaria hygrometrica.* Changes of the arrangement of chloroplast and of quasi transluctance recorded in blue light. Ligth intensity 50 kluxes. *B — Elodea canadensis.* Changes of the arrangement of chloroplasts and of quasi transluctance recorded in blue light. Light intensity 30 kluxes

were different when the light intensity was about 100 000 luxes which caused the aggregation of chloroplasts. Fig. 8 representing the aggregation of chloroplasts by schematic diagrams shows that in the course of the aggregation process the tendency towards a decrease of transmission was very distinct. Since the aggregation of chloroplasts also occurs in terrestrial plants it may be an additional factor influencing the changes of the overall transmission of leaves.

On the whole analogical effects are to be found also in other water plants with assimilating mesophyll consisting of a single or a few layers. Fig. 9 shows by way of examples the changes of quasi transluctance in relation to the changes of chloroplast arrangements in *Funaria hygrometrica* and *Elodea canadensis.* In contrast to these species the leaves of *Fontinalis antypiretica* having no phototactic movements manifested an almost constant quasi transluctance that changed by not more than 2 per cent in spite of prolonged exposure to strong light (Fig. 10).

The results here reported indicate that quasi transluctance measured by the simple method of placing a photovoltaic cell in the plane of the microscope image must be regarded as only an approximate measure of the chloroplast arrangements. The difficulties of appying this method for the determination of chloroplast arrangement consist in the high variability of quasi transluctance for the two main chloroplast arrangements

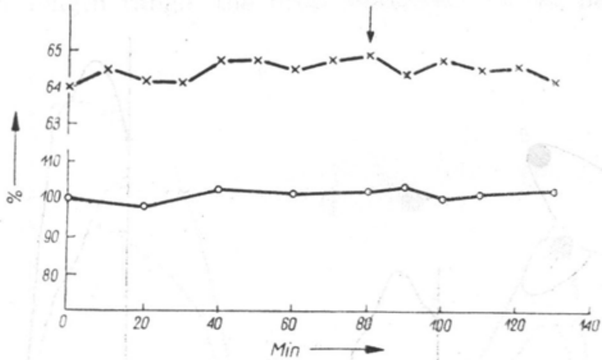

Fig. 10. *Fonitinalis antipiretica.* Changes in the number of chloroplasts in the flat arrangement and of quasi transluctance in blue light. The difference in the number of chloroplasts are caused by errors in the counts

in different species and even in different plants of the same species, the absence of strict parallelism between quasi transluctance and the changes in the chloroplast arrangements, and the additional disturbances arising in the case of unusual chloroplast arrangements (aggregation).

II

More accurate measurements of the optical properties of leaves with chloroplasts in the flat or profile arrangement were carried out on *Lemna trisulca.* The leaves of this plant consisting near the tip of single layer mesophyll are built at the centre of multi-layer assimilating tissue with numerous air spaces between the cells. Thus, the tip part of these leaves is very typical for the thinest leaves (of *Funaria* type), whereas the centre part resembles more the leaves of terrestrial plants. Fig. 11 illustrates the results of measurements of semi integral transluctance made at three points of a leaf: *A* in the single-layer part of a very young leaf, *B* in the single-layer part of a fully grown leaf, and *C* in the multi-layer part of a medium leaf. The lower transmission at point *B* as compared to *A* seemed to be due to the greater amount of the pigment (darker chloroplasts), and the lower transmission at point *C* as compared to *A* was caused by greater reflection owing to the presence of many intercellular spaces. However, in all instances semi integral transluctance was distinctly lower for the profile than for the flat arrangement. The greatest differences occured in the short-wave part of the spectrum and in red light, i.e. for the range of strong absorption, whereas in the 500 to 600 mμ wave-length range the differences were insignificant.

The full characteristic of the changes in the optical properties of leaves taking place after the regroupment of chloroplasts is shown in

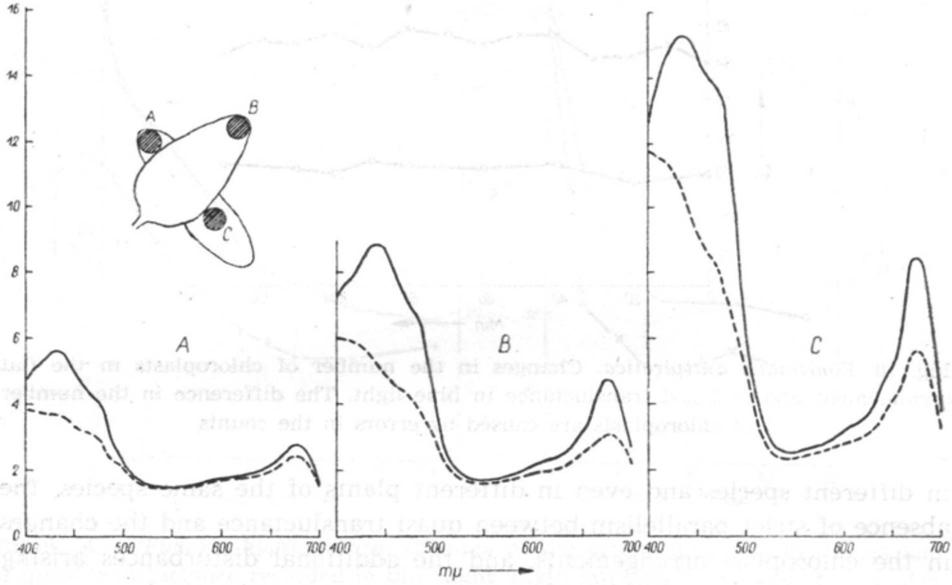

Fig. 11. *Lemna trisulca*. The dependence of semi integral transluctance on the wave length of light and the arrangement of chloroplasts for three segments of *Lemna trisculca* fronds: *A* — single-layer part of a young leaf, *B* — single-layer part of fully grown leaf, C — multi-layer part of leaf. Y-axis — reciprocal of semi integral transluctance, X-axis — wave-length

fig. 12 and 14. In the single-layer part of a leaf (fig. 12) reflection R_s was relatively small and amounted to about 3 per cent in the strong absorption range and to about 8 per cent in the middle of the spectrum. After the displacement of chloroplasts to the side cell walls reflection distinctly increased — to about 10 per cent — only in the middle part of the spectrum, whereas in the wave-length range of strong absorption the increase of reflection was insignificant (by about 0.5 per cent). Integral transluctance T_s increased very distinctly after the rearrangement of chloroplasts into the profile position. i.e. from 14 to 27 per cent in blue and from 27 to 39 per cent in red light. The differences of integral transluctance decreased with the decrease of absorption by pigments, so that at wave-lengths of about 550 mμ integral transluctance could even be somewhat lower in the profile than in the flat arrangement of chloroplast. This effect was associated distinctly with the changes of reflection in this wave-length range. The changes of absorptance accounting for the changes of reflection and light transmission indicate a marked drop of absorption in the profile arrangement for the whole range of the spectrum, though the drop was most pronounced in the most strongly absorbed wave-length range: the drop amounted to 15 per cent in the

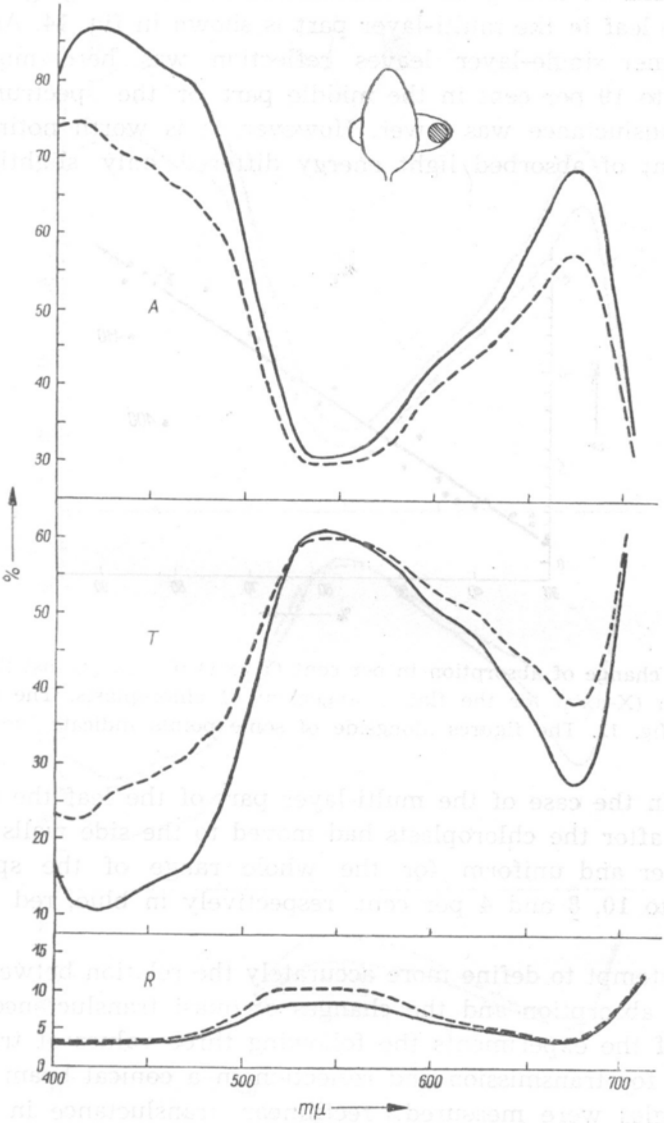

Fig. 12. Reflection (*R*), transmission (*T*) and absorption (*A*) spectra of the single--layer part of a *Lemna trisulca* leaf for the flat (—) and profile (- - -) chloroplast arrangements. X-axis — wave length; Y-axis — *R*; *T* and *A* in percentage

short-wave part of the spectrum, to 12 per cent in red light, and only to 2 per cent in the middle of the spectrum. The decrease of absorption in the profile arrangement was almost directly proportional to the initial absorption for epistrophe (Fig. 13), the only exception being the change of absorption in the 400 to 420 mμ wave-range which was much smaller than what could have to be expected from the initial absorption.

The results of analogical measurements of the changing optical properties of a leaf in the multi-layer part is shown in fig. 14. As compared to the thinner single-layer leaves reflection was here much higher, amounting to 19 per cent in the middle part of the spectrum, whereas integral transluctance was lower. However, it is worth noting that the total amount of absorbed light energy differed only slightly in both

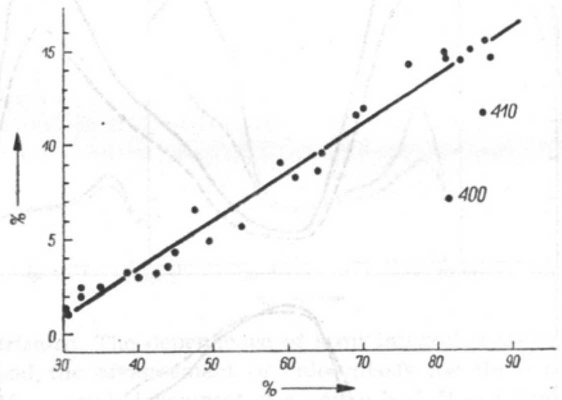

Fig. 13. The change of absorption in per cent (Y-axis) plotted against the magnitude of absorption (X-axis) for the flat arrangement of chloroplasts. The data are the same as in fig. 12. The figures alongside of some points indicate the wave-length

instances. In the case of the multi-layer part of the leaf the decrease of absorption after the chloroplasts had moved to the side walls was somewhat smaller and uniform for the whole range of the spectrum. It amounted to 10, 8 and 4 per cent respectively in blue, red and green light.

In an attempt to define more accurately the relation between the true changes of absorption and the changes of quasi transluctance examined in part I of the experiments the following three values of transluctance accounting for transmission and reflection in a conical beam of light of various angles were measured: rectilinear transluctance in a beam of light parting at 7 degrees and containing almost exclusively parallel rays of light I_p, quasi transluctance for an optical system catching rays with the maximum divergence angle of 25 degrees, and integral transluctance for an opal plate. The results of these measurements carried out on the same surface of a single-layer leaf are shown in fig. 15. It is to be seen from this figure that, besides the different results of the absolute measurements of transluctance, the spectral course of the curves varies somewhat in all three instances. These differences indicate that the

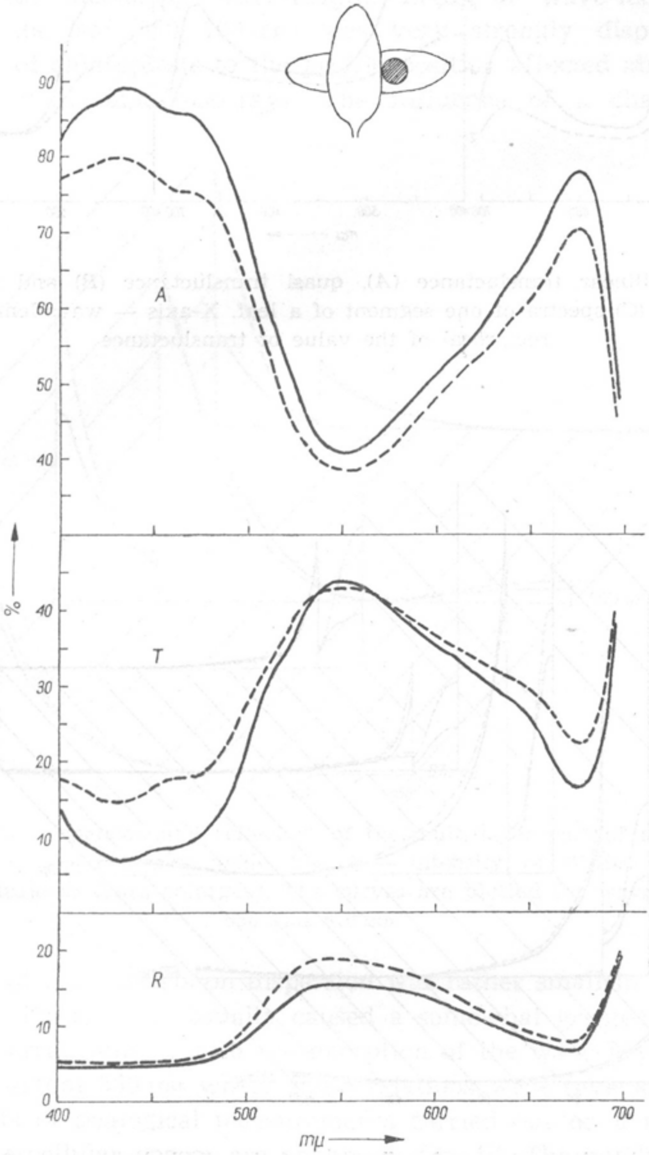

Fig. 14. Reflection, transmission and absorption spectra for the multi-layer part of a *Lemna trisulca* leaf. Details as in fig. 12

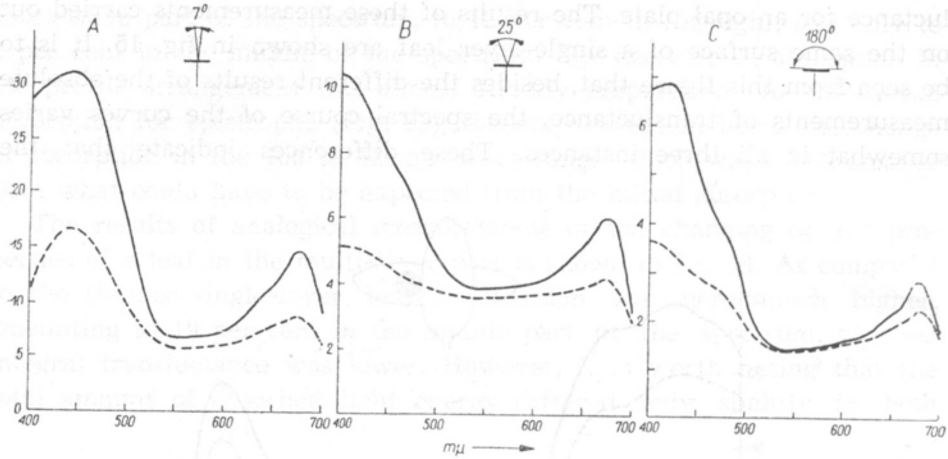

Fig. 15. Rectilinear transluctance (*A*), quasi transluctance (*B*) and semi integral transluctance (*C*) spectra of one segment of a leaf. X-axis — wave-length, Y-axis — reciprocal of the value of transluctance

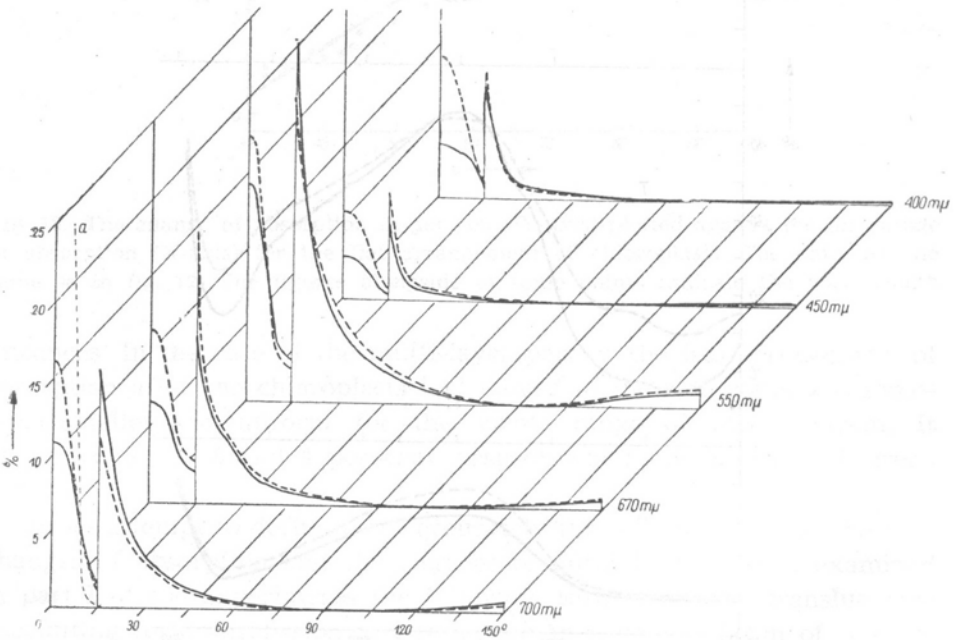

Fig. 16. Angular dispersion and reflection of the single-layer part of a *Lemna trisulca* leaf. X-axis — dispersion angle, Y-axis — ratio of the intensity of diffuse light to the maximum intensity of undiffuse light (from 15 degrees Y-scale 10 times enlarged)

angular dispersion of light must have been different for various wave-lengths.

The results of recordings of angular dispersion for various wave-lengths carried out on the surface of another leaf are shown in fig. 16. As is to be seen from the curves the dispersion being associated with the wave-length was small in the short-wave range and on the whole increased with increasing wave-length. Light of wave-lengths little absorbed by the leaf (550, 700 mμ) was very strongly dispersed. The displacement of chloroplasts to the profile position affected above all the transmission of the undiffuse rays. The influence of a change in the

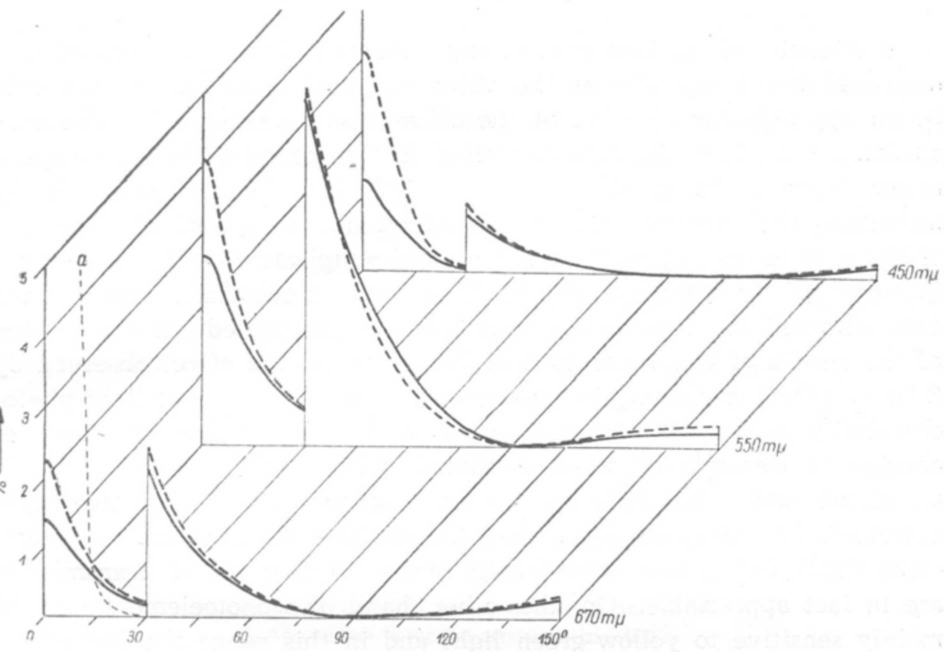

Fig. 17. Angular dispersion and reflection of the multi-layer part of a *Lemna trisulca* leaf. X-axis — dispersion angle, Y-axis — intensity of diffuse light (from 30 degrees X-scale 10 times enlarged). The curves are plotted for wave length 450, 550 and 670 mμ

arrangement of chloroplasts on dispersion was rather small in the single--layer leaves. Parastrophe usually caused a somewhat greater dispersion then the flat arrangement, with the exception of the wave-length 700 mμ and to some extent 550 mμ where these relations were reversed.

The results of analogical measurements carried out on a multi-layer leaf with intercellular spaces are shown in fig. 17. The conditions with regard to transluctance and reflectance of this leaf are shown in fig. 14. Owing to the different anatomical structure of the leaf transmission was much weaker, and light dispersion much stronger than in a single-layer leaf. However, the conditions in both kinds of leaves were basically similar. Dispersion increased with the increase of wave length and was especially high in light most weakly absorbed by a leaf. The change of the arrangement of chloroplasts affected most strongly the transmission of undispersed rays. The profile arrangement of chloroplasts increased dispersion, which was small in blue light, stronger in red light, and increased in light of wave length 550 mμ within the angle of 40 degrees but dropped when the divergence of rays was more than 40 degrees.

DISCUSSION

The results of the first part of experiments indicate that transmission measured microscopically as the value of quasi transluctance can only be an approximate criterion of the chloroplast arrangements. The conclusion arising from the data assembled in the second part of the experiments is that the spectral sensivity of light detector very strongly influences the experimental results. Although, the optical characteristic of leaves of terrestrial plants is much more complicated owing to the great influence of the water content of these leaves, neverthelles some of the data obtained for terrestrial plants may be interpreted on the ground of the results of this investigation. For instance, the effect observed by Biebl (1955) consisting in the increase of transmission for light photochemically active on sensitized paper and the simultaneous lack of changes in transmission when measured with a photoelement may be associated with the different spectral sensivity of these two light detectors. Photographic paper may be sensitive to short waves (violet-blue light) and in this wave-length range the changes of transmission are in fact appreciable. On the other hand the photoelement may be mainly sensitive to yellow-green light and in this range the changes of transmission are much smaller. In view of these results Babuskin's (1955) method of determining the arrangement of chloroplasts from the density of exposed sensitized paper seems to be of insufficient accuracy for investigations on the action spectrum.

In all the measurements of this investigation a decrease of light absorption has been observed when the chloroplasts passed from the flat to the profile arrangement. In single-layer leaves this decrease is very well marked in strongly absorbed light and much less apparent in yellow-green light. In multi-layer leaves the differences of light absorption are only in a small degree dependent on the spectral composition of light. The data obtained for this last kind of leaves approach more the results reported by Seybold (1956) for terrestrial plants; this worker observed a decrease of absorption by about 3 to 5 per cent more or less uniform over the whole spectrum. The differences of absorption between the flat and the profile arrangement of chloroplasts are with satisfactory approximation directly proportional to the total absorption within a given part of the spectrum. The decrease of absorption is distinctly smaller only in the short-wave end of the spectrum (below 420 mμ). A similar effect, though less distinctly marked, is also visible on the curves plotted by Seybold (1956) and is probably associated with the rising absorption by other elements of a cell in the short wave-length range. Metzner (1930) demonstrated the weak absorption of long wave ultraviolet radiation by the cytoplasm and nuclei and the much greater absorption by the cell sap of the epidermis and mesophyll of many plants. The observations of Seybold and Weissweiler (1942) show that the leaves of *Pelargonium zonale* containing assimilating pigments absorb about 20 per cent of medium and long wave light, whereas at 400 mμ their absorption increases to about 50 per cent. For almost the whole range of the spectrum the photosynthetic pigments have the greatest influence on absorption and thus the rearrangements of chloroplasts are associated with the changes of absorption proportional to the light absorbed in these pigments. In the short wave range absorption of light in other parts of a cell which are not rearranged tends to play a greater role and this seems to be the explanation of the relatively small influence of chloroplast displacements on the changes of absorption.

Table 2

Decrease of the absorption of light when chloroplasts pass to the profile arrangement

(Zurzycki 1953, mean values from table 4. Absorption in the flat arrangement = 100%

Log E of a chloroplast	Decrease of absorption %
3.5	1.79
4	6.52
4.5	16.5
5	33.4

This author (Zurzycki 1953) has theoretically calculated the changes of light absorption from the decrease of the surface area of chloroplasts directly exposed to light and the simultaneous increase of the thickness of the absorbing layer. In the case of *Lemna trisulca* the decrease of the surface exposed to light amounts to 50—60 per cent. The

Table 3

Decrease of the absorption of light after chloroplasts passed to the profile arrangement
(Absorption in the flat arrangement = 100%)

Wave lenght mμ	Decrease of absorption in parastrophe %
440	18.8
480	18.4
550	4.5
600	7.5
670	17.8

calculated changes of light absorption for some given extinction of a chloroplast are assembled in table 2. The value of log E for a chloroplast ranges 3.5 to 4.4. The changes of absorption obtained experimentally and shown in fig. 12 are listed in table 3. As is to be seen the data in these two tables are in very good agreement except for the middle part of the spectrum where the changes of absorption are mainly due to reflection, which factor has not been included in the theoretical calculations. This agreement between the theoretical and experimental values of light absorption at least so far as the order of the magnitudes involved is concerned, proves that the theoretical principles underlying the calculations (Zurzycki 1953) are correct. One of these assumptions has been the uniform distribution of light throughout the whole crossection of the cell. It seems, therefore, that the refraction of light at the edges of a cell has much less significance than has been believed hitherto (Senn 1908, Zurzycki, 1953, also Haupt 1959). When comparing the results of this investigation with the influence of the displacement of chloroplasts on the rate of photosynthesis (Zurzycki 1955) it is to be seen that, though the decrease of absorption in the profile with regard to the flat arrangements is only 12 per cent, the effect of the chloroplast displacement on photosynthesis is much greater. Some experimental data illustrating this point are assembled in table 4.

The differences in the rate of photosynthesis are of the order of 50 per cent and they thus exceed several times the changes of light absorption. The question now arising is whether this discrepancy is caused by the additional influence exerted by the induction period of photosynthesis or by the different photosynthetic capacity with regard to the amount of absorbed energy of chloroplasts in the profile than in the flat arrangement. The latter supposition may be supported by the

Table 4

Changes of the rate of photosynthesis after chloroplasts passed to the profile arrangement (photosynthesis for the flat arrangement epistrophe = 100%)

No. of experiment	Respiration 10^{-3} μl/10 min	True photosynthesis		% reduction of photosynthesis rate
		epistrophe	parastrophe	
L 8	— 5.63	0.82	1.61	49.0
L11	—11.21	5.50	9.61	42.8
L20	—14.10	8.23	17.60	53.3

lamellar structure of chloroplasts and the partial orientation of the pigment molecules in this structure (Goedheer 1957).

The angular dispersion in leaves of *Lemna trisulca* is much smaller than in the leaves of terrestrial plants (Seybold 1933, Metzner 1957). Here again the multi-layer parts of the *Lemna* leaves occupy the intermediate position between the very weakly dispersing leaves having a single-layer mesophyll tissue and the leaves of terrestrial plants. According to Pokrowski (1925) light reflected from a leaf consists partly of light reflected from the external surface and partly of light reflected from the inner surfaces of the leaf. This latter component consists of light more strongly dispersed in various directions. The comparison of the light reflection and dispersion of single-layer *Lemna* leaves, of multi-layer *Lemna* leaves and of the leaves of terrestrial plants shows how relative amount of diffuse light increases owing to the growing complexity of the anatomic structure and of the intercellular system.

In the measurements of angular dispersion no maximum has been recorded, though the existence of such a maximum has seemed probable in connection with the interference of light in the chloroplasts when they are in the profile arrangement (Menke 1957). The interference effect is either very weak or very indistinct owing to the differences in the positions of the particular chloroplasts.

Laboratory of Plant Physiology
Jagellonian University
Cracow

(Entered: 26.3.61 r.)

SUMMARY

1. A simple microscopic apparatus provided with a photovoltaic cell has made possible the demonstration of a certain parallelism between the changes in the quasi transluctance of a leaf and the rearrangement of chloroplasts. However, the changes of quasi transluctance cannot be taken as an accurate criterion of the chloroplast arrangements.

2. The changes in the true absorption of leaves when the chloroplasts pass from the flat to the profile arrangement depend on the total absorption; in *Lemna trisulca* leaves with a single-layer mesophyll they range from about 1 per cent in green light to about 12 per cent in red light and 15 per cent in blue light. The corresponding relative changes expressed in percentages of the absorption in leaves with a flat arrangement of chloroplasts are 19, 18 and 4.5 for blue, red and green light respectively. The average decrease of absorption for the profile arrangement as compared to the flat arrangement may be accepted to be 10 to 12 per cent for the wave-length range 400 to 700 mμ.

3. The leaves of *Lemna trisulca* with a multi-layer mesophyll manifest a lower transluctance, greater reflection and greater dispersion than the leaves with a single--layer mesophyll. In the multi-layer leaves the changes of absorption when the chloroplasts pass to the profile arrangement are not so strongly dependent upon the wave length as in leaves with a single layer.

4. The comparison of the experimental results with the theoretically calculated changes of absorption and with the measurements of the rate of photosynthesis point to the small significance at any rate in leaves with a single-layer mesophyll, of the local reduction of light intensity on the side cell walls. The reduction of the rate of photosynthesis when the chloroplasts are in the profile arrangement seems to be greater than could be expected from the changes in the absorption of light energy.

LITERATURE CITED

Babuskin L. N., 1955, Spektrofototaksisa chloroplastow, DAN SSSR, 103: 333—5.

Biebl R., 1954, Lichttransmission und Chloroplastenbewegung. Flora 141: 163—177.

Biebl R., 1955. Tagesgänge der Lichttransmission verschiedener Blättern, Flora 142: 290—294.

Detlefsen E., 1888, Die Lichtabsorption in assimilierenden Blättern, Arb. Bot. Inst. Würzburg 3: 534.

Goedheer J. C., 1957, Optical Properties and in Vivo Orientation of Photosynthetic Pigments, Diss. Utrecht.

Haupt W., 1959, Chloroplastenbewegung, in Encyclopedia of Plant Physiology ed. by W. Ruhland. Vol. 17/1: 278—317.

Menke W., 1957, Lichtinterferenzen an Chloroplasten, Z. Naturf. 12 b: 407—411.

Metzner P., 1930, Über das Optische Verhalten der Pflanzengewebe im langwelligen ultravioletten Licht, Planta 10; 281—313.

Metzner P., 1957, Zur Optik der Blattoberflächen, Die Kulturpflanze 5: 221—239.

Pokrowski G. J., 1925, Über die Abhängigkeit der Koeffizienten der diffusen Reflexion vom Einfallswinkel des Lichtes, Z. Physik 32: 563.

Schanderl H. und W. Kaempert, 1933, Über Strahlungdurchlässigkeit von Blättern und Blattgeweben, Planta 18: 700—750.

Shibata K., 1958, Spectrophotometry of intact biological materials, Jour. of Biochem. 45: 599—623.

Shibata K., 1959, Spectrophotometry of Translucent Biological Materials — Opal Glass Transmission Method, in Methods of Biochemical Analysis ed. by D. Glick, 7: 77—109.

Seybold A., 1933, Über die optische Eigenschaften der Laubblätter III, Planta 20: 577—601.

S e y b o l d A., 1955, Beiträge zur Optik der Laubblätter, Beitr. Biol. Pfl. 31: 499—513.
S e y b o l d A., 1956, Hat die Chloroplastenverlagerung in Laubblättern eine Bedeutung?, Naturwiss. 43: 90—91.
S e y b o l d A. und W e i s s w e i l e r A., 1942, Spektrophotometrische Messungen an grünen Pflanzen und an Chlorophylllösungen, Bot. Arch. 43: 253—290.
S t a h l E., 1880. Über den Einfluss von Richtung und Stärke der Beleuchtung auf einige Bewegungserscheinungen im Pflanzenreiche, Bot. Ztg. 38: 297.
Z u r z y c k i J., 1953, Arrangement of chloroplasts and light absorption in plant cell, Acta Soc. Bot. Pol. 22: 299—320.
Z u r z y c k i J., 1955, Chloroplast arrangement as a factor in photosynthesis, Acta Soc. Bot. Pol. 24: 27—63.
Z u r z y c k i J., 1957, The destructive effect of intense light on the photosynthetic apparatus, Acta Soc. Bot. Pol. 26: 157—175.