The destructive effect of intense light on the photosynthetic apparatus

Działanie destrukcyjne silnego światła na aparat fotosyntetyczny

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1. INTRODUCTION

The destructive effect of strong light on the photosynthetic apparatus in plants has long been known. Many researches have demonstrated various degrees of photosynthesis inhibition by strong light and the usually reversible nature of this inhibition which is abolished when light returns to middle intensities (Steemann-Nielsen 1942-1952, Meyer and Burr 1940 and others). On the other hand, many results indicate that under the influence of strong light chlorophyll is decomposed and gradually disappears from the living cell (e. g. Iwanowski 1913, Montfort and Zollner 1942, Montfort 1950). Finally, it is well known that the chloroplasts in cells of many plants are displaced, rearranged or undergo other changes according to the intensity of incident light (Senn 1908, Biebl 1954, and others).

Unfortunately all investigations on the three aspects of the influence of strong light on the photosynthetic apparatus were carried out on various plant species and were unrelated between them. Consequently it is impossible to draw any general conclusions on the influence of strong light on the three processes simultaneously.

The present investigation has been undertaken with the aim to fill this gap by a series of observations on the relations between the chloroplast arrangement, the inactivation of photosynthesis and the destruction of chlorophyll on one hand and the light intensity and the time of exposure on the other. One species only and light with the same spectral composition were used for all experiments.

2. MATERIAL AND METHODS

For the experiments young leaves of *Lemna trisulca* were prepared in the same manner as previously described (Zurzycki 1954). The size

of the leaves was 3—7 mm². Microscopic observations were made on the part of leaves with a single or double mesophyll layer.

The source of light in the experiments was a projection lamp of the Osram E 30 type supplied with 215 ± 1 V electric current. The light reached an illuminated leaf through two convergent lenses. To regulate the intensity of light the distance from the lamp and the system of lenses to the object was altered. Light intensity was measured with a Lange photometer equipped with neutral filters.

For exposure to light, the leaves were mounted between a slide and a cover glass held together in several points at the edges with paraffin. During the exposure the slide with the leaves were placed in a flat vessel in strong current of flowing water to prevent heating of the object.

The arrangement of chloroplasts in cells was defined by qualitative description.

The degree of photosynthesis inactivation was estimated by measuring the rate of photosynthesis in a leaf at light intensity of 2500 lux before the illumination with strong light and again after exposure to this light. For the measurements the micro-method described earlier (Zurzycki 1954) was used. To find out if and in what extent the drop of photosynthesis was temporary, the leaves after exposure were kept in diffuse day light and the measurements were repeated at several intervals. The rate of photosynthesis after exposure was defined as the percentage of the rate before illumination taken as being 100 per cent.

The destruction of chlorophyll was defined by measuring light absorption in the ether extract from the leaves used for experiments. The extract was made by squashing the leaves (4—6 leaves about 4—7 mm²

Table 1

Light absorbed by extract in %		Absorption in sample II in respect to ab- sorption in sample I 100%
Sample I	Sample II	
35,0	32,5	92,8
36,0	35,5	98,6
41,5	44,0	106,0
39,5	38,8	98,2
45,4	45,3	100,2
Mean		99,16

each being used for one measurement) in ether, filtering and adding ether to 2 ml. Measurement of absorption were made with a Pulfrich photometer using absorption cell of 1,5 ml capacity and S 61 filter. The differences in chlorophyll concentration were defined relatively to the per-

centage change of absorption, the absorption by the extract from an unexposed control sample being taken as 100 per cent. The most difficult problem was the choice of two similar samples of which one would be used as a control and the other for exposure to strong light. The most effective procedure was found to be the preparing of numerous leaflets and then choosing several pairs which would resemble one another as closely as possible in respect to size, shape, intensity of colour etc. Although this method had the disadvantage of not being free from personal judgement the choice of 4 to 6 leaves for one sample equalized to some extent the possible differences. When the choice was made carefully the procedure gave very consistently uniform results, which can be seen from the examples in Table I where light absorption in extracts from several pairs of samples chosen in the manner just described and not exposed to strong light are listed.

3. RESULTS

3.1. MORPHOLOGICAL CHANGES OF CHLOROPLASTS IN CELLS EXPOSED TO LIGHT OF 100 000 LUX

Before the experiments the leaves were kept in diffuse day light and consequently the starting position of chloroplasts was always the flat arrangement, epistrophe (fig. 1—1). After exposure to light of 100 klux already after 15 minutes almost all chloroplasts are on the lateral cell walls in the profile position. Not more than 5—15 per cent of chloroplasts remain still in the flat position. After 30 min of exposure parastrophe is complete. If there are not too many chloroplasts in a cell there is enough space for all of them to be pressed with the flat face against the lateral walls (fig. 1—2). In exceptional cases when the chloroplasts are numerous the arrangement is not typical. Those chloroplasts for which there is no room on the lateral walls lie alongside one of the walls touching it not with the flat surface but with the edge and projecting into the cell. The tendency to assume the profile position is very strongly marked in light of this intensity, whereas in lower light intensities e.g. 30 klux — those chloroplasts for which there is no room on the lateral walls lie in the flat position on the outer wall and only move away from the centre.

The typical profile arrangement is maintained for 1,5 to 2 hours of exposure to strong light. After this time the chloroplasts begin to form aggregations at first in some few cells only and then more and more commonly. The aggregations of chloroplasts usually lie in one or several corners of a cell but do not form typical systrophe (aggregation around a nucleus) because as a rule there are two and more aggregations in one cell (fig. 1—3). After 3 to 4 hours all or almost all chloroplasts are assembled in aggregations and the greater part of the lateral cell walls

are without chloroplasts (fig. 1—4). The arrangement of aggregations is usually such that in two neighbouring cells the aggregations lie opposite one another on either side of the cell membrane.

The conditions as described above remain unchanged for the next 4 hours of exposure. After that time some chloroplasts slip out of the

Fig. 1. Schematic diagrams of typical morphological changes of chloroplasts in cells of *Lemna trisulca* exposed to strong light.

aggregations and disperse along the cell walls. Their colour at this stage is distinctly less intense, though just before dispersing they were still normally coloured (fig. 1—5). As time passes all the chloroplasts scatter, the aggregations disappear and the chloroplasts become pale green and finally quite colourless (fig. 1—6). The refraction coefficient of chloroplasts increases and consequently both their outlines and the outlines of starch grains inside become very clearly visible. The diameter of chlo-

PLATE I

1

4

2

5

3

6

Morphological changes of chloroplasts in cells of *Lemna trisulca* exposed to strong light

Fig. 1. Epistrophe arrangement of chloroplasts in diffuse light

Fig. 2. Parastrophe arrangement of chloroplasts after exposure for 30 minutes to the light of 100 klux

Fig. 3. Aggregations of chloroplasts after 2 hours exposure to strong light

Fig. 4. Chloroplasts aggregations after 3—4 hours exposure to strong light

Fig. 5. The beginning of the dispersion of the chloroplasts; at the same time they begin to be paler

Fig. 6. The last stage when the chloroplasts are dispersed and colourless.

roplasts diminishes. Approximately at the same time when all chloroplasts completely lose their pigmentation the cells die which is evidenced by the loss of the ability for plasmolysis.

Between different leaves there are slight variations in respect to the sensitivity to strong light. Similarly, variability in this respect is displayed by the different parts of one leaf. The time that all the stages here described last refer to the central part of the leaf consisting of one or two mesophyll layers. The rims of leaves are usually more sensitive, whereas in the basic and central part consisting of many layers and with large intercellular spaces the processes are somewhat slower. For instance, when the whole leaf is already almost entirely decolorized, aggregations of absolutely normally coloured chloroplasts still persist in the multilayer parts giving a greenish hue.

3.2. MORPHOLOGICAL CHANGES IN CHLOROPLASTS AT OTHER LIGHT INTENSITIES

The changes in the morphology and arrangement of chloroplasts characteristic for light intensity of 100 klux also occur at other intensities. However in light of 30 klux only parastrophe is induced and exposures lasting even 12 hours do not cause the formation of aggregation. At

Fig. 2. Relation between the stage of chloroplasts in a cell and exposure time and light intensity. Abscissae — light intensity in klux, ordinates — exposure time in hours. The manner of shading is explained by fig. 1.

60 klux the change are much slower and exposure during 12 hours only causes the formation of aggregation. At higher light intensities of 130 and 160 klux the successive stages in response are more rapid. The relation between the stage of chloroplasts and the intensity and time of illumination is shown schematically in fig. 2.

Exposure times to light of a given intensity necessary to produce the various changes in about half of the material under observation are marked with circles. The graphical representation of the types of chloroplast arrangements are showed here in the same manner as in fig. 1. It appears from the curves that if the aggregation of chloroplasts is to be caused the intensity of light must exceed 30 to 50 klux. Light of 100 klux is lethal for cells after about 10 hours of exposure and exposure for 4 hours is sufficient to obtain the same effect at 160 klux. Of course these results refer to the light used in the present experiments only. For light with a different spectral composition the corresponding results may have different values, but the general character of the relationship will probably remain unchanged.

3.3. INACTIVATION OF PHOTOSYNTHESIS IN LIGHT OF 100 000 LUX

Even a short illumination with light of 100 000 lux produces a distinct depression in the rate of photosynthesis. The inhibition may be complete if the leaf is exposed for 5 to 6 hours. The results from a series of mea-

Fig. 3. The course of photosynthesis and respiration in a leaf before and after exposure for 5 hours to light of 100 klux. Abscissae—time in mins, ordinates — the production or consumption of oxygen in $10^{-3} \mu\text{l}$.

surements of the gaseous metabolism in a leaf before and after exposure for 6 hours are shown in fig. 3. It seems, therefore, that strong light damages selectively only the photosynthetic apparatus.

The destruction of the photosynthetic mechanism proceeds very rapidly. Fig. 4 shows how the inactivation of photosynthesis proceeds with the time of exposure. Already after 15 minutes of exposure to light of 100 klux photosynthesis is inhibited in about 40 per cent and after half an hour of exposure the rate of photosynthesis is reduced by more than half. Exposure to light of this intensity for 4 to 6 hours produces a complete disability for photosynthesis. The broken line curves show the chan-

ges in the ability for assimilation which occur after various exposure times in intense light and the subsequent transfer to the weak light. It appears from the curve that even a relatively strong depression of photosynthesis (up to 40 per cent) is entirely reversible 2 to 4 hours after the action of strong light is interrupted. Stronger inhibitions caused by longer illumination are only partly reversible and even after several hours in dim light photosynthesis does not return to its normal level.

Fig. 4. Inactivation of photosynthesis in light of 100 klux in relation to exposure time. Abscissae — time of exposure in hours, ordinates — relative intensity of photosynthesis. The broken lines show the course of the reactivation of the ability for photosynthesis.

Finally, when the inhibition reached 80 per cent and more the ability for photosynthesis is no longer reactivated and for many hours the intensity of photosynthesis remains at the same level.

3.4. INACTIVATION OF PHOTOSYNTHESIS AT OTHER LIGHT INTENSITIES

The effect of other light intensities on the rate of photosynthesis is illustrated by curves 2 and 3 in fig. 6. They show the inactivation percentage of photosynthesis after exposure for 0.5 and 3 hours to varying intensities of light. It follows from the assembled data that photosynthesis is slightly inhibited after long exposure only if light intensity is about 50 klux. The inhibition sets on more rapidly as light intensity is increased and at 160 klux exposure for half an hour causes an almost complete destruction of the ability for photosynthesis.

It is worth noting that the arrangements of chloroplasts (curve 1 fig. 6) respond to far lower light intensities than those having a harmful effect on the photosynthetic apparatus. Partial parastrophe is induced already by light of 3 klux and in light of 10 to 15 klux parastrophe is

complete, whereas the inactivating effect of light on the process of photosynthesis is observed at intensities 3 to 5 times as great i. e. at about 50 klux.

3.5. DESTRUCTION OF CHLOROPHYLL IN LIGHT OF 100 000 LUX

Of the three responses here considered the decomposition of chlorophyll as determined with colorimetric measurements displays the least sensitivity to light. The results from the corresponding measurements are illustrated by fig. 5. The continuous-line curve shows the changes of

Fig. 5. The destruction of chlorophyll in light of 100 klux in relation to exposure time. The broken lines mark the changes in chlorophyll after exposure to strong light is interrupted. Dotted line shows the inactivation of photosynthesis.

absorption in the ether extract after various exposure times. The very slight changes in the concentration of chlorophyll caused when leaves are exposed for 2 hours can still be considered as being within the limits of error. Only after 4 hours of exposure the changes in chlorophyll concentration exceed 10 per cent. It is thus seen that the rate of the changes is much slower than the inactivation of photosynthesis (in fig. 5 the later changes are marked by the dotted-line curve) and this is best illustrated by the fact that the disability for photosynthesis is complete when only 10 to 20 per cent of chlorophyll is decomposed. The destruction of chlorophyll proceed gradually as the exposure time is prolonged exceeding 50 per cent of the initial value after 10 hours.

It has been mentioned earlier in the paper (3.1.) that exposure for 10 hours causes the complete or almost complete bleaching of the chloroplasts, however colorimetric measurement indicates that after the same

time only half of the chlorophyll is decomposed. The apparent discord of these results is understood when it is remembered that microscopic examination were made on the segments of leaves consisting of 1 or 2 layers, whereas the multilayer part of leaves retain the undestroyed chlorophyll pigment somewhat longer and this obviously influences the results of colorimetric measurements.

In principle the decomposition of chlorophyll is irreversible and when the action of strong light is stopped the chlorophyll concentration does not change for at least the next 20 hours. Although the relevant measurements (broken-line curves) show a slight increase in chlorophyll concentration the differences are small and do not exceed the limit of error.

3.6. DESTRUCTION OF CHLOROPHYLL IN OTHER LIGHT INTENSITIES

The course of chlorophyll destruction after 0,5 and 3 hours exposure to light of various intensities is shown by curves 5 and 4 respectively in fig. 6. As can be seen from the curve a noticeable destruction of chlo-

Fig. 6. Influence of light intensity on: curve 1 — the arrangement of chloroplasts after exposure for 0,5 hour; curve 2 — inactivation of photosynthesis after exposure for 3 hours; curve 3 — inactivation of photosynthesis after exposure for 0,5 hour; curve 4 — destruction of chlorophyll after exposure for 3 hours; curve 5 — destruction of chlorophyll after exposure for 0,5 hour.

rophyll after half an hour exposure is caused only by light of 130 klux and more, to obtain a similar effect at 100 klux exposure must last 3 hours. However, as the intensity of light increases the rate of the decomposition processes increases rather rapidly. Nevertheless, in all light intensities used in the present experiments the decomposition of chlorophyll is a process much slower than the inactivation of photosynthesis.

3.7. DESTRUCTION OF CHLOROPHYLL IN A SINGLE CELL

The data on the inactivation of photosynthesis and the destruction of chlorophyll are of a statistical nature and refer to the mean ability for photosynthesis in one leaf or the mean changes in chlorophyll concentration of several leaves. However, when the differentiation and the lack of uniformity in the response of the various cells to light stimulation are considered it seems likely that these changes may develop along somewhat different lines in the particular cells. There is no method for measurement photosynthesis in one or several leaf cells but some information on the concentration of chlorophyll in so small fragments of tissue may be

Fig. 7. Changes in the transparence of a part of a *Lemna* leaf to light during exposure to light of about 150 klux. Abscissae — exposure time, ordinates — relative transparence in per cent.

obtained from measurements of light absorption in living leaves. The equipment for these measurement which is based on the principle developed by Biebl (1954) and only adapted for measurements on microscopic objects will be described elsewhere. Briefly it consists of a selenium photo-cell placed on the microscope at the point where the image is formed, thus making possible the measurement of the relative transparence of the leaf to blue-red light (gentian violet filter) which is strongly absorbed by chlorophyll. Fig. 7 shows the changes in transparency of a homogeneous segment from leaf of *Lemna* (the diameter of the segment was 440μ) exposed to light of about 150 klux. At the bottom of the figure

the simultaneous changes in the morphology of chloroplasts in the segment are marked in with the symbols used previously.

The relative transparence is at first less than 60 per cent (chloroplasts in epistrophe) but rapidly increases to about 72 per cent which is associated with the displacement of chloroplasts to the profile arrangement on the lateral cell walls. Shortly afterwards there is another small increase in absorption caused by the assembling of chloroplasts in aggregations and then as long as the chloroplasts do not disperse the transparence does not change. A rapid rise in transparence starts after 1,5 to 2 hours. Microscopis observations shows that at the same time the changes defined as stage 5 (fig. 1) occur i. e. the dispersion of chloroplasts from aggregations and their gradual decolorization. When all chloroplasts scatter throughout the cell the transparence of the object stabilizes and remains unchanged for a long time. Because the changes in transparence shown by the curve and occuring during the first hour of exposure are always related with the corresponding changes in the displacement of chloroplasts, even if the light causing the displacements is not harmful for the photosynthetic apparatus (Z u r z y c k i unpublished) it is to be concluded from the measurements that the rate of chlorophyll decomposition is not uniform through the exposure time. Moreover, the decomposition process is related with the morphological changes in the arrangement of chloroplasts. As long as the chloroplasts are assembled in the aggregations the decomposition of chlorophyll is relatively slow but the rate of decomposition distinctly rises at the moment the chloroplasts begin to scatter and bleach. This results is contradictory to those reported earlier in the paper (3.5.) when it was stated that chlorophyll is decomposed gradually as the time of exposure to light is prolonged. However, the contradiction is only apparent because the course of chlorophyll destruction obtained from measurement of the changes in the concentration of the chlorophyll extract is based on statistical evidence and does not allow for the individual variability of leaves and, first of all, the variability between the different parts of one leaf.

From the microscopic measurements on the transparence of leaves it is impossible to decide whether in the first stages (stages 1 — 4 in fig. 1), before the chloroplasts scatter from aggregations and bleach, chlorophyll does decompose though more slowly and less intensely than at a later stage, or whether it is then stable. By comparing the changes in the morphology of chloroplasts with the destruction of chlorophyll the former supposition seems to be a correct one. A measurable drop in the chlorophyll level occurs at the time when even in the most sensitive parts of a leaf there is still no scattering and decolorizing of chloroplasts. This indicates that the destruction of chlorophyll starts at an early stage of exposure.

However, within the particular cells the destruction process is very slow at first and its rate does not increase until the chloroplasts pass into stage 5 (fig. 1).

4. DISCUSSION

The sensitivity of plants to very strong light intensities varies greatly. In some plants the destructive changes in the photosynthetic apparatus appear at relatively low light intensities, whereas in others even direct sun light acting for a long time does not produce them. Sensitivity in this respect depends primarily on onto- nad phylogenetic adaptation (Harder 1930, 1933, Montfort 1937, 1950, Rabinowitch 1945). The plant used as material for the present investigation must be grouped with the species rather sensitive to light, as fairly strong destructive processes are already induced by light of relatively low intensity.

When light intensity is sufficiently strong, though before it passes the critical limit (10 — 50 klux), the arrangement of chloroplasts in the cells changes from flat to profile. The profile arrangement is an adaptation tending to reduce the absorption of light. The mechanism regulating absorption operates on three lines:

a) When the chloroplasts lie on the lateral walls they occupy the parts of a cell at least partly shaded by local refractions of light (Senn 1908). This becomes especially well visible in the multilayer mesophyll where the green aggregations of chloroplasts which persist longest are those lying behind intercellular spaces strongly refracting light.

b) The profile arrangement reduces the total surface of chloroplasts directly exposed to light and at the same time increases the thickness of the mass of chloroplasts. It was demonstrated earlier that such a change produces reduced absorption (Zurzycki 1954).

c) The change in the position of chloroplasts in respect to the incident light rays is accompanied with a partial change in the spectral composition of light absorbed, because of the orientation of some pigment molecules in the chloroplasts (Goedheer 1954).

Strong light acting for some time caused the chloroplasts to assemble into large aggregations. The resulting arrangement is not a typical systrophe, as they are usually two or three such aggregations in one cell. Similar as in the case of the systrophe arrangement the responsible factor seems to be of a chemical nature. This is indicated by the frequent symmetry in the arrangement of the aggregations on both sides of the cellular membrane separating two neighbouring cells. The biological significance of such arrangements is not understood but there is no doubt that simultaneously the absorption of light increases (c. f. paragraph 3.7.)

It is possible that the aggregation of chloroplasts is already the outcome of the beginning disfunction of the cell, similarly as the systrophe is frequently indicative of slight damage (Küster 1910, Germ 1931). In the next stage — in which the chloroplasts disperse and decolorize — distinct symptoms of degeneration appear. This is indicated both by the irreversibility of the process and the appearance in the cell sap of calcium oxalate crystals, the presence of which is usually associated with extensive damage in cells (Nadson and Rochlin-Gleichgewicht 1928, Biebl 1954).

Morphological changes of chloroplasts under the influence of strong light have been described many times. However, on the whole these descriptions refer to the final effect after long exposures to light (Montfort 1937, Montfort and Neydel 1928, Gessner 1940, Münding 1952, Biebl 1954). Detailed investigations (Münding 1952, Biebl 1954) indicate that the damage caused in the cell by strong light may vary greatly. The chloroplasts may stay green, decolorize or change colour, they may agglutinate or remain unagglutinated. The forms observed in *Lemna* indicate that in the course of the destruction process the chloroplast arrangements may change radically. The final condition of chloroplasts in *Lemna* resembles most the type described by Biebl (1954) in *Hookeria lucens* characterized by the disperse arrangement and the loss of colour without the intermittent yellowish stage. Agglutinations so common when chloroplasts are damaged (Lepeschkin 1937) are not observed in *Lemna*. A somewhat similar course of changes in chloroplast arrangements, but without the intermittent aggregation stage was observed by Gessner (1940) in *Trichomanes radicans* during 9 hours of exposure to sun light.

The depression of photosynthesis under influence of strong light is reported by numerous workers (Ewart 1896-8, Arnold 1931, Harder 1930, 1933, Montfort and Neydel 1928, Emerson 1935, Montfort 1937-50, Stålfelt 1939, Meyer and Burr 1940, Steemann - Nielsen 1942-52). In very strong light the depression of assimilation may be so great that the compensation point is exceeded and oxygen instead of being given out is taken in (Montfort and Neydel 1928, Fockler 1937, Montfort 1937, 1941). This effect is associated with the one observed sometimes in leaves of terrestrial plants — i. e. the disappearance of starch — which is known as the solarization of leaves (Ursprung 1917, Holman 1930). A similar complete inhibition of photosynthesis was observed in the present experiment on *Lemna*. However, even when photosynthesis stops completely respiration is not affected to any significant extent. This indicates the selective effect of light on the photosynthetic apparatus.

Similar results were obtained by Steemann-Nielsen in his studies on the inactivation of photosynthesis.

It has been shown by Steemann-Nielsen in his detailed investigation (1942—1952) that two kinds of inactivation can be distinguished. One produces no depression of photosynthesis in intense light but becomes manifest after the return to weak light and the other causes a drop in the rate of photosynthesis already during exposure to intense light. In this investigation the rate of photosynthesis during exposure to intense light was not measured and consequently the data on the inactivation of photosynthesis refer to the combined effects of both inactivation types. Both types of inactivation are reversible (Stålfelt 1939, Montfort 1941, Steemann-Nielsen 1942, 1949) or when exposure to intense light lasts too long they may be partly or entirely irreversible (Focker 1938, Montfort 1937). *Lemna* resembles in its behaviour the results obtained by Montfort (1941) for *Ottelia* where inactivation after 4 hours was fully reversible, whereas a depression of photosynthesis exceeding 40 per cent was not.

The destructive effect of strong light on chlorophyll has also been known for a long time (Batalin 1874, Iwanowski 1917, Emerson 1935, Montfort 1941, 1950, Montfort and Zollner 1942, Münding 1952). In agreement with the earlier investigation the decomposition in *Lemna* is not uniform throughout the leaf but proceeds zonally. The destruction of chlorophyll in this species is irreversible which support the results reported by Iwanowski (1917) for other material. Quantitative comparisons in this respect with the results obtained by other workers are difficult because of the differences in light used for the experiments. However, on the whole the rate at which chlorophyll decomposes is analogous with the decomposition rate recorded by Iwanowski in *Elodea*, by Münding in *Vallisneria* and by Montfort in umbrophilic aquatic plants. If the classification introduced by Montfort and Zollner (1942) is to be accepted then *Lemna* must be classed in group IV i. e. with the plants very sensitive to intense light.

When the effects here described are compared it is seen that there are correlations between some of them and no correlations between others. In relatively weak light there is a correlation between the arrangement of chloroplasts and photosynthesis (Zurzycki 1954). The saturation point of photosynthesis coincides approximately with the point when the chloroplasts reach complete parastrophe. Both lie at about 10 klux. The inactivation of photosynthesis begins, even when exposures are prolonged, in light intensities approximately 5 times higher (50 klux) which is associated with the appearance of the first aggregation of chloroplasts.

In more intense light there is no correlation between the manner in which the inactivation process proceeds with time and the changes in chloroplast arrangements. The inactivation of photosynthesis is rather rapid and independent of the simultaneous displacement of chloroplasts. It seems that inactivation is somewhat reduced when the chloroplasts are in parastrophe, but it has been impossible to demonstrate this experimentally. The beginning of the formation of chloroplast aggregations is already connected with very strong and in principle irreversible inactivation. By the time the typical aggregations are fully formed photosynthesis is inhibited completely.

At the same time the destruction of chlorophyll is still very slight. This corroborates earlier results according to which photosynthesis is always more sensitive than chlorophyll (Emerson 1935, Montfort 1941, Montfort and Zollner 1942). However the decomposition of chlorophyll always takes place before the chloroplasts begin to disperse from the aggregations and lose colour. Though only at this latter stage the decomposition becomes more intense. The death of a cell does not occur till the chloroplasts have finished scattering, that is at the time when the greater part of chlorophyll is already decomposed. This supports earlier observations on the decomposition of chlorophyll which indicates that this process develops in a living cell (Montfort 1941, 1950, Montfort and Zollner 1942, Münding 1952).

5. SUMMARY

1. Three aspects of the changes in the photosynthetic apparatus were examined: a) the morphological state of chloroplasts, b) ability for photosynthesis, and c) the concentration of chlorophyll in respect to light intensity and time of exposure to intense light. The fact that the measurement referred to one species (*Lemna trisulca*) and to light with a constant spectral characteristic made possible a quantitative comparison of how light affects the above three factors.

2. The destructive effect of light begins at the intensity of about 50 klux i. e. in light of intensity 5 times higher than at the saturation point of photosynthesis. In very strong light the destruction may cause the death of a cell after several hours of exposure.

3. The typical course of changes in the morphology of chloroplasts after exposure to strong light is as follows: flat arrangement — profile arrangement — the formation of aggregation on the lateral walls — the dispersion of chloroplasts from the aggregation and decolorization. The time that every one of this stages lasts depends on light intensity and the local sensitivity of the tissue.

4. Light intensity exceeding 50 klux causes the inactivation of photosynthesis. The inhibition of photosynthesis increases with the increase of light intensity and exposure time. The inhibition of photosynthesis may be complete without any major disturbances in respiration. When the inactivation does not exceed 50 per cent it is entirely or to a high degree reversible.

5. The destruction of chlorophyll is the slowest of the three processes. It proceeds relatively slowly till aggregations of chloroplasts are formed, but is greatly accelerated in the next stage when the chloroplasts scatter.

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STRESZCZENIE

Badania niniejsze odnoszą się do szkodliwego działania światła o wysokim natężeniu na aparat fotosyntezy. Dotychczasowe obserwacje w tej dziedzinie, prowadzone na różnych obiektach roślinnych przy użyciu różnego rodzaju światła i zwracające uwagę na pewne oderwane problemy, nie pozwalają na wyrobienie sobie syntetycznego obrazu zmian morfologicznych i fizjologicznych, które zachodzą podczas naświetlenia silnym światłem oraz przebiegu tych zmian w czasie. Doświadczenia niniejsze przeprowadzone były na jednym tylko obiekcie roślinnym (*Lemna trisulca*) przy użyciu światła o różnym czasie ekspozycji i różnej intensywności, ale o stałym składzie spektralnym. Głównie zwracano uwagę na: a) zmiany morfologiczne zachodzące w układzie i zabarwieniu chloroplastów (metoda: obserwacja mikroskopowa), b) zmiany w zdolności do fotosyntezy (metoda: pomiar fotosyntezy przy użyciu mikrometody autora przed i po naświetleniu), c) zmiany w ilości chlorofilu w liściu (metoda: pomiar absorpcji światła w ekstrakcie eterowym z liścia w fotometrze Pulfricha).

A. Światło o natężeniu przekraczającym 50 klux wywołuje po pewnym czasie widoczne w obrazie mikroskopowym zmiany destrukcyjne w stanie chloroplastów. Zmiany te przebiegają w określonej kolejności: najpierw następuje skupienie chloroplastów w kilka agregatów na ścianach bocznych, potem stopniowe rozchodzenie się ich z tych skupień po całej komórce i równoczesne wyrażenie odbarwianie. Równocześnie z całkowitym odbarwianiem następuje śmierć komórki (ryc. 1). Szybkość tych przemian zależy od intensywności oświetlenia (np. dla 60 klux skupienia chloroplastów pojawiają się po około 6 godz., a w 160 klux już po 1 godz.) oraz od specyficznej wrażliwości tkanki (najbardziej wrażliwy jest brzeg liścia). Zależność stanu chloroplastów od intensywności i czasu naświetlenia ilustruje ryc. 2.

B. W silnym świetle bardzo szybko maleje i wreszcie zupełnie zanika zdolność do fotosyntezy. Np. w oświetleniu 100 klux po 4—6 godzinach liść zupełnie traci zdolność do fotosyntezy. Nawet półgodzinne naświetlanie tą intensywnością redukuje do połowy dawną zdolność do asymilacji (ryc. 3). O ile obniżenie tej zdolności nie jest zbyt silne (nie prze-

kracza 80%), utrata zdolności do fotosyntezy jest całkowicie lub przynajmniej częściowo odwracalna. Korelacja z opisanymi powyżej zmianami morfologicznymi pozwala stwierdzić, że gdy następuje początek skupiania się chloroplastów w agregaty, fotosynteza jest już bardzo silnie zahamowana, a w momencie wystąpienia typowych skupień zdolność do fotosyntezy zanika zupełnie.

C. Silne światło działa rozkładająco na chlorofil. Spadek stężenia chlorofilu w liściu przebiega jednak znacznie wolniej niż opisane poprzednio procesy. I tak np. w oświetleniu 100 klux po 10 godz. zostaje rozłożona zaledwie połowa chlorofilu. Zastosowanie specjalnej metody pomiaru przepuszczalności dla światła wycinka liścia obserwowanego przez mikroskop pozwoliło zebrać dokładniejsze dane o przebiegu rozkładu chlorofilu w czasie. Rozkład ten następuje od pierwszych chwil naświetlenia, ale przebiega początkowo stosunkowo powoli, natomiast rozpoczyna się z większą szybkością od chwili, gdy chloroplasty zaczynają rozchodzić się ze skupień i wykazywać wyraźną utratę zabarwienia, a więc od chwili, gdy komórka już dawno utraciła zdolność do asymilacji. Cały lub prawie cały chlorofil rozkłada się jeszcze w ciągu życia komórki. Rozkład chlorofilu jest nieodwracalny przynajmniej w ciągu kilkudziesięciu godzin.

Porównanie szybkości przebiegu opisanych zmian w różnych intensywnościach światła (ryc. 6) pozwoliło na stwierdzenie, że u wybitnie cieniulubnej rośliny, jaką jest *Lemna trisulca*, intensywności światła, które działając przez kilka godzin wywierają szkodliwy wpływ na aparat fotosyntezy, muszą przekraczać 50 klux. Ponieważ u badanego obiektu punkt wysycenia fotosyntezy jak również punkt osiągnięcia całkowitej parastrofii leży przy 10—15 klux, działanie szkodliwe światła rozpoczyna się zatem od intensywności 3—5 razy silniejszych.

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