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The roles of the palisade and spongy parenchymas of leaves in photosynthesis

by

W. STARZECKI

1. INTRODUCTION

It is generally assumed that in a leaf the palisade parenchyma is the main assimilating tissue. This opinion is based on following facts: first, this parenchyma is situated below the upper epidermis of the leaf and is therefore the tissue which is directly penetrated by light. In the second place the majority of the chloroplasts is grouped in this tissue. In some species the number of chloroplasts in the palisade cells per unit of leaf area exceeds 6 times the corresponding number found in the spongy parenchyma (Haberlandt 1914).

It has been maintained thus far that from a physiological point of view the spongy parenchyma plays a minor role in the leaf. The feeble light energy which reaches this tissue and a lower content of chloroplasts are factors causing a less economical utilization of light. There are even physiologists who deny the spongy parenchyma any participation in the CO_2 assimilation; e.g. Schroeder (1924) and Romell (1927) do not take this tissue in consideration in their theoretical studies on the diffusion gradient of carbon dioxyde in the leaf.

Thus, the actual opinions on the role of the assimilating parenchyma are based on two premises: the quantity of light penetrating in the tissues in question and their chloroplasts content. The role assigned to these tissues is based rather on theoretical speculations than on experimental evidence. The possibility, however, of inverting the roles played by these tissues in the assimilation process in favour of the spongy parenchyma cannot be excluded and for this reason the structure and functions accomplished by the palisade and spongy parenchymas should be submitted to a new and detailed analysis free from preconceived ideas.

In the first place let us examine the environmental conditions upon which the development of these tissues depends. A typical structure with a multilayered palisade parenchyma and a well developed spongy tissue is characteristic of leaves growing in well illuminated environments. Leaves growing in shadowy conditions have, as a rule, a well developed spongy parenchyma, whereas, the palisade tissue, if present, is developed in an untypical way with considerably smaller and looser cells. An exception are evergreen species which even in shadowy conditions present a well developed palisade parenchyma.

Within one species, great differences are observed in the development of the assimilating parenchyma in dependence on light conditions (Morton and Gams 1925, Wylie 1948, Starzecki 1958). Specimens deriving from outside the cave have a well developed palisade and spongy parenchymas, whereas, specimens from the inside of a cave are deprived of the first tissue.

In leaves grown in various light conditions structural differences are observed even in the same specimen. Hasselman (1904) made a detailed anatomical study on leaves of *Coryllus avellana* deriving from various parts of the bush. He found that leaves from the southern exposure were much thicker and had two layers in the palisade parenchyma, whereas leaves from the inside of the bush, where the light intensity was reduced to 1/50 of the full value, were considerably thinner and had a very loose unilayered palisade parenchyma. Many other examples of this kind could be mentioned.

Thus, there can be no doubt that in shadow leaves, deprived of palisades, the spongy parenchyma is the tissue whose principal function consists in the assimilation of carbon dioxide. It must be further assumed that in spite of the reduced number of chloroplasts, this tissue fulfils this function with adequate efficiency. The similarity of anatomical structures shown by the spongy parenchymas in shadow and in sun leaves suggests that in the last mentioned leaves the spongy parenchyma is also the principal assimilating tissue, whereas, the palisades play some other, as yet unknown role.

The hypothesis that the spongy parenchyma is the main assimilating tissue in all leaves is in contradiction with the generally adopted view. However, this hypothesis is corroborated by the results of a detailed comparison of the two tissues in question made from different points of views including the supplies of carbon dioxyde, water and light energy, the migration of assimilates, the arrangement of chloroplasts and their displacement in the cells.

The exchange of carbon dioxide and oxygen between the atmosphere and the leaf and in the leaf itself proceeds on the way of diffusion. In a tissue well fitted for this kind of gas exchange the following conditions should be fulfilled: a greater diffusion surface and a structure assuring a rapid equalization of internal partial gas pressures.

The gas exchange between the leaf and the atmosphere takes place through the stomata. As early as in 1900 Brown and Escombe have shown that the stomata, in spite of their small dimensions, do not appreciably depress the intensity of gas exchange between the leaf and its external environment. It can be assumed that owing to its position in the leaf, the spongy parenchyma is well provided with CO_2. Basing on this fact Schroeder (1924) and Rommel (1927) assumed that the CO_2 concentration in the leaf in the vicinity of the stomata is 100 percent its content in the atmosphere.

It is obvious that numerous and great intercellular spaces assure a rapid equalization of the differences of the CO_2 concentration in the tissues. A comparison of the anatomic structure of both the assimilating parenchymas suggests that the spongy tissue is privileged in this respect. According to Schroeder the intercellular spaces in the spongy parenchyma of *Sambucus nigra* leaves occupy on a cross section 40,4% of the total area and only 5,8% in the palisade tissue.

In the leaf the greatest resistance to diffusion is offered by the cell membranes and the protoplasm of the assimilating cells. Only an increase of the area of the cell wall separating the intercellular spaces and the protoplasm can assure a better penetration of CO_2 into the cell. In this respect the spongy parenchyma is also a privileged tissue because the greater part of the cell surface is in contact with the intercellular spaces. On the contrary, in the palisades these spaces form narrow channels which, on the one hand, render more difficult the equalization of partial pressures within the tissue, and on the other hand, because of their small dimensions, depress the amounts of carbon dioxide diffusing into the cells.

It results from the considerations on the diffusion gradient of Schroeder (1924) and Romell (1927) that in the upper layer of the palisade tissue the CO_2 concentration approaches the value of the CO_2 compensation point which according to Gabrielsen (1949) is about 0,01% (by volume) for leaves of *Sambucus nigra*. In their discussion, however, the authors did not take into consideration the fact that the presence of spongy parenchyma causes an additional drop of the CO_2 concentration in the leaf. This fact must, doubtlessly, contribute to lower the numerical values obtained by them. From these facts it can be inferred that in a multilayered parenchyma the upper layers either do not take part in photosynthesis at all, because of an insufficient provision with CO_2, or, if they do, their contribution is very small.

The supply of water and the migration of assimilates is assured by a branched and complicated system of conductive tissues. In this connection it is important to point out, that the smallest veins, whose function consists in supplying water to the adjacent cells or in removing the assimilates, are always situated in the spongy parenchyma beneath the palisade cells. This suggests that this parenchyma is the principal tissue concerned with the water supply or the migration of assimilates.

It is obvious that the higher water requirement of the spongy parenchyma is a consequence of its higher rate of transpiration which in turn is caused by the immediate vicinity of the stomata (in a hypostomatal leaf).

The phloem is either situated on the lower side of the vein or in bicollateral bundles on its two sides. In the first case the assimilates formed in palisade cells must migrate through the spongy parenchyma before reaching the phloem. This is compatible with the supposition that the production of assimilates is greater in the spongy parenchyma than in the palisades. In the bicollateral bundles the distance between the palisades and the phloem is much shorter, but the conductive area of the phloem near the palisades is smaller than the corresponding area of the phloem situated on the opposite side of the vein. Thus, also in this case it may be supposed that the production of organic matter in the palisade cells is smaller than in the spongy parenchyma.

The role played in photosynthesis by luminous energy requires a detailed discussion of the light conditions prevailing in a leaf illuminated from above.

In the first place let us examine the minimal light intensity necessary to assure the maximal assimilation rate. In this respect the plant species differ greatly according to their helio or skiophytic character. It results from ecological studies (Szennikow 1951, Rabinowitch 1951) that the light saturation point of photosynthesis varies from 200 to 2000 lux for plants from shadowy environments and from 2000 to 8000 lux for well illuminated sites. It may be recalled that the increase of the light intensity above the saturation point does not lead to any further increase of the assimilation rate, but may even cause a more or less pronounced drop.

The intensity of the light emitted by the sun and the sky radiation attains 100 kilolux at the sea level. Thus, it exceeds 50—500 times the maximal light requirement of skiophytes and 14—50 times the requirement of heliophytes. It must be stressed, however, that the light saturation values quoted above refer to light emitted by artificial sources whose composition differs notably from that of the sunlight or sky radiation. The percentage of short wave radiations (characterized by a strong action on plants) is much higher in natural radiation than in artificial light. (Montfort 1950, Brodführer 1955, Rüsch and Müller 1957). In the mountains the light intensity is still higher and the percentage of ultraviolet radiation increases considerably.

It results from several investigations that high light intensities cause a decrease of photosynthesis and a concomitant drop of the chlorophyll content. The inactivation of photosynthesis however, proceeds at a much more rapid rate than the destruction of the pigments. (Schoder 1932, Emerson 1935, Gessner 1940, Steemann-Nilsen 1949, Zurzycki 1957, Sironval and Kandler 1958). Steemann-Nilsen (1952) distinguishes two types of inactivation of photosynthesis resulting from the exposition of a plant to light of high intensity. In the first case the drop of the assimilation rate manifests itself only after the return of the plant to lower light intensity.

It results from the studies of Zurzycki (1957) that the drop of the assimilation rate observed after the return of the plant to feeble light may be transitory and depends on the length of exposure of the plant to light of high intensity. Fronds of *Lemna trisulca* which were exposed to strong light (100 kilolux) for 1 hour showed after their return to low light a small increase of the assimilation rate, which however, never reached the original value. An irreversible drop of photosynthesis was induced in fronds illuminated for 3 hours. The amount of decomposed chlorophyll in fronds of *Lemna trisulca* increased with the length of exposure to intense light.

Similar results were obtained when light of lower intensity but with a higher content of ultra-violet radiation was used (Montford 1950).

The results of studies of Emerson (1935) and Gessner (1940) are an essential contribution to the problem under discussion. They found that a specially harmful influence of light on photosynthesis is observed when plants are illuminated in an atmosphere free of carbon dioxide.

Moreover, high light intensities cause an increase of respiration rate (Montfort 1950, Sironval and Kandler 1958), and a decline of starch content in the leaves (Ursprung 1917, Holman 1930).

It results from the above discussion that in an unshadowed environment the plants receive amounts of light energy exceeding their requirements several times. It has also been stressed that an excess of radiation energy has a harmful effect on the photosynthetic apparatus. These two factors must be taken into consideration in a discussion on the roles played by the palisade and spongy parenchymas in the leaf.

It is obvious, that owing to its position, the palisade parenchyma is the tissue overloaded with light and exposed to its damaging action. Moreover, the circumstance that this tissue is poorly supplied with CO_2 increases the destructive action of light. On the contrary the spongy parenchyma, protected by the palisades, receives light of feeble or moderate intensity deprived of noxious influence, but sufficient to assure a normal photosynthetic activity. The conclusion drawn from these remarks is that in sun leaves also the spongy parenchyma is the principal assimilation tissue, whereas, the palisades play the role of a light filter protecting the spongy tissue against the destructive effect of intense light.

This conclusion is corroborated by the results of researches on the arrangement of chloroplasts in the tissue in question. The positions occupied by the chloroplasts in the spongy tissue in feeble and intense light respectively are the epistrophic and the parastrophic positions. The reaction of the palisade chloroplasts to the light intensity is different. In faint light they place themselves on the side walls and form cluster in intense light in the lower part of the cells (Senn 1908, Devide 1959/60). The position of the palisade chloroplasts in feeble light permits a greater amount of light energy to reach the spongy parenchyma, whereas, in strong light the cluster of chloroplasts form a screen protecting the pigments of the spongy cells against destruction.

The position occupied by the palisade chloroplasts in feeble light is probably an adaptation to the CO_2 conditions prevailing in the palisade tissue. By placing themselves along the side walls in contact with the intercellular spaces the chloroplasts occupy the position with the best access of CO_2 molecules.

There can be no doubt that in natural conditions the palisade chloroplasts are the plasts which are directly exposed to the destructive action of strong light. For instance Zurzycki (1957) observed that the decoloration of *Lemna trisulca* exposed to intense light proceeded from the edge of the frond with one layer of cells toward its centre; the central part, where the upper layers protected the lower layers, the green colour maintained for a longer time. Montfort, who examined the action of UV radiation on leaves of spinach, found that the first chloroplasts that were damaged were those localized in the palisades.

The author (Starzecki 1958) compared the rates of respiration and assimilation shown by leaflets of specimens of the fern *Asplenium trichomanes* growing before and inside a cave at the distance of 22 m. The thickness of the leaflets decreased about 4 times, whereas, the respiration and assimilation rates decreased 6,5 and 2—2,5 times respectively. The considerable decrease of the respiration intensity is a consequence of the complete disparition of the palisades in the specimens deriving from the inside of the cave. On the contrary it is difficult to reconcile the relatively small decrease of the assimilation rate with the generally adopted view concerning the roles played in photosynthesis by the two parenchymas. These difficulties are removed if it is assumed that not the palisades, but the spongy parenchyma is the main assimilating tissue.

It appears from the above discussion that several anatomical, ecological and physiological arguments can be invoked to strengthen the hypothesis that the spongy parenchyma is the main assimilating tissue and the palisades play the role of a screen absorbing the excess of light energy and protecting the chloroplasts in the spongy tissue against the damaging action of intense light.

The hypothesis which, as yet, is based only on indirect evidence, requires experimental confirmation. The second part of this paper presents the results of experiments undertaken with the aim of determining the function accomplished in photosynthesis by the spongy and palisade parenchymas.

2. MATERIAL

The experiments were performed on discs cut from leaves of 9 species: *Salix elegantissima, Populus alba, Betula verrucosa, Fraxinus excelsior, Robinia pseudoacacia, Vitis vinifera, Symphoricarpus albus, Rubus* sp. and *Tropaeolum majus*. Discs, 4,3 mm diameter were cut out from leaf blades of three leaves of various age. Leaves nearest the apical point from which samples for three replications could be taken were regarded as young leaves. Leaves with a well developed leaf blade of dark green colour with no mechanical injuries and no traces of chlorose, yellowing or browning, were regarded as old leaves. Leaves completely or almost completely developed, placed on the twig about the middle between the young and old leaves but of a considerably brighter green colour than the latter were assumed to be leaves of middle age.

The leaves samples were taken from well illuminated twigs of the same specimen. As a rule all the leaves were collected from one twig. However, it was not always possible to observe this condition, especially as far as trees are concerned, where young and middle aged leaves were provided by young shoots. In these cases samples were cut out from old leaves picked from places closest to young shoots. The discs were cut from parts of the blade free from principal veins. Before measurements were performed the discs were kept on moistened filtration paper placed on moistened cotton wool in weighing bottles (Starzecki 1961).

3. METHOD

The most direct method leading to the determination of the functions accomplished by the tissues in question would consist in measuring the assimilation rate of the palisade and spongy parenchymas separated from each other. For two reasons, however, these tissues were not separated in this way. The fact that an easy separation of the two tissues is limited to a few species is a circumstance that narrows considerably the choice of the plant material. In the second place the separation of the tissues in question is a drastic operation that modifies to a great extent the conditions existing in the intact leaf.

The following device permits however to estimate with sufficient accuracy the photosynthetic activity of the two tissues in question. If a leaf is supplied with light of low intensity then almost the whole luminous energy is absorbed by the pigments of the cells that are proximate to the light source. In these conditions the intensity of photosynthesis of the whole leaf is practically determined by photosynthetic activity of the tissue which is less remote from this source. Thus, in order to decide which of the two assimilating tissues utilizes better the light energy and consequently is photosynthetically more active, the leaf or its fragment was in turn illuminated with light of the same intensity directed towards its lower or its upper side.

It is known that a part of the incident light is reflected by the leaf surface. There are however, great differences not only in the amounts of light reflected by the upper or lower side of a leaf but also in its spectral composition. For this reason a correction for light reflection was introduced in the calculations of the assimilation rate. The value of this correction was determined by means of a specially adapted Ulbricht's sphere.

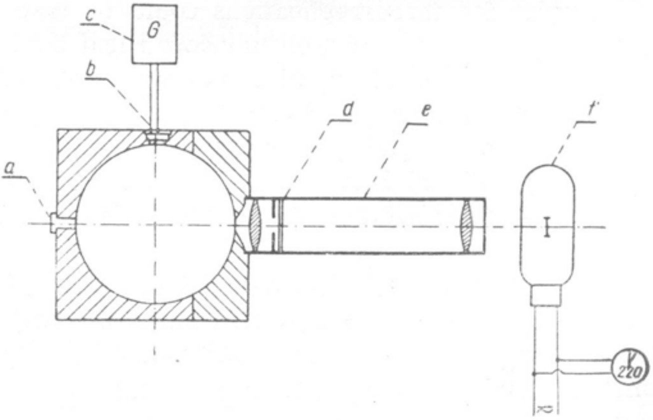

Fig. 1. Scheme of the arrangement for measurements of light reflected by the leaf surface: *a* — inserted measurement piece, *b* — photoelement, *c* — galvanometer, *d* — filter RG$_1$, *e* — optical equipment, *f* — projection lamp

The sphere made of gypsum, was composed of two parts (fig. 1). The inner diameter of the sphere measured 140 mm and its inner surface was coated with magnesium oxyde. The sphere was provided with one vertical and two horizontal apertures. One of the side apertures was equipped with a two lenses system by means of which a light beam emitted by an electric lamp (250 W, 220 V) penetrated in the cavity of the sphere. A selenium element, 22 mm diameter, placed in the upper aperture was connected with a galvanometer (Kipp, A 70, 50 PER).

The aperture opposite the light source was destined for the calibration of the apparatus and for measurements by means of an inserted piece (fig. 2). The piece "a" was used to determine the zero point of the apparatus. The surface which was directed towards the inside of the sphere was covered with coal black. The brim of the piece "b" was also coated with coal black, but its central part 4,3 mm, in diameter, was covered with magnesium oxide.

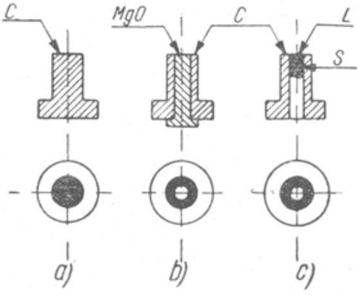

Fig. 2. Inserted pieces for measurement of light reflected by the leaf: *a* — to determine the 0 point, coated with coal black (C), *b* — to determine the maximal reflexion of light with a ring coated (C) with magnesium oxide (MgO), *c* — to determine the light reflexion by the upper and lower surface of the leaf (C — ring coated with coal black, *L* — disc of a leaf blade, *S* — sponge saturated with water)

Let V_0 and V_{100} be the deviations of the galvanometer read when either the piece "a" or the piece "b" was inserted in the corresponding aperture of the sphere. Then the difference

$$V = V_{100} - V_0 \tag{1}$$

is the maximal difference of galvanometric deviations, V_{100} corresponding to (approximately) total reflection and V_0 to maximal absorption of the light energy by the surface of the pieces.

The piece "c" was used for the determination of the light energy reflected by the leaf surface. The leaf disc was inserted in the piece and this piece was in turn introduced in the corresponding aperture of the sphere. A small sponge saturated with water was applied to the external side of the disc and protected it from drying. With each disc two measurements were performed, the first with its upper surface directed towards the inside of the sphere and the second with its lower surface oriented in the same direction. If V_u and V_l are the corresponding readings of the galvanometer then the differences

$$U = V_u - V_0 \text{ and } L = V_l - V_0 \tag{2}$$

are the measures of the reflexion power of the upper, and lower surface of the leaf. The percentages of light reflected by the upper surface of the leaf, respectively to the lower one, were calculated according to the formula:

$$\% \mathrm{u} = \frac{100\,\mathrm{U}}{\mathrm{V}} \quad \text{and} \quad \% \mathrm{l} = \frac{100\,\mathrm{L}}{\mathrm{V}}. \tag{3}$$

In turn these formulas permitted to calculate the relative intensities $\%\ I_u$ and $\%\ I_l$ of light really penetrating into the tissues of leaves illuminated either from above or from below:

$$\%\, I_u = 100 - \%\, u \quad \text{and} \quad \%\, I_l = 100 - \%\, l. \tag{4}$$

In order to eliminate the errors resulting from the unequal reflexion of light of different wave lengths by the upper and lower leaf surfaces the measurements of the reflexion capacity and photosynthesis were performed in approximately monochromatic light obtained by filtering white light through a Schott RG_1 glass filter and a liquid filter with Mohr's salt.

A microrespirometer described in previous papers (Zurzycki 1955, Starzecki 1961) was used for determination of the intensity of photosynthesis. The respirometer was equipped with two prisms and a light source movable on a perpendicular axis. This arrangement enabled to illuminate directly either the upper or the lower surface of the leaf disc.

4. RESULTS

In the first series of experiments the assimilation rates of discs cut from young leaves, middle aged and old ones of 9 species were determined. The light intensity varied within large limits (from zero to 50×10^3 erg/cm^2 sec) and the corresponding light curves of photosynthesis were obtained. The results are presented in fig. 3 and fig. 4 and in the table I.

It appears from the graphs that two distinct light curves are obtained, representing the assimilation rate of discs illuminated from above or below. In the first case the bulk of the luminous energy is absorbed by the palisade parenchyma, in the second case the spongy parenchyma is the light privileged tissue. For high light intensities both these tissues become light saturated and in conformity with expectiation the difference $I_u - I_l$ which is considerable for moderate light, decreases to zero with increasing light intensity.

For young leaves the fact that $I_u > I_l'$ indicates that the palisades form the photosynthetically more active tissue of the leaf; on the contrary from the relation $I_l > I_u'$, observed in other cases, it must be concluded that in middle aged and old leaves the spongy parenchyma is the principal assimilating tissue; the role of the palisades consists mainly in the absorption of the excess of light energy.

Marked differences between I_u and I_l appear only if the incident light energy I_0 is corrected for the amount that is reflected from the lower and upper surfaces of the leaves. In the graph 5, by way of example, are shown the results of measurements concerning the reflection capacity of leaves of *Populus alba*. The upper and lower sides of the leaf of this species show great differences in this respect: 12 and 52% of the incident light energy are reflected respectively by the upper and lower sides of an old leaf. The corresponding data for a young leaf are 14 and 74%. It is obvious that the amounts of reflected light are considerable and cannot be neglected. The differences I_u — I_l would be reduced to small values if the corrections for the reflected light were omitted.

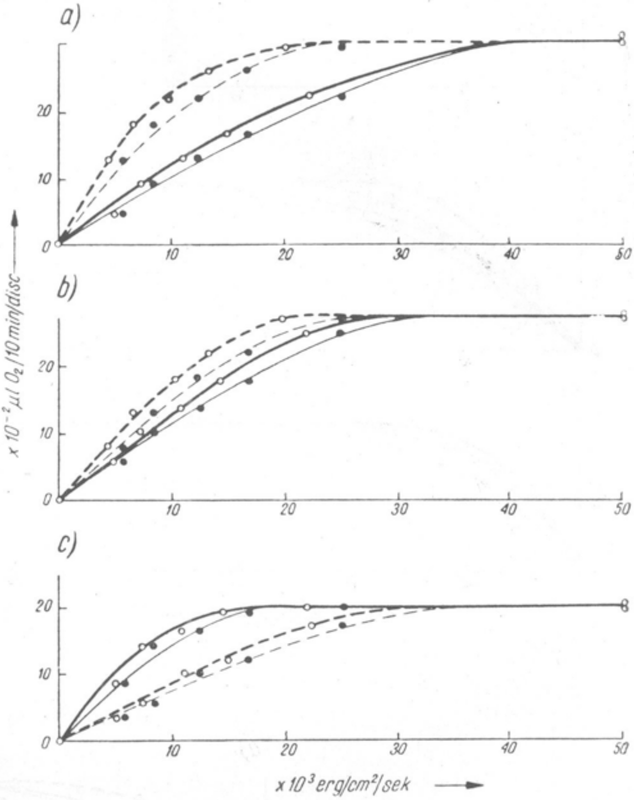

Fig. 3. Curve of photosynthesis for *Vitis vinifera* illuminated from above (continuous line) and from below (interrupted line) after correction for light reflexion (thick line); *a* — old leaf, *b* — middle aged leaf, *c* — young leaf

In order to compare the results obtained with leaves of various ages and different species the activity ratio U: L was calculated. This ratio is the ratio of two ordinates I_u and I_l (corresponding to the same abscissa)

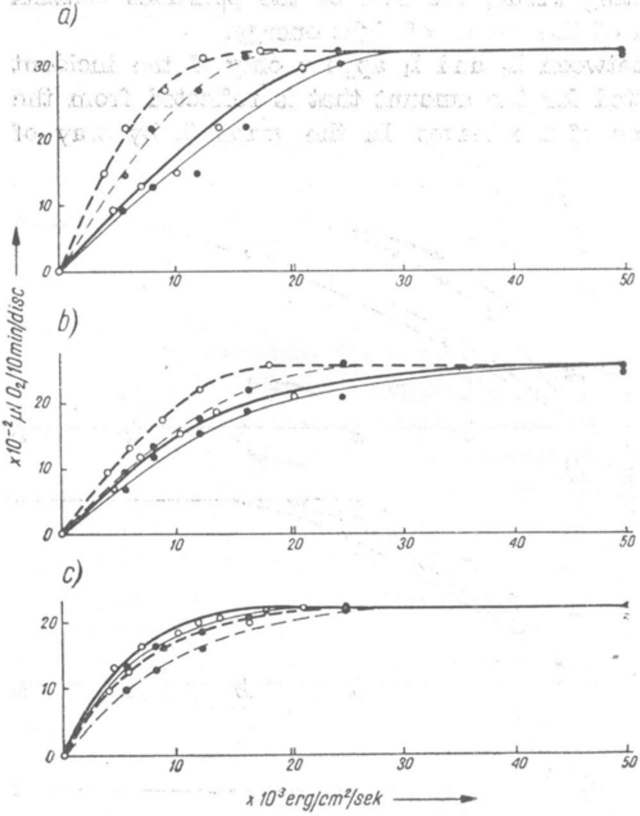

Fig. 4. Curves of photosynthesis for *Symphoricarpus albus* illuminated from above (continuous line), from below (interrupted line); after correction for light reflexion (thick line): *a* — old leaf, b — middle aged leaf, c — young leaf

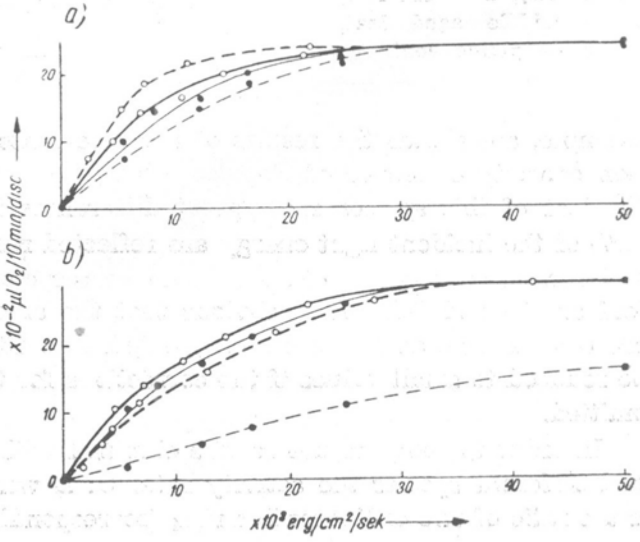

Fig. 5. Curves of photosynthesis for *Populus alba* illuminated from above (continuous line) from below (interrupted line); after correction for light reflexion (thick line): *a* — old leaf, b — middle aged leaf, c — young leaf

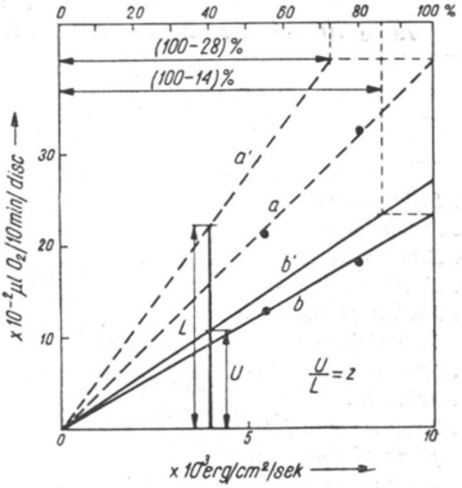

Fig. 6. Example of a graphic determination of U/L of an old leaf of *Symphoricarpus albus*

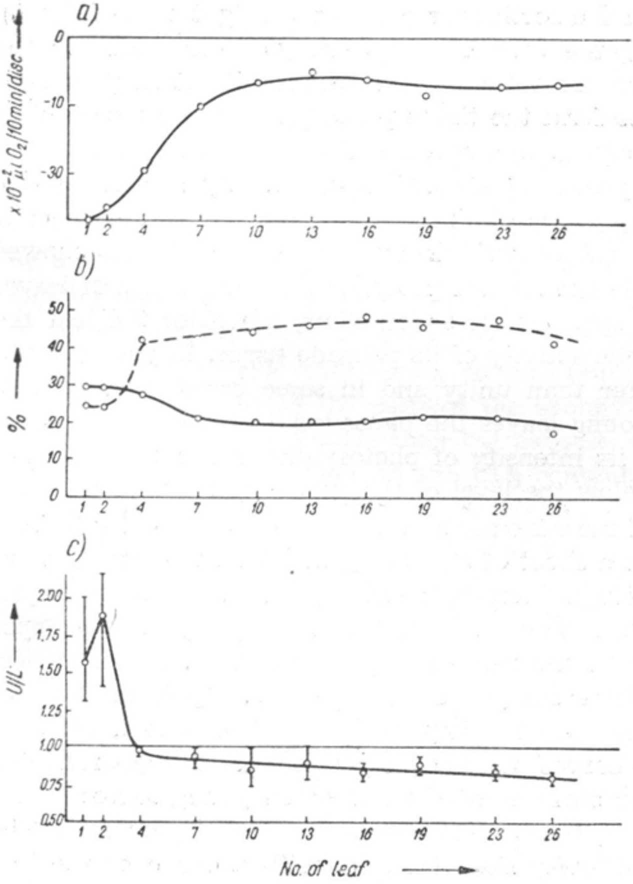

Fig. 7. Changes in dependence on the age of the leaf of *Salix elegantissima*: *a* — deviations, *b* — light reflection by the upper side of the leaf (continuous line), by the lower side (interrupted line), *c* — values U/L

Table I

Value U/L of investigated species for old, middle aged and young leaves

	U/L		
	Old leaf	Middle aged leaf	Young leaf
1. *Salix elegantissima*	0.46	0.82	1.73
2. *Populus alba*	0.77	0.83	1,28
3. *Betula verrucosa*	0,49	0,87	1,23
4. *Fraxinus excelsior*	0,51	0,60	1,87
5. *Robinia pseudoacacia*	0,56	0,90	1,50
6. *Vitis vinifera*	0,51	0,66	2,00
7. *Symphoricarpus albus*	0,58	0,74	1,15
8. *Rubus* sp.	0,54	0,85	1,15
9. *Tropaeolum majus*	0,46	0,79	2,50
Maximal value	0,77	0,90	2,50
Minimal value	0,46	0,60	1,15
Middle value	0,54	0,79	1,70

whose end points are on the rectilinear parts of the light curves (fig. 6); I_u and I_l are obviously the incident light energies corrected for the amounts reflected. When the ratio U: L exceeds unity it may be concluded that the palisades form the tissue photosynthetically more active than the spongy parenchyma. The reverse is true for $U : L < 1$.

In the table I are presented the ratios of activity shown by old, middle aged and young leaves of the examined species. Values below 1 were obtained for all old and middle aged leaves. Old leaves showed lower values than middle aged leaves provided that both kinds of leaves derived from the same specimen of shoot. Thus, the older the leaf the smaller the photosynthetic activity of its palisade tissue. In young leaves the ratio U: L is greater than unity and in some cases even exceeds 2; this means that in young leaves the palisades form the main assimilating tissue. However, its intensity of photosynthesis declines with age and eventually drops below the level of the spongy parenchyma.

The second series of measurements was made on discs cut out from 10 leaves collected from a shoot of *Salix elegantissima*. The leaves were labelled according to their position on the shoot, No. 1 being the youngest and Nr. 26 the oldest leaf. The results are shown in fig. 7. The upper curve refers to changes in the rate of respiration which is the highest in young leaves and decreases gradually with age. Up from the leaf Nr. 10 the rate does not change any more and keeps to a constant level. The intermediate curves present the percentages of light reflected by the upper and lower surfaces of the leaves. In young leaves (Nr. 1 and 2) the upper side reflects more light than the lower side. The reflection ability of the lower side indreases with the age of the leaf and that of the upper side decreases. Up from the leaf Nr. 7 the amounts of light reflected by the lower surface are about 25% higher than those of the upper surface. The lower curve in fig. 7 presents the ratios of activity U : L. The values for leaves 1 and 2 are much higher than unity. The value closest to unity is found in the leaf Nr. 4. In the remaining leaves the ratio U : L decreases gradually concomitantly with the increasing age of the leaves.

5. DISCUSSION

The generally admitted opinion is that in a leaf the palisade parenchyma is the main assimilating tissue, and the spongy parenchyma forms a tissue of secondary importance. This opinion, however, has been gradually weakened in the course of a detailed analysis presented in the first part of this paper and concerned with the structure and the function of the leaf.

This analysis suggests the view, that in a leaf there is only one assimilatory tissue which — in dependence on the light conditions — differenciates more or less into a palisade and a spongy parenchyma layer. This view is corroborated by the fact that in leaves of some plant species all transitory forms from a two layered to an unilayered assimilating tissue can be observed according to the light intensity prevailing during the development of the leaf.

It was emphasized that in shadow leaves the spongy parenchyma is the unique and efficient assimilating tissue and it was inferred from this fact that also in sun leaves (with palisade tissue) the spongy parenchyma is probably the principal assimilating tissue.

It is also probable that owing to differences in the dimensions of the intercellular spaces the cells of the spongy tissue are much better provided with carbon dioxide than the palisade cells. It can be assumed that considerable depression of the CO_2 content may occur in a multilayered palisade tissue.

The localization and the structure of the conductive tissues suggest that (1°) the assimilates are exported in the first place from the spongy tissue, (2°) the spongy cells are better supplied with water than the palisade cells.

The position occupied in the leaf by the palisade tissue facilitates the light destruction of chlorophyll and the inactivation of photosynthesis. It was also shown that the reduction of the palisade layer induces a relatively small decrease of the assimilation rate, when compared with the corresponding diminution of the leaf thickness.

It was inferred from these facts and observations that the function of the palisade tissue is not restricted to the CO_2 assimilation in light of high intensity.

The following facts were quoted to support this hypothesis:

1. The relatively compact structure of the palisade tissue is better compatible with the assumption that this tissue is principally a light filter.

2. It must be expected that the higher the light intensity the greater must be the thickness of the light filter. For this reason the palisade layer is the better developed the stronger the light intensity to which the leaf in question is exposed during its development.

3. The disproportion observed between the scarce CO_2 supply of the palisade cells and their very numerous chloroplasts is explained if it is assumed that the cells form a light filter.

4. The displacements of the chloroplasts in the palisade cells are a factor regulating (to a certain extent) the intensity of the light reaching the spongy parenchyma.

5. Even the shadow leaves of evergreen plants posses a palisade parenchyma; this particularity is explained by assuming that these leaves, which are active during several years must be better protected against the destructive action of light than normal one year leaves.

It is, however, obvious that the evidence presented in the first part of this paper is an indirect one; only suitably devised experiments can bring a decisive proof of the correctness of our hypothesis. Results of such experiments are summarised in the second part. In our opinion their most satisfactory explanation consists in admiting that the photosynthetic efficiency of the palisade tissues decreases with age of the leaf. This is well demonstrated by the inversion shown by the ratio L : U in the course of the development of the leaves. In young leaves where the palisade tissue is still photosynthetically active this ratio exceeds unity in contrast to older leaves where it is < 1. This inversion indicates that in young leaves the palisade parenchyma plays the double role of a light filter and an assimilating tissue. Later, however, the harmful action of strong light to which the palisade tissue is exposed and the insufficient CO_2 supply are the factors leading to a gradual inactivation of the chloroplasts of this tissue in older leaves. The degree of inactivation increases with the age of the leaf or the intensity of the incident light.

The influence of the light intensity on the inactivation of chloroplasts is confirmed by a comparison of the results obtained on leaves of the same species in the years 1959 and 1960. The summer in the last year was exceptionally cloudy and rainy; consequently the mean intensity of the sun light was below the normal level, and in conformity with expectation the values of the ratio U : L (for old leaves) were lower in 1959 than in 1960. For instance, in 1959 they amounted to 0,46 and 0,51 for *Salix elegantissima* and *Vitis vinifera* respectively, whereas in 1960 the corresponding values were 0,79 and 0,82.

The hypothesis presented in this paper and concerned with the role played by the assimilating parenchymas is completely different from the generally adopted view. The author is aware that the above presented experimental evidence does not constitute a definite proof of its correctness, and that further investigations are necessary. Their results will be shortly published.

6. SUMMARY

In the first part of the paper the role ascribed to the assimilating parenchymas in leaves (palisade and spongy parenchymas) made the object of a critical revision based on a detailed analysis of the structure and function of the leaf and a hypothesis concerning the roles played by these tissues in the process of photosynthesis was advanced. This hypothesis is in opposition to the generally adopted view, which considers the palisade parenchyma as the principal assimilating tissue.

The second part presents the results of experiments tending to elucidate the physiological functions of the tissues in question and to corroborate the author's hypothesis. In these experiments the assimilation rates of fragments of leaves of several species and various ages were determined. The fragments were alternatively illuminated from above or from below with light of the same intensity. A correction was made for the light energy reflected by the upper or lower surface of the leaf fragments.

The principal points of the hypothesis formulated by the author are as follows:

1. In a leaf there is only one assimilating tissue which according to the light conditions — may differentiate, or not, into two distinct layers (palisade and spongy tissue, or spongy tissue only).
2. The spongy parenchyma is the principal assimilating tissue, even in mature leaves with a well developed palisade parenchyma. In comparison with this tissue, the spongy tissue is characterized by a higher photosynthetic activity, a better provision of carbon dioxide, a more efficient migration of assimilates a better supply of water and optimal light conditions.
3. The experiments have shown with a high degree of probability that in mature leaves the photosynthetic contribution of the palisade tissue is smaller than the corresponding contribution of the spongy tissue and it was concluded that in these leaves the palisade parenchyma plays the role of a light filter protecting the pigments in the spongy tissue against the destructive action of intense light. Only in young growing leaves the palisade parenchyma plays visibly a double role of a light filter and an assimilating tissue.

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