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ULTRASTRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF PLASTIDS IN THE LEAVES OF *LIGUSTRUM OVALIFOLIUM* HASSK. VAR. *AUREUM**

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Introduction

It is known that the so-called aurea varieties of some plants are highly light sensitive (Monfort 1953, Monfort and Kress-Richter 1950, Bauer 1956, Sagromsky 1956, Egle 1960). The work of all these authors has shown that the leaves of such aurea plants are able to green only in dim light, while in strong sunlight they become more or less yellow. In these yellow leaves the concentration of the chlorophylls is drastically reduced, and so is also the thylakoid system in their plastids (Wrischer et al. 1975 a, b). On the other hand Willstätter and Stoll (1918) have already noticed that under high light conditions the yellow leaves of some aurea plants have a very high photosynthetic rate ("Assimilationszahl").

In order to obtain some new data about structure and function of plastids in one of such aurea plants (*Ligustrum ovalifolium* var. *aureum*) the pigment contents and the photosynthetic activities of the leaves have been measured and morphometric analyses of the plastids have been carried out on ultrathin sections. The obtained results have then been compared and correlated.

Material and Methods

For the experiments green leaves (grown in the shade) and yellow leaves (grown in sunlight) of *Ligustrum ovalifolium* Hassk. var. *aureum* (Krüssmann 1962) were used. All investigations were carried out during the summer months.

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The pigments were extracted by 80 % aqueous acetone and the quantitative determination was done as described earlier (Wrischer et al. 1975 a).

The photosynthetic activity was measured manometrically with a Warburg apparatus as oxygen yield, as previously mentioned (Wrischer et al. 1975 a).

For electron microscopic examinations pieces of leaves were fixed in 1 % glutaraldehyde, postfixed in 1 % OsO_4, and, after dehydration, embedded in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined in a Siemens Elmiskop I.

Semithin sections (about 0.5 μm thick) of the same material were stained with toluidine blue and examined in the light microscope.

Morphometric measurements were performed on micrographs (of 30,000 times final magnification) on which a 5 mm square lattice was additionally coppied. The area of the plastid section surface occupied by different plastid components was estimated from the number of hits which the crossings of the lattice, covering a certain plastid structure, were forming (Weibel 1969, Atkinson et al. 1974, Sitte 1974).

Results

The leaves of *L. ovalifolium* var. *aureum* (in further text briefly: aurea-privet) which are growing at the periphery of the shrub and are constantly exposed to the sunlight are markedly golden yellow, while leaves which are partly shaded, e. g. by other leaves, are yellow-green. Only leaves growing in deep shade inside the shrub are green.

The data about the content of the pigments and the photosynthetic activity are presented in Table 1.

Table 1. Total chlorophyll, relative amount of carotenoids, and photosynthetic activity in green leaves (grown in shade) and yellow leaves (grown in sunlight).

	Total chlorophyll (mg/g fr. wt.)	δA^{car}_{480}	Photosynthetic activity (μg O_2/mg fr. wt./h)	Photosynthetic efficiency (μg O_2/mg chlorophyll/h)
Green leaves	1.33 (100 %)	3.562 (100 %)	0.051	38.346
Yellow leaves	0.064 (4.78 %)	1.518 (42.3 %)	0.013	203.125

Whereas the concentration of the chlorophylls in yellow leaves reaches only 4.78 % of that in green leaves, the concentration of the total carotenoids drops in yellow leaves to only 42.3 % if compared with the quantity present in green leaves. The ratio of chlorophyll (*a*) to chlorophyll (*b*) is in both leaf types similar: in yellow leaves it is 2.2 and in green ones 2.5.

The total photosynthetic activity measured (at 5900 ±500 lx) as O_2 yield (in μg O_2/g fresh leaf weight per hour) is in yellow leaves low, but if calculated to the content of the chlorophyll it is more than 5 times higher than in green leaves grown in the shade (Table 1).

The plastids of yellow leaves which grow in strong sunlight, contain a very reduced membrane system. It consists of short single thylakoids, which are grouped into rudimentary grana in some places only (Figs. 2, 5). Some of these aggregated and often curved thylakoids are very densely packed, so that single membranes are hardly discernible (Figs. 3, 4). Portions of some thylakoids are also dilated in some places and large empty vesicles are numerous as well (Figs. 2—5).

In the stroma besides ribosomes, plastoglobules are always present. In completely yellow leaves starch has never been found. It develops, however, in these plastids when the illumination is appropriatelly reduced, e.g. at the end of a period of partly cloudy days (Fig. 5).

If the leaves are exposed for a long time to very intensive sunlight, they bleach. The plastids of the bleached leaves contain an extremely reduced number of membrane structures and resemble the plastids of bleached leaves in other aurea plants (Wrischer et al. 1975 a, b).

In green leaves, which were grown in the shade, the chloroplasts posess grana, with even more than 30 thylakoids in places, and large starch grains as well (Fig. 6).

Besides these striking differences in the plastid structure between yellow and green leaves, there are no other differences in the morphology of other cell components. In the nuclei large crystalline inclusions, probably of proteinaceous nature, can very often be observed (Fig. 5).

Table 2. The percentage of total plastid section surface contributed by different plastid components.

		Green leaves	Yellow leaves
Thylakoids	All thylakoids	36.3 %	14.1 %
	Grana thylakoids	27.5 %	5.5 %
Starch		18.3 %	
Plastoglobules		2.2 %	2.8 %
Stroma		43.2 %	83.1 %

The results of the morphometry are presented in Fig. 1 and Tables 2 and 3. The contribution of different components to the plastid section surface in green and in yellow leaves is presented in Fig. 1 and the percentage of the plastid section surface occupied by these components in Table 2. In Table 3 the quantities of thylakoids in plastids of both leaf types are compared. The plastids were photographed at random. According to the study of Weibel (1969, p. 243) the relation of section surfaces equals to the volume relation. Only structures which could be interpreted as deriving directly from the thylakoid apparatus were

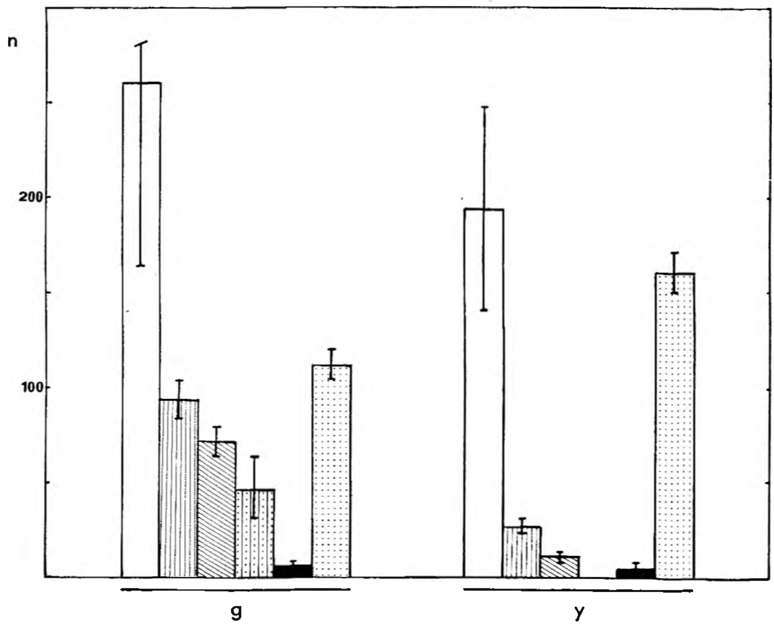


Fig. 1. The number of hits (mean value with standard error indicated) on plastid components counted by crossings forming a lattice of 5 mm squares overlying micrographs (at final magnification of 30,000 times).

Legend: g = green leaves; y = yellow leaves; n = number of hits; white columns = plastids; columns with vertical lines = all thylakoids; columns with diagonal lines = grana thylakoids; columns with lines and dots = starch; black columns = plastoglobules; columns with dots = stroma.

Fig. 2. Plastid from the yellow leaf of *Ligustrum ovalifolium* var. *aureum*. The thylakoid system is strongly reduced. 25,000 : 1.

Figs. 3 and 4. Portions of two plastids from the yellow leaf showing single thylakoids, tightly appressed and curved grana thylakoids, and vesicles; in the stroma ribosomes and some plastoglobules. 80,000 : 1.

Fig. 5. Portion of a cell from a yellow-green leaf. Although the thylakoid system of the plastid is reduced, starch is present in the stroma; in the nucleus several crystalline inclusions. 32,000 : 1.

Fig. 6. Portion of a chloroplast from the green leaf with large grana. 65,000 : 1.

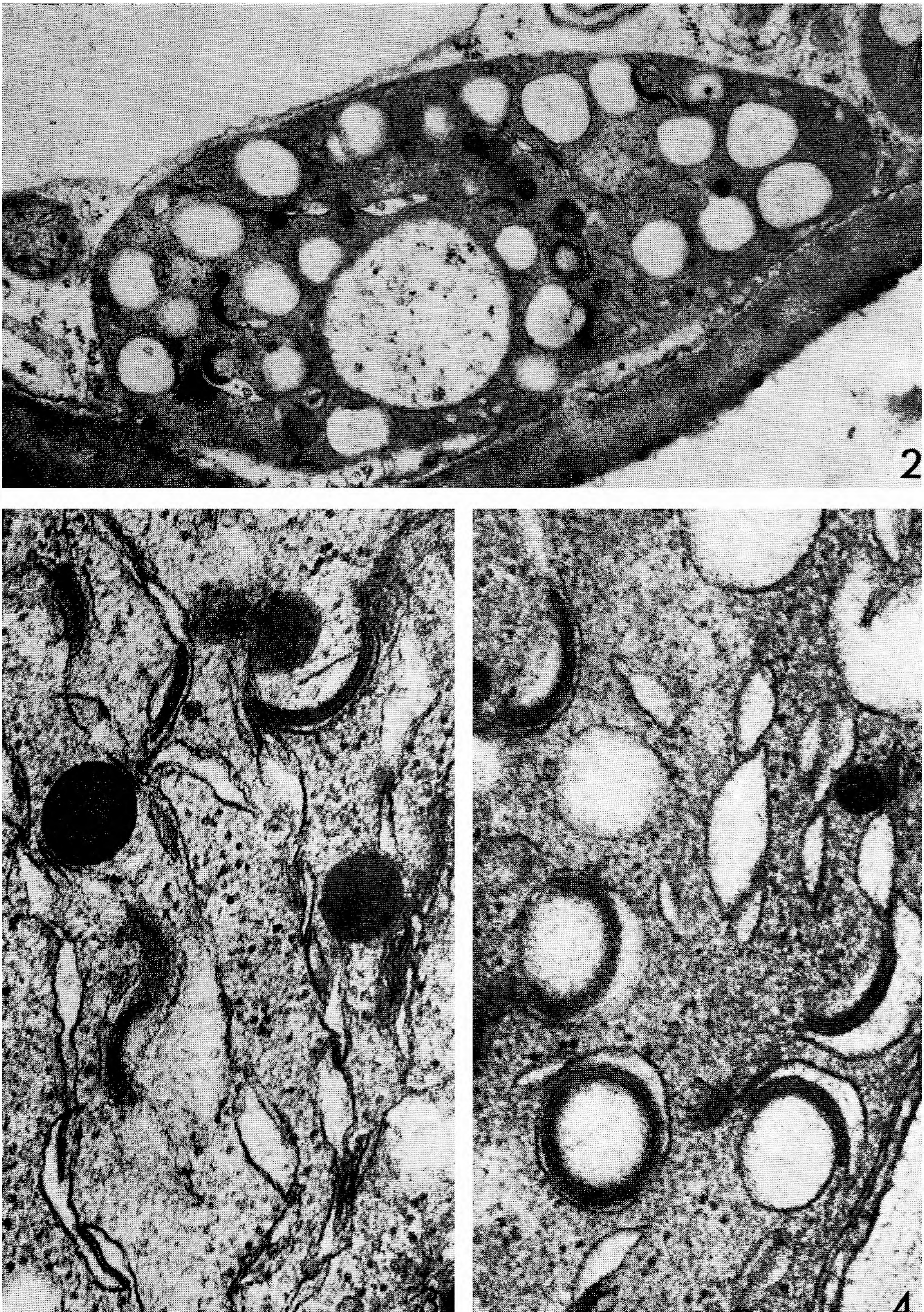
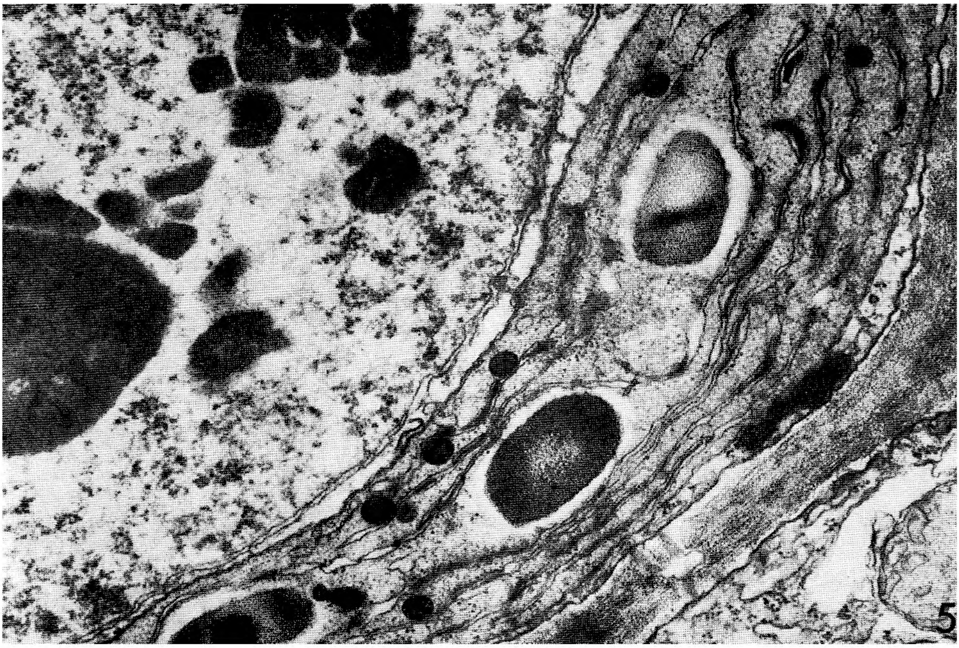


Fig. 2—4.



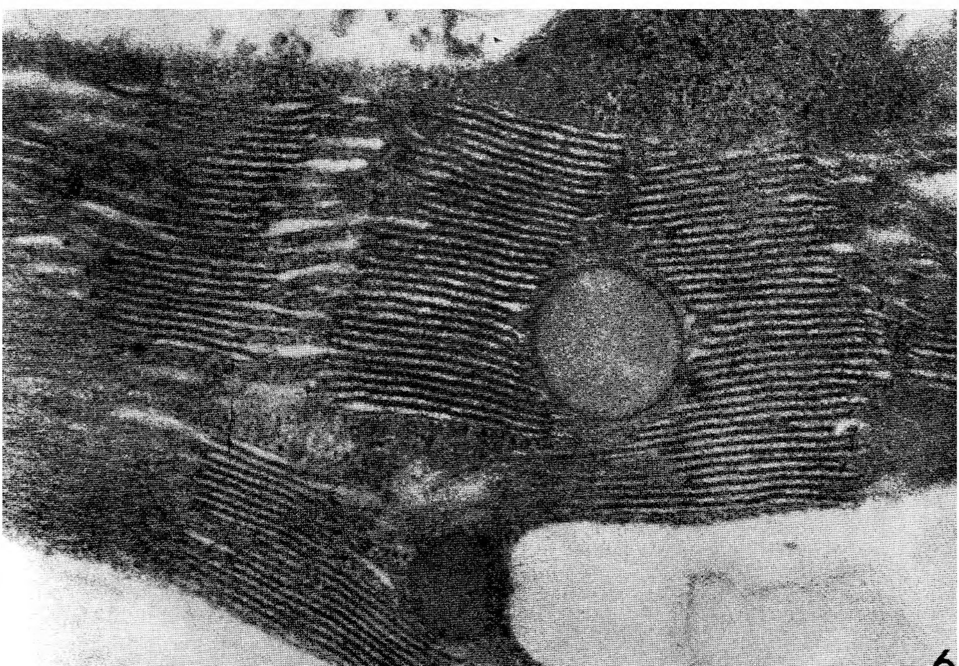


Fig. 5—6.

treated as thylakoids. For this reason the invaginations of the inner membrane of the plastid envelope were not considered. Parts of the thylakoids aggregated into groups of two or more were interpreted as belonging to the grana. According to the reasons explained by Sitte (1974), the whole space surrounding the contracted starch grain was estimated as starch. The area occupied by plastid stroma was estimated as the rest of the whole plastid section surface when all other inclusions were subtracted.

From the data presented in Fig. 1 and Tables 2 and 3 it is obvious that in yellow leaves the quantity of thylakoids is very low, if compared to that in green leaves. Especially low is the value of the thylakoids forming grana. If the contributions of the thylakoids of both leaf types, presented in Fig. 1, are compared, it can be calculated that in plastids of yellow leaves the percentage of all thylakoids reaches 29 % of that in the plastids of green leaves (taken as 100 %), whereas the percentage of the thylakoids arranged into grana makes only 15 % of grana thylakoids in green leaves (if this value is again taken as 100 %).

Table 3. The quantity of thylakoids (in %) in plastids of yellow leaves as compared to the quantity of thylakoids in plastids of green leaves. In brackets: The percentage of thylakoids after correction explained in the text.

	Green leaves	Yellow leaves
All thylakoids	100 %	29 % (21 %)
Grana thylakoids	100 %	15 % (11 %)

Light microscopic examination of semi-thin sections shows that — due to somewhat larger cells in yellow leaves — there are less plastids per surface unit of leaf section in yellow leaves (72.7 %) than in the green ones. If this correction is also taken into account, in yellow leaves the previously mentioned percentages drop from 29 % to 21 % for the whole thylakoid system and from 15 % to 11 % for the grana thylakoids (Table 3).

Discussion

The results obtained on *L. ovalifolium* var. *aureum* by pigment analysis and by fine structural studies are well in agreement with earlier observations of the same authors on two other aurea plants (Wrischer et al. 1975 a, b). The concentration of pigments, especially of chlorophylls, and the number of thylakoids per plastid are very reduced in these leaves when they are growing in strong sunlight. On the other hand, the results of the present investigation show that the photosynthetic activity — if calculated on the chlorophyll basis — is in yellow leaves several times higher than in the green ones. Willstätter and Stoll (1918) have already noticed this phenomenon in some aurea plants examined, and later the same was observed in two yellow mutants of tobacco (Schmid and Gaffron 1969, Lemoine 1974). Besides that, there are several other chlorophyll mutants known to have a 2 to 8 times higher photosynthetic rate than normal leaves of the wild type plants (e.g. Highkin et al. 1969, Keck et al. 1970, Benedict et al. 1972, Müller 1972, Crang and Noble 1974, Więckowski and Ficek 1974).

If the results of pigment analysis, morphometry, and photosynthetic measurements are compared, it may be concluded that in yellow leaves the concentration of the chlorophylls is lower than it would be expected accordingly to the quantity of thylakoids present in the plastids. This is the case even if only grana thylakoids are taken into consideration. On the other hand, the high photosynthetic efficiency of yellow leaves does not correlate with their low concent in chlorophyll. These data indicate that the composition of the thylakoid constituents in both leaf types must be different. The idea of a smaller photosynthetic unit, which was proposed for some chlorophyll mutants (Schmid and Gaffron 1969, Wild 1969, Müller 1972), should perhaps be also taken into consideration for the yellow leaves of the here mentioned aurea plant. In any case, this different photosynthetic efficiency of shaded and sun-exposed leaves of the aurea-privet enables this plant to use the available sunlight suitably.

The morphometric analysis shows that the percentage of the section surface occupied by the stroma is much higher in plastids of yellow leaves than in those of green leaves. This indicates that high illumination — while strongly inhibiting the formation of thylakoids, especially the formation of grana — at the same time does not inhibit the formation of the stroma material in the same measure. It is known, that in some chlorophyll mutants the synthesis of some enzymes of the Calvin cycle, which are located in the plastid stroma, is not inhibited by strong light (Björkman 1968, Benedict and Kohel 1969).

There are, however, indications that strong light does not have the same inhibitory effect in all aurea plants (Bauer 1956). According to the changes which were observed in the fine structure of the thylakoid system, it seems that there must exist at least two types of aurea plants. While in *Acer negundo* var. *odessanum* strong illumination inhibits the formation of thylakoids in the plastids, so that their number remains constantly low (Wrischer et al. 1975 a), in *Fraxinus excelsior* var. *aurea* (Wrischer et al. 1975 b) and in *L. ovalifolium* var. *aureum* this seems not to be the case. In these two plants some membrane components start to differentiate in strong light, but for some reason — perhaps because they are functionally deficient — they become disorganized again. Tightly appressed membranes and membrane coils are the consequence of these degenerative processes. The question is however, which of these membranes are still photosynthetically active. For the moment no exact answer can be given. Cytochemical analyses which are in course, will perhaps throw more light on this problem.

Summary

The leaves of *Ligustrum ovalifolium* Hassk. var. *aureum*, which are for a long time exposed to strong sunlight, do not green, but turn yellow. In these yellow leaves the plastids have a very reduced thylakoid system consisting of single thylakoids, vesicles, and membrane coils, and contain very few grana. Leaves grown in the shade are, on the contrary, more or less green and their plastids posess large grana. Morphometric

measurements show that the plastids of yellow leaves contain only 11 % of grana thylakoids present in plastids of green leaves.

While in yellow leaves the concentration of pigments, especially of chlorophylls, is very low (4.78 % of that in green leaves), their photosynthetic activity (calculated to the chlorophyll content) is more than 5 times higher than in green leaves.

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SADRŽAJ

ULTRASTRUKTURNE I FUNKCIONALNE KARAKTERISTIKE PLASTIDA U LISTOVIMA BILJKE *LIGUSTRUM OVALIFOLIUM* HASSK. VAR. *AUREUM*

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Listovi biljke *Ligustrum ovalifolium* Hassk. var. *aureum*, koji su dulje vremena izloženi intenzivnoj sunčanoj svjetlosti, ne ozelene već postanu žuti. Plastidi u takvim listovima imaju vrlo reducirani tilakoidni sistem, koji se sastoji pretežno od pojedinačnih tilakoida i vezikula, dok su grana malobrojna. Naprotiv listovi izrasli u sjeni zeleni su i njihovi plastidi posjeduju velika grana. Morfometrijska mjerenja pokazuju da plastidi žutih listova sadržavaju samo 11 % grana-tilakoida plastida zelenih listova.

Dok je koncentracija pigmenata, naročito klorofila, u žutim listovima vrlo niska (4,78 % od one u zelenim listovima), njihova je fotosintetska aktivnost (preračunata na istu količinu klorofila) naprotiv preko pet puta veća od one u zelenim listovima.

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