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STRUCTURAL AND FUNCTIONAL CHANGES IN CHLOROPLASTS OF SENESCENT LEAVES OF *SOPHORA JAPONICA* L.

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The leaves of *Sophora japonica* remain green throughout the autumn then fall off late in November or the beginning of December without change in their colour. Their ultrastructure, pigment content and photosynthetic activity were investigated in two week intervals from mid September till the beginning of December. The ultrastructure of the chloroplast was quite normal during this time. The gradual increase of plastoglobules and the presence of some interthylakoidal inclusions showed that senescence was still in progress. The content of pigments and the photosynthetic activity of the leaves were also normal. The enhanced senescence did not start until late autumn in leaves which had already fallen off the trees.

Introduction

Autumnal leaf senescence of deciduous trees usually consists in their progressive yellowing and ends in their detachment from the twigs. The yellowing comprises a degradation of all components of the photosynthetic apparatus (Thomas and Stoddart 1980). In this process, particularly the content of chlorophylls is drastically reduced, and is followed by the loss of chloroplast proteins and RNA. At the end of senescence even the DNA disappears from the chloroplast stroma (Sodmergen et al. 1991).

There are some deciduous trees which do not yellow during senescence. One of these is *Sophora japonica*. Its leaves retain their green colour throughout the autumn. In late autumn or early winter – under favourable meteorological conditions – they fall off green. The behaviour of these leaves seemed interesting. Therefore their ultrastructure, pigment content, and photosynthetic activity were investigated.

Materials and Methods

The investigations were performed on leaves of the trees of *Sophora japonica* L. growing under garden conditions. The leaf tissue was taken for analyses every two weeks from mid September to the beginning of December during two seasons.

For fine structural analyses small pieces of tissue were fixed in 1% glutaraldehyde in cacodylate buffer (pH 7.2) with the addition of 1% caffeine (Modrušan and Wrischer 1987). After washing in buffer the material was postfixed for 2 h in 1% OsO_4 in the same buffer, dehydrated and embedded in araldite. Thin sections were stained with uranyl acetate and lead citrate and examined in a Zeiss-Opton 10 electron microscope. For light microscope examination, besides fresh material, semithin sections of embedded tissue stained with toluidine blue were used.

The pigments were extracted in 80% acetone and measured spectrophotometrically. Photosynthetic activity of leaf pieces was measured with an O_2 electrode (Hansatech Ltd., England). The reaction mixture contained 0.1 mol phosphate buffer (pH 7.2–7.4) and 0.1 mol sodium bicarbonate (Modrušan and Wrischer 1987). The samples were illuminated at saturated illumination with a halogen lamp.

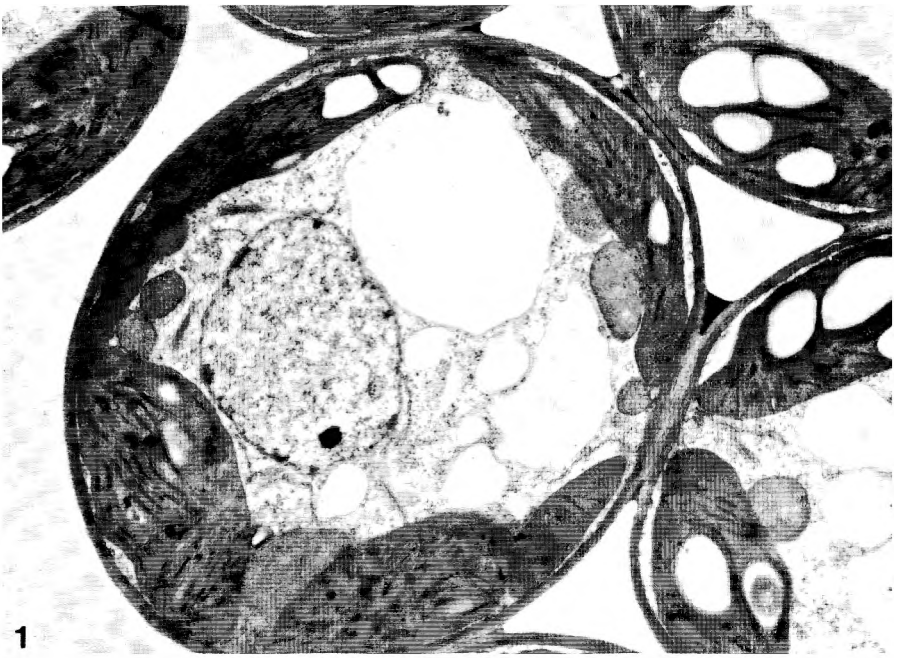
Results

The leaves of *Sophora japonica* examined in mid September by light microscopy (as fresh tissue or as semithin sections) were very thin and had large intercellular spaces. There were about 15 chloroplasts per longitudinal section of a palisade cell. The ultrastructure of these leaves was completely normal. The dimensions of chloroplasts were about 6.9×2.8 μm and grana stacks had from 3 to many thylakoids. The stroma contained numerous ribosomes, large strach grains and 3–7 small plastoglobules (less than 0.1 μm in diameter) per plastid section (Fig. 1). In the light zones of the stroma fine filaments – plastid nucleoides – were present.

At the beginning of October some changes appeared in the chloroplast ultrastructure. The quantity of thylakoids seemed unchanged, but dark osmiophilic inclusions appeared between two grana thylakoids (i. e. interthylakoidally) (Fig. 2). At the same time plastoglobules increased in number and size. Per chloroplast section there were about 10 plastoglobules having 0.3 μm in diameter. Starch was present in the stroma which was filled with ribosomes.

Fig. 1. Leaf cell with nucleus, chloroplasts, mitochondria and peroxisomes (mid September). 6,300:1.

Fig. 2. Portion of a chloroplast with interthylakoidal inclusions (arrows) and plastoglobules (beginning of October). 64,000:1.



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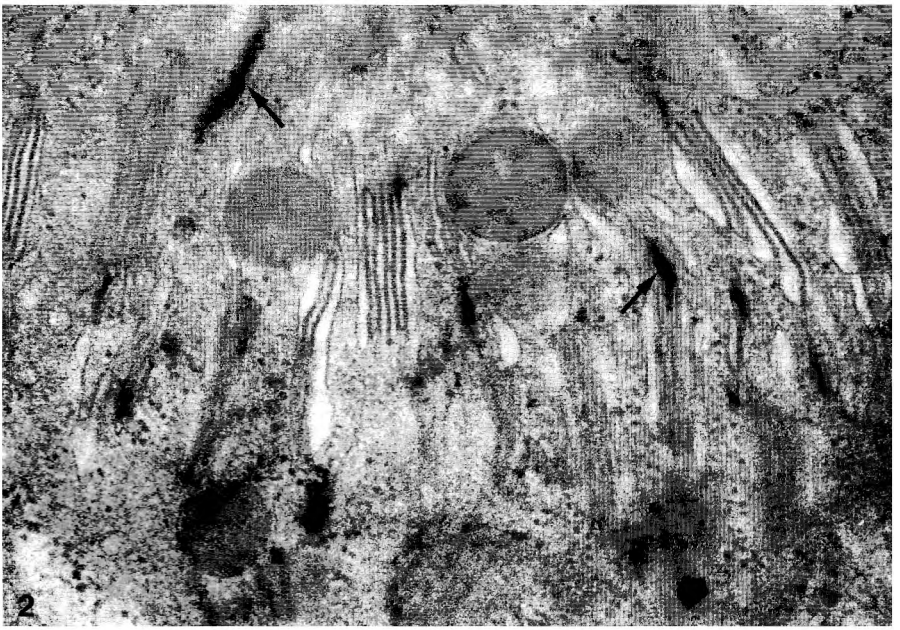

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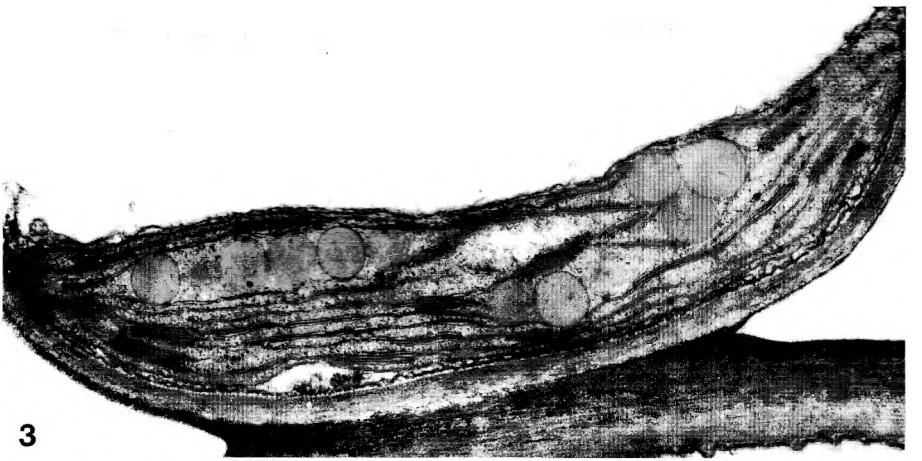

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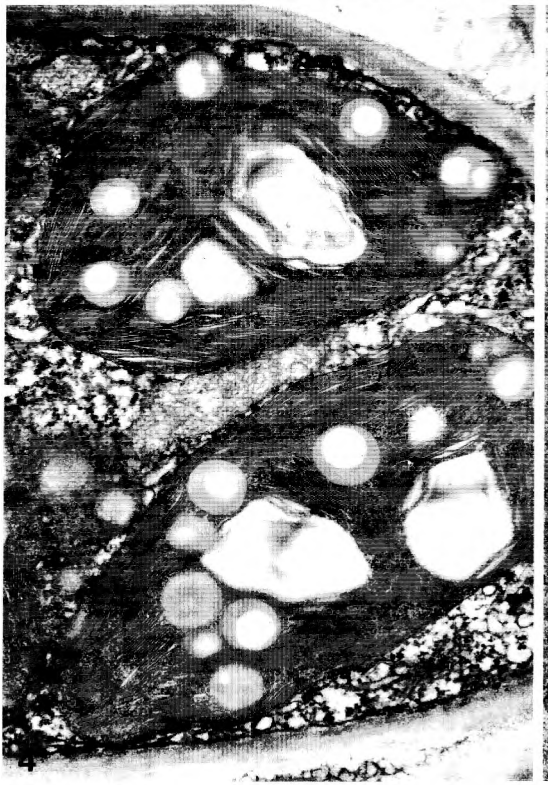

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Interthylakoidal inclusions, often observed in chloroplasts of leaves early in October, became rare in those of late October and the beginning of November. At the same time plastoglobules further enlarged, their diameters reaching 0.4 μm. As the autumn passed the chloroplasts became smaller. In late autumn they had on average only 4.7 × 1.5 μm (Fig. 3).

In favourable meteorological conditions (i. e. when the temperatures remained above zero) the leaves did not fall off before late November or early December. In leaves soon after their falling off there were still rather well preserved chloroplasts (Fig. 4) and in the stroma, besides large plastoglobules, aggregates of dark small particles – probably phytoferritin – were present (Fig. 5). Light zones in the stroma contained fine filaments, the plastid nucleoides.

Other organelles in leaf cells retained a normal ultrastructure throughout the autumn. The mitochondria were particularly well developed and often located near the chloroplasts together with large peroxisomes (Fig. 1).

The chlorophyll content of the leaves examined in October and November was rather high (Table 1). About the time when they fell off (at the end of November or beginning of December) the leaves still contained about 70% of the chlorophyll present in the October leaves. In leaves recently fallen off there was 1.2 mg of chlorophyll per g fresh leaf weight. The content of carotenoids was low in all leaves examined and did not vary much throughout the autumn months (Table 1).

The photosynthetic activity (measured as production of oxygen) was fairly high in autumn leaves (Table 1). In late autumn, in leaves soon after their falling off the photosynthetic activity was only 30% lower than in those still attached to the twigs.

Table 1. Total chlorophyll, total carotenoids, and photosynthetic activity of autumnal (October – November) leaves of *Sophora japonica* (mean values of several measurements)

Total chlorophyll (mg/g fr. wt.)	Total carotenoids (mg/g fr. wt.)	Photosynthetic activity (μmol O_2/g fr. wt./h)	Photosynthetic efficiency μmol O_2/mg chlorophyll/h)
2.13	0.49	103.25	48.46

Fig. 3. Chloroplast with grana and stroma thylakoids and large plastoglobules (beginning of December). 20,000:1.

Fig. 4. Chloroplasts with a well preserved thylakoid system, starch grains, and large plastoglobules of a fallen off leaf (end of November). 15,000:1.

Fig. 5. Aggregates of phytoferritin in the chloroplast stroma of a fallen off leaf (end of November). 150,000:1.

Discussion

The autumnal yellowing of the leaves is a very complex and dramatic process (Thomas and Stoddart 1980, Rüdiger and Schoch 1989). It comprises destruction of chlorophylls, proteins and nucleic acids (Maunders and Brown 1983, Nii et al. 1988, Matile et al. 1989). Structural changes in the chloroplasts are also conspicuous. Thylakoids become disorganized and large lipids containing plastoglobules appear in the stroma (Ljubešić 1968, Dodge 1970). The process ends with a complete disintegration of the thylakoid system and ruptures of the chloroplast envelopes (Hurkman 1970).

In autumnal leaves of *Sophora,* the senescence is considerably slowed down, and particularly the pigmentation remains quite normal. Similar preservations of pigments were detected also in autumn leaves of some other plants. In individuals of *Fraxinus excelsior* e. g. the leaves remained green until their detachment in late autumn (unpublished data). However, this type of decelerated senescence does not resemble that described for leaves of the nonyellowing mutant of *Festuca pratensis* (Thomas 1977, 1982). Although thylakoids containing chlorophyll were present in senescing chloroplasts of this *Festuca* mutant plant, there were no true grana thylakoids and the photosynthetic activity was lost.

Only the presence of large plastoglobules indicated that in autumn leaves of *Sophora* senescence was still in progress. Dark inclusions located between grana thylakoids in some of the chloroplasts probably originated from degraded membranes. Due to the strong osmiophily these inclusions were obviously lipidic. Similar interthylakoidal structures were found in yellowing leaves of some »aurea« mutants, which became exposed to light of high intensity and were forced to degrade a part of their photosynthetic membranes (Wrischer et al. 1975, 1976).

A well preserved thylakoid system, a complete set of membrane protein complexes and of stroma proteins (unpublished data), as well as a high chlorophyll content are prerequisites for the leaves of *Sophora* to keep efficient photosynthesis until late into the autumn. The senescence is enhanced only in the leaves fallen off is late autumn. In these leaves the breakdown of chlorophylls is intensified, while the appearance of phytoferritin aggregates indicates a rapid degradation of the thylakoids (Ljubešić 1976).

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SAŽETAK

STRUKTURNE I FUNKCIONALNE PROMJENE U KLOROPLASTIMA LISTOVA SOFORE (*SOPHORA JAPONICA* L.) TIJEKOM STARENJA

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Listovi sofore ostaju zeleni kroz čitavu jesen, tako da krajem studenog ili početkom prosinca otpadnu bez bitne promjene u boji. Ultrastruktura, sadržaj pigmenata i fotosintetska aktivnost tih listova praćeni su kroz dvije sezone u razmacima od dva tjedna i to od sredine rujna do početka prosinca. Tijekom tog vremena ultrastruktura kloroplasta ostaje razmjerno normalna. Postupno povećavanje dimenzije plastoglobula i prisutnost intertilakoidnih uklopina ukazuje na starenje kloroplasta. Sadržaj pigmenata i fotosintetska aktivnost listova također su normalni tijekom jesenjih mjeseci. Ubrzano starenje kloroplasta (destrukcija klorofila i pojava nakupina fitoferitina) započinje tek nakon njihovog otpadanja krajem jeseni ili početkom zime.

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