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SEASONAL CHANGES IN THE CHLOROPLASTS OF CHERRY-LAUREL LEAVES

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Changes in fine structure and pigment content of chloroplasts in the leaves of cherry-laurel (*Prunus laurocerasus* L.) were studied during late autumn, winter and spring. The leaves, which grew up in autumn, could not finish their development and remained light-green in winter. Their chloroplasts were juvenile. Only the areas around the nectaries at the base of the leaves were dark-green. In these areas the chloroplasts had a well-developed thylakoid system, similar to that in fully grown leaves. In early spring the young leaves continued their differentiation; they became dark-green and developed normal chloroplasts. The differences in coloration of leaf laminas now disappeared. Such differences were again detectable in young leaves newly formed in spring. The reasons for the faster rate of development of the chloroplasts around the nectaries are discussed, and the high content of carbohydrates in the regions around the nectaries appears to be the most probable explanation.

Introduction

Thick, leathery perennial leaves of cherry-laurel (*Prunus laurocerasus* L.) are fairly well adapted to winter conditions of the Central European continental climate. As a rule they overwinter and perish only in exceptionally strong winters. Because of short days and low temperatures the youngest leaves, which grew up in autumn, do not complete their growth. They remain small and light-green. We noticed that at the beginning of the winter season in these young leaves the tissue around the nectaries at the leaf base developed dark-green. With the start of warmer and longer days in the early spring these differences in colour slowly disappeared. The cause of the appearance of these dark-green patches on light-green leaves was the subject of our study. We investigated the chloroplast fine structure and the pigment content of light-green leaves and of dark-green tissue around the nectaries during late autumn, winter, and spring. The fine structure and the pigment content of dark-green old leaves and senescent yellow ones were examined for comparison.

Materials and Methods

The experiments were carried out on leaves of cherry-laurel (*Prunus laurocerasus* L.) growing in the garden of the Rudjer Bošković Institute, Zagreb. The leaves were examined during late autumn, winter, and spring.

Small pieces of leaves were fixed in 1% glutaraldehyde in cacodylate buffer (pH 7.2). The material was washed in buffer and postfixed in 1% OsO_4. Fixation was followed by dehydration in graded ethanol series and embedded in araldite.

Hand made sections through fresh leaves were examined in light microscope. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in an Opton EM 10 electron microscope. The stacking degree of thylakoids was determined by measuring the total length of stroma and grana thylakoids and the length of stacked regions with a kilometer tracer (Meier and Lichtenthaler 1981).

The pigments were extracted in 80% aceton and measured spectrophotometrically. Chlorophylls and carotenoids were quantitatively determined according to Goodwin (1972). Carotenoids were separated by thin-layer chromatography, dissolved in petrol eter-ethyl acetate-diethylamine (58 : 30 : 12) and estimated according to Stahl (1969).

Results

In late autumn and in winter the young not completely grown up cherry-laurel leaves were light-green and only the areas around the nectaries (2 — 3 mm in diameter) at the leaf base were dark-green. In early spring these leaves continued their development. They slowly became dark-green and now the differences in coloration of the leaf laminas disappeared. In fully grown leaves, which obtained a dark-green colour already in autumn, the areas around the nectaries were never conspicuous.

The light-microscopic study of hand made sections through fresh leaves showed structural differences among leaves and leaf regions. In the dark-green areas around the nectaries of young winter leaves only the palisade cells were dark green, while the spongy parenchyma cells had a colour similar to the remaining leaf tissue. The secretory cells and the glandular parenchyma of the nectaries were uncoloured. According to their anatomy these nectaries belong to the »nectaries with palisade epidermis« (Zimmermann 1932).

The thickness of the leaves varied considerably. The light-green winter leaves were on the average 0.40 mm and the dark-green areas 0.45 mm thick. The fully grown, dark-green winter leaves had a thickness of 0.43 mm. In spring the thickness of both young and grown up leaves increased somewhat.

In winter the content of the total chlorophyll of young leaves was rather low — 0.9 mg/g fr. wt. (fresh weight). At the same time the dark-green areas around the nectaries contained noticeably more chlorophyll (1.2 mg/g fr. wt.). In spring the chlorophyll content in these leaves increased to 1.4 mg/g fr. wt., and is now as high as that of the tissue around the nectaries. The greening of the young winter leaves can be stimulated by exposing pieces of leaves on wet filter-paper for several days to continuous illumination (3000 lux) and a temperature of 24°C.

The chlorophyll content of the dark-green fully grown winter leaves was 1.2 mg/g fr. wt. and in spring it slightly increased to 1.5 mg/g fr. wt. In old senescent yellow leaves the chlorophyll content was very low (0.5 mg/g fr. wt.).

In all examined leaves the changes in the concentration of total carotenoids followed those of the chlorophylls. The results of thin layer chromatography indicate that the relations among various carotenoids are similar to those in the leaves of other higher plants. The content of β-carotene is rather low in all winter cherry-laurel leaves.

In late autumn and winter, chloroplasts of light-green young leaves were elongated and had an average length x width of 5.5 × 2.1 μm. The thylakoids were arranged into small grana with only 2 — 4 thylakoids, so that the stacking degree of the thylakoids was 26%. In the stroma there were some small plastoglobules about 0.2 μm in diameter (Fig. 1). Chloroplasts of the dark-green palisade tissue around the nectaries were roundish (average length × width: 4.5 × 2.4 μm). Their grana contained 5—6 thylakoids, although in some chloroplasts grana with 15 or more thylakoids were found. The stacking degree of the thylakoids was 67%. Scarce plastoglobules were about 0.2 μm in diameter (Fig. 2).

In early spring the leaves, whose development had been stopped in autumn, continued their growth and differentiation. At first, chloroplasts became roundish (average length × width: 4.0 × 2.4 μm) and the number of thylakoids pro granum increased to 5—6, so that the stacking degree of the thylakoids reached 55% (Fig. 4). The fine structure of the chloroplasts in palisade tissue around the nectaries did not change much and was similar to that of the chloroplasts of the remaining leaf tissue (Fig. 5). The stacking degree of their thylakoids was 64%. A month later in these still young leaves the chloroplasts differentiated further. Their average length × width was 5.5 × 2.1 μm. The number of the thylakoids also increased. Grana contained on the average 7 thylakoids and the stacking degree of the thylakoids reached 81%. In the stroma there were large starch grains.

In the elongated chloroplasts (average length × width: 5.7 × 2.1 μm) of the fully grown winter leaves, grana contained 5—6 thylakoids. The stacking degree of the thylakoids was 65%. Characteristic of these chloroplasts are large plastoglobules 0.4—0.5 μm in diameter (Fig. 3). In early spring the stacking degree of the thylakoids in the chloroplasts of these fully grown leaves increased to 77%. At the same time the dimensions of the plastoglobules remained unchanged.

In young leaves which grew up in spring the differences in coloration and structure between the palisade tissue around the nectaries and that of the surrounding leaf tissue were again detectable. The roundish chloroplasts (average length × width: 3.8 × 2.3 μm) of the dark-green tissue around the nectaries contained grana with 5—6 or even more thylakoids and their stacking degree was 77%. The chlorophyll content of these chloroplasts was rather high: 1.9 mg/g fr. wt. The chloroplasts in the rest of the leaf tissue were somewhat smaller, having an average length × width of 3.4 × 1.8 μm. The stacking degree of the thylakoids was 50% and there were only 2—4 thylakoids per granum. The chlorophyll content of the chloroplasts was 1.5 mg/g fr. wt.

In the chloroplasts of senescent yellow leaves there were very large plastoglobules, some measuring up to 1 μm in diameter. In the stroma, crystalline aggregates of phytoferritin were present (Figs. 6, 7). The tissue around the nectaries was always of the same yellow colour and structure as the rest of the leaf. The senescent leaves changed irreversibly and usually decayed in spring.

Discussion

Unfavourable external conditions (low temperatures, short days) in autumn and winter stop the development of young cherry-laurel leaves leaving their chloroplasts in a juvenile stage. In these leaves only the tissue around the nectaries is dark-green and contains chloroplasts which are in an advanced stage of development. Their grana are well formed and the stacking degree of the thylakoids is high. These dark-green leaf areas look very similar to the so called green islands on leaves infected by certain insects or fungi (Harding et al. 1968, Camp and Whittingham 1975). There are, however, functional differences between these two cases. While in »green islands« the senescence of the chloro-

Fig. 1. Chloroplast from a young light-green winter leaf 20,000 : 1.

Fig. 2. Chloroplast from dark-green palisade tissue around the nectary of a young winter leaf. 25,000 : 1.

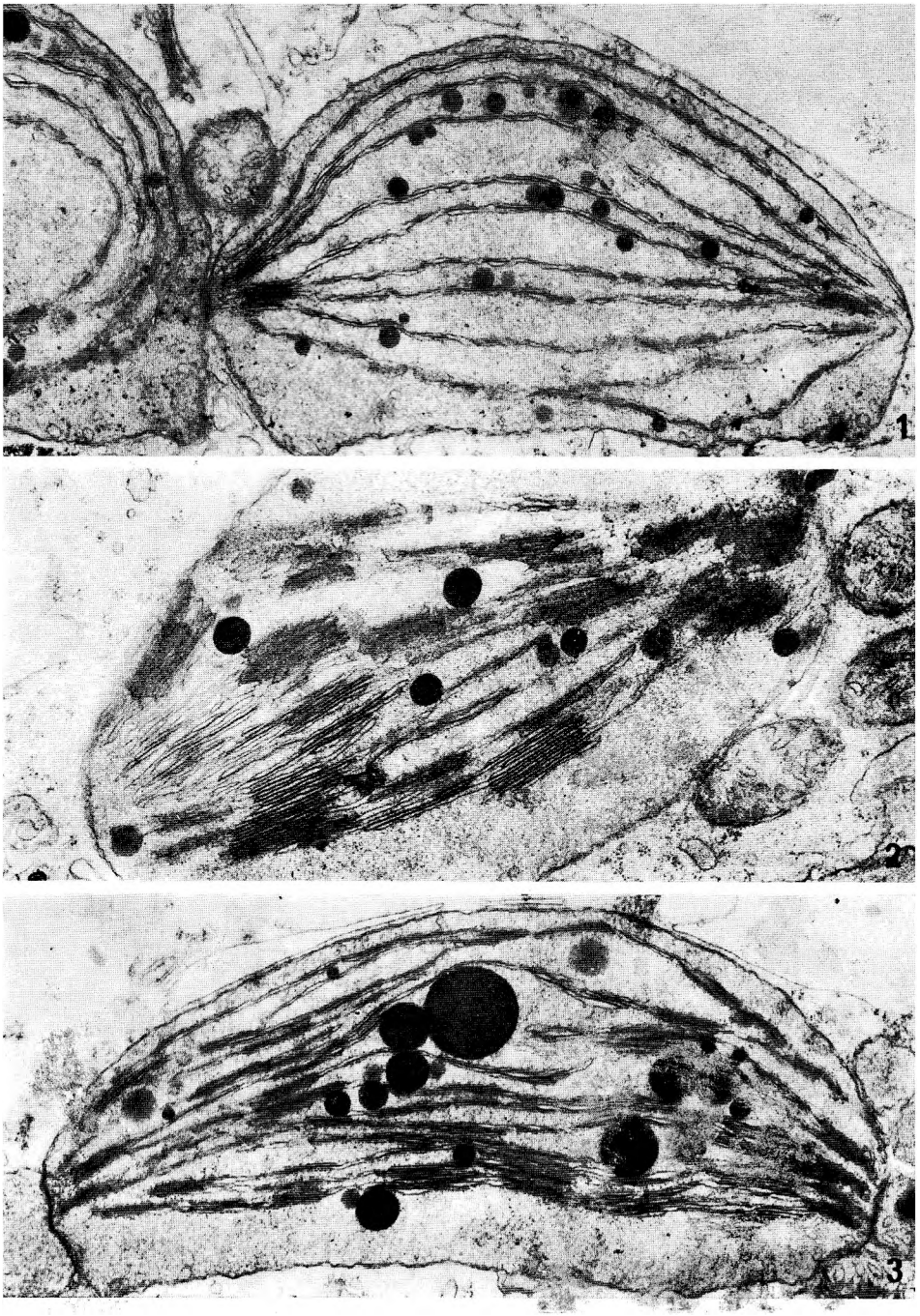
Fig. 3. Chloroplast from a grown up dark-green winter leaf. 25,000 : 1.

Fig. 4. Chloroplast from an overwintered young leaf examined in early spring. 25,000 : 1.

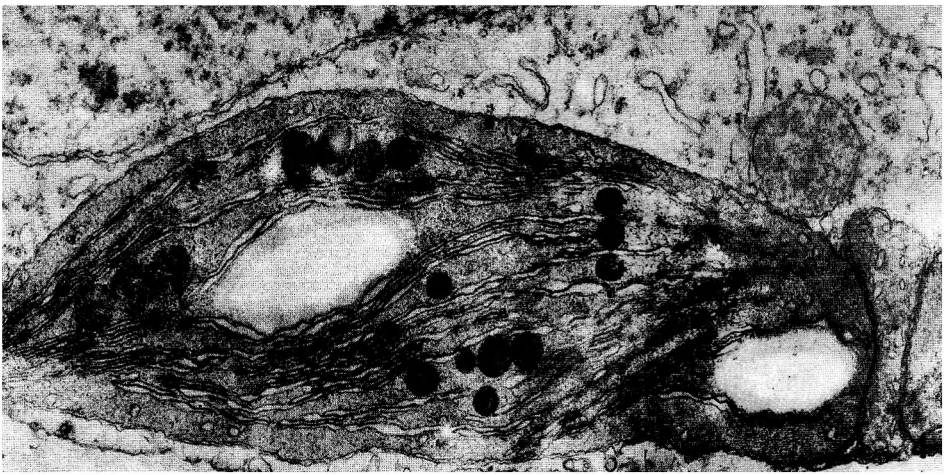
Fig. 5. Chloroplast from the palisade tissue around the nectary from a young overwintered leaf examined in early spring. 25,000 : 1.

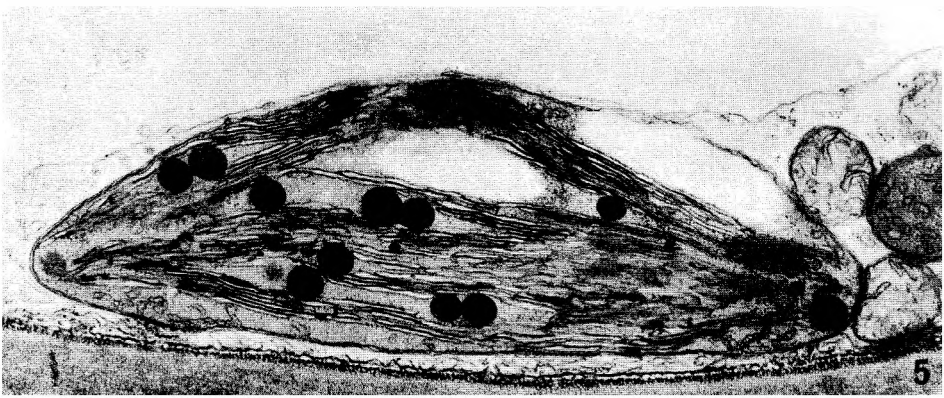
Fig. 6. Chloroplast from a senescent yellow leaf. 20,000 : 1.

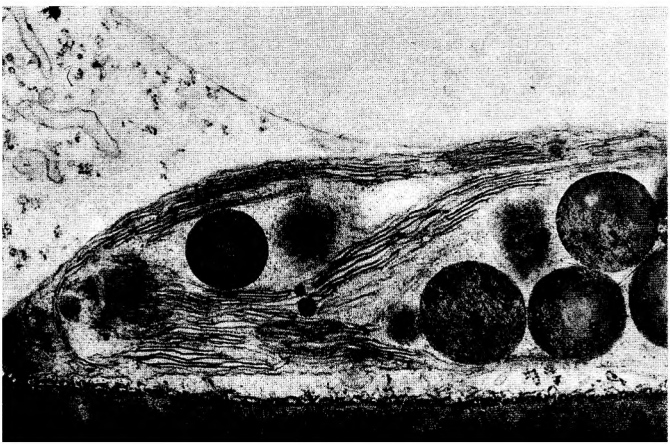
Fig. 7. Aggregates of phytoferritin in the stroma of a senescent yellow leaf. 45,000 : 1.



Figs. 1—3.







Figs. 4—7.

plasts is delayed (when compared with those in the surrounding leaf tissue), in dark-green areas around the nectaries of cherry-laurel leaves chloroplast differentiation is, on the contrary, enhanced. An increase in the content of certain plant hormons has been reported for the tissue of »green islands« and other regreened tissues. This increase in hormon content should delay chloroplast senescence (Witsch 1965, Camp and Whittingham 1975).

The nectaries of cherry-laurel excrete sugars — mostly fructose and glucose (Helder 1958) — but there are no data about the presence of plant hormons in nectar. The nectaries are actively secreting till late into the autumn. It is therefore very likely that a supply of nutrients from the glandular tissue hastens or even prolongs the developmental period of the chloroplasts in the surrounding palisade cells.

When cherry-laurel leaves grow up, the differences in pigmentation on leaf laminas are no more detectable. The chloroplasts now contain large plastoglobules, a sign of their maturation (Wrischer et al. 1986). Still larger plastoglobules appear in senescent yellowing leaves together with conspicuous crystalloids of phytoferritin, indicating that destructive processes in photosynthetic membranes are in progress (Ljubešić 1976).

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SAŽETAK

SEZONSKE PROMJENE KLOROPLASTA U LISTOVIMA LOVORVIŠNJE

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Istraženi su ultrastruktura kloroplasta i sastav pigmenata listova lovorvišnje (*Prunus laurocerasus* L.) tijekom kasne jeseni, zime i ranog proljeća. Listovi izrasli u jesen ne uspijevaju do početka zime stvoriti potpuno razvijene kloroplaste te takvi listovi ostaju tijekom zime svijetlozelene boje. Međutim tkivo oko nektarija, koji se nalaze pri bazi takvih listova, izrazito je zelene boje. Elektronskomikroskopska istraživanja pokazala su da svijetlozeleni listovi sadržavaju kloroplaste s nepotpuno razvijenim tilakoidnim sustavom, a tamnozeleno tkivo oko nektarija, te tamnozeleni stariji listovi, normalno razvijene kloroplaste. S početkom vegetacijske sezone, u rano proljeće, svijetlozeleni listovi cijelom površinom poprime tamnozelenu boju i razviju normalne kloroplaste. Na mladim listovima izraslim u proljeće ponovo se uočuju razlike u pigmentaciji. Razmotreni su mogući razlozi bržeg razvoja kloroplasta oko nektarija u kasnu jesen, od kojih je najvjerojatniji visok sadržaj ugljikohidrata u području nektarija.

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