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CHANGE IN LEAF PIGMENTS DURING AUTUMN COLOURATION

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Abstract

Over three autumns (1972, 1973, 1974) we investigated the transformation of pigments in the leaves of four tree-like shrubs and one sub-shrub. The pigments were separated with thin-layer chromatography. The changes in pigments in the green, yellowish-green, and yellow leaves were measured and compared with one another.

(1) It seems to be a general tendency that during the autumn colouration of leaves chlorophyll *a* decomposes faster than chlorophyll *b*. We attribute this phenomenon to the stronger ligh sensitivity of the chlorophyll-*a* molecule.
(2) In the withering leaves of some species, the decomposition of chlorophyll *b* may also be faster or the same as that of chlorophyll *a*. Thus, during the autumn colouration of leaves, we can observe three types of chlorophyll *a*/*b* ratio development. We attribute this peculiarity of the „species" to the varying strength of the chlorophyll-membrane bond and to the varying presence of anthocyan.
(3) The degradation of chlorophyll *a* is followed fastest by carotene.
(4) In the course of the autumn decomposition lutein manifested itself as stablest of the carotenoids. The amount of epoxi-carotenoids in the withering leaves may rise or fall.

Introduction

In the course of ontogeny the amount and ratio of chlorophylls, hydrocarbon-, hydroxi- and epoxi-carotenes characteristically changes in the leaf. The autumn pigment changes most frequently indicate transformation and degradation. But new carotenoid synthesis may also occur in the ageing leaves.

Willstätter and STOLL (1918) made a scientifically historical statement concerning autumn pigment degradation. TSWETT (1911) published that in the autumn leaves carotene is almost completely transformed into xanthophylls.

KUHN and BROCKMANN (1932) established that the „autumn pigments" in nautre are not carotenes but esterified xanthophylls.

According to KARRER and WALKER (1934), autumn pigments are the products of the degradation of carotenes and xanthophylls. SCHERTZ (1929) was the first to really observe a seasonal changes.

But he did not separate chlorophylls and carotenoids. WOLF (1956) studied the chlorophyll a/b ratio in the autumn leaves. Concerning the autumn change in the pigment content of leaves, there are but few literary data to be found. According to STRAIN's comprehensive studies (1938, 1959) on autumn leaves during autumn colouration the amount of carotenes decreases and that of xanthophylls increases.

In the pericarp of the green paprika (capsicum) of leaf origin, in addition to the carotenoid transformations which are similar to those in foliage-leaves, there are also differences. CHOLNOKY (1937) and CHOLNOKY *et al* (1955) established that in the green berry-fruit of the paprika during ripening, the chlorophyll content decreases steadily, and the carotenoid (capsanthin, capsarubin, etc.) content increases. β-carotene is transformed into neoxanthin, α-carotene into lutein, and these epoxidate while ripening occurs.

With thin-layer chromatography the transformation of pigments in the leaves of four ligneous plants and one sub-shrub plant was investigated over three autumns (1972, 1973, 1974). After determining and evaluating hundreds of measured results, we looked for answers to the following problems:

1. Is the degradation of chlorophylls uniform or does the ratio of chlorophyll a/b change?

2. What is the connection between the amounts of chlorophylls and carotenoids in the course of pigment decomposition?

3. How do carotenes, hydroxi- and apoxi-carotenes develop in the autumn?

Materials and Methods

In our investigations we used the green, yellowish-green, and yellow leaves of Cercis siliquastrum L., the green-leaved common birch (*Betula pendula* ROTH.), *Rubus idaeus L.*, *Betula pendula* var. *purpurea* (ANDRÉ) SCHNEID., and *Robinia pseudo-acacia L.* The entirely developed, normal leaves were gathered from the garden of the Ady-square building of the University, always at the same time, 8 o'clock a.m. During sampling, we took into consideration that the leaves should possibly be the same age and come from the same position on the shoots. From the leaves disks of 1 cm diameter were cut out and extracted by the method of MARÓTI—GABNAI (1971). Then the pigments were separated and measured.

Results

During the autumn leaf colouration, the pigments in the leaves of the species investigated changed in different ways. In the green, yellowish-green, and yellow leaves the chlorophyll content relating to the dry matter develops in the following way:

Table 1. Chlorophyll mg/100 g dry-matter content of leaves of different ages and colours.

Species	Pigments	green leaf		yellowish-green leaf		yellow leaf	
		mg/100 g	chl. a/b	mg/100 g	chl. a/b	mg/100 g	chl. a/b
Cercis s.	Chl. a.	299,3	4,34	128,2	3,90	17,6	3,45
	Chl. b.	69,0	—	32,9	—	5,1	—
Betula pendula	Chl. a.	348,0	4,71	87,4	4,48	3,7	0,84
	Chl. b.	73,9	—	19,5	—	4,4	—
Betula p. v. *purpurea*	Chl. a.	328,0	5,14	104,8	6,02	20,6	5,72
	Chl. b.	63,8	—	17,4	—	3,6	—
Robinia p.	Chl. a.	499,7	4,46	144,7	3,95	81,6	4,10
	Chl. b.	112,1	—	36,6	—	19,9	—
Rubus idaeus	Chl. a.	352,9	4,45	145,7	4,73	23,6	4,00
	Chl. b.	79,3	—	30,8	—	5,9	—

During the degradation of leaves, the chlorophylls in all samples decompose considerably. In the species investigated, the degradation of chlorophyll *a* and chlorophyll *b* is of different degree. On the basis of the change in the *a*/*b* ratio of chlorophylls, three types could be separated:

a) Chlorophyll *a* decomposes faster than chlorophyll *b*, therefore the *a*/*b* ratio decreases. This may be observed in the leaves of *Cercis* s. and *Betula pendula*. (Fig. 1).

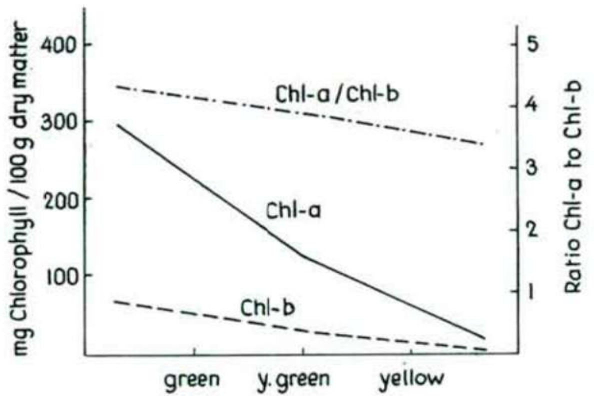

Fig. 1. Change in the chlorophyll content and the a/b ratio in the different leaves of *Cercis* s.

b) Initially, in the withering leaves, the decomposition of chlorophyll *b* is faster, therefore the chlorophyll *a*/*b* ratio at first increases and then decreases. (Fig. 2).

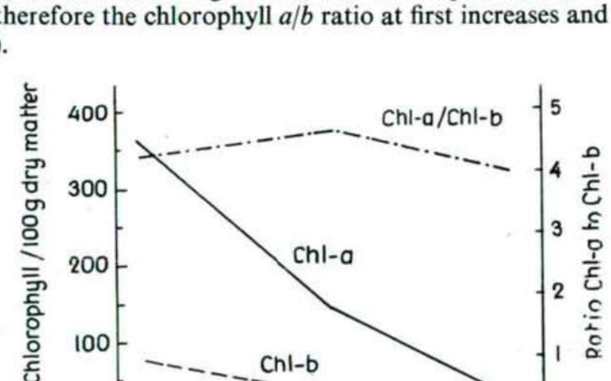

Fig. 2. Change in the chlorophyll content and the *a*/*b* ratio in the leaves of different colours of *Robus idaeus*.

c) Initially, the decomposition of chlorophyll *a* is faster, then it becomes slower in a later phase of withering. Consequently, at first the chlorophyll *a*/*b* ratio decreases, then somewhat increases. (Fig. 3).

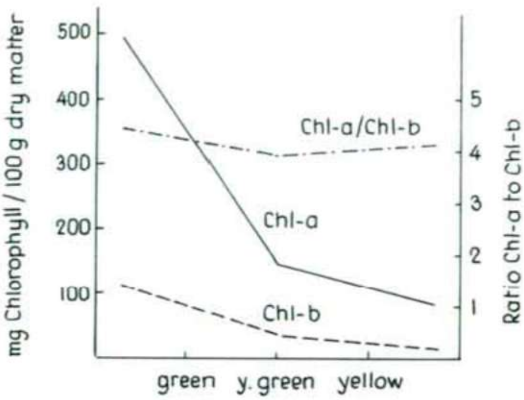

Fig. 3. Change in the chlorophyll content and the *a/b* ratio in the leaves of different colours of *Robinia pseudoacacia*.

In the course of the autumn colouration of leaves, apart from chlorophylls, the change in carotenoids was also measured. We studied mainly the development of the ratio of carotenes, hydro- and epoxi-carotenoids.

1. Most carotenes and hydroxi-carotenes (lutein + zeaxanthin) are contained in the photosynthetically active green leaves. These pigments do not accumulate in the course of withering but decompose. The amount of carotene decreases in approximately direct tario to the decomposition of chlorophyll *b* (Table 2).

Table 2. Change in the amount of carotenes during autumn colouration of leaves. The values are relative to mg carotene/100 g dry matter. The carotene content of the green leaf was taken as 100 per cent.

Species-investigated	green leaf	yellowish-green leaf		yellow leaf	
	mg/100 g	mg/100 g	per cent	mg/100 g	per cent
Cercis s.	32,7	12,8	39,1	3,4	10,4
Betula p.	30,2	12,1	40,1	10,3	34,1
Rubus i.	34,8	13,2	37,9	8,1	23,3
Betula p. v. *purpurea*	41,7	20,1	48,2	12,8	30,6
Robonia p.	66,8	35,5	53,1	27,4	41,0

2. During autumn colouration, the amount of hydroxi-carotenes diminishes less than the carotene content. The degradation of lutein in the leaf of *Betula p.* var. *purpurea* is remarkably small (Tab. 2).

Table 3. Change of the lutein +zeaxanthin content in the leaves of different colours. The values refer to mg pigment/100 g dry matter. The hydroxi-carotene content of the green leaf was taken as 100 per cent.

Species investigated	green leaf	yellowish-green leaf		yellow leaf	
	mg/100 g	mg/100 g	per cent	mg/100 g	per cent
Cercis s.	52,3	40,5	77,4	17,2	32,9
Betula p.	79,0	56,5	71,5	35,2	44,6
Rubus i.	51,0	29,7	58,2	23,6	46,3
Betula p. var. pur.	69,5	51,5	74,1	49,3	70,9
Robinia p.	108,2	64,8	59,9	58,5	54,1

3. The change in the epoxi-carotenes (antheraxanthin, violaxanthin, neoxanthin, during autumn colouration of leaves, takes place in a way characteristic of the single species (Table 4).

a) Most violaxanthin and neoxanthin are contained in the green leaf, and these pigments also decompose gradually. This may be observed in the leaves of *Cercis s., Rubus i., Betula p.,* and *Robinia p.* (Table 4).

Table 4. Change in the amount of epoxi-carotenes during the autumn colouration of leaves. The values refer to mg epoxi-carotene/100 g dry matter. The antheraxanthin, violaxanthin, and neoxanthin content of the green leaf was taken as 100 per cent.

	Pigments	green leaf	yellowish-green leaf		yellow leaf	
		mg/100 g	mg/100 g	per cent	mg/100 g	per cent
	antherax.	6,5	5,1	78,5	2,4	36,9
Cercis s.	violax.	23,4	11,5	49,1	1,9	8,1
	neox.	18,8	11,0	58,5	3,4	18,1
	antherax.	7,2	9,3	129,1	11,9	165,3
Betula p.	violax.	21,0	10,3	49,0	13,7	65,2
	neox.	18,1	11,6	64,1	8,0	44,2
	antherax.	7,8	4,9	62,8	4,7	60,3
Rubus i.	violax.	33,4	12,4	37,1	5,2	15,6
	neox.	17,3	8,9	51,4	5,3	30,6
Betula p. var.	antherax.	3,4	6,9	202,9	11,8	347,0
purpurea	violax.	23,0	29,7	129,1	21,3	92,6
	neox.	10,5	12,6	120,0	8,8	83,8
	antherax.	9,7	10,5	108,2	14,8	152,6
Robinia p.	violax.	35,5	27,2	76,6	19,4	54,6
	neox.	24,5	24,3	99,2	17,9	73,1

b) Antheraxanthin accumulates in the yellowish-green and yellow leaves of the *Betula p.* var. *purpurea, Betula p.,* and *Robinia p.* species.
c) The total epoxi-carotene accumulates in the yellowish-green leaves of *Betula p.* var. *purpurea.*

Discussion of results

Willstätter and STOLL (1918) found that in thel leaves should precede „in the autumn" the ratio of chlorophyll *a/b* does not change. Sevaral authors, however, namely: RUDOLF (1934), NAGEL (1940), SEYBOLD (1943), EGLE (1944) Jeffrey and GRIFFITH (1947), STRAIN (1949), WOLF (1956), GOODWIN (1958), established that chlorophyll *a* decomposes faster than chlorophyll *b,* in green leaves as a result of various conditions. SANGER (1971) demonstrated that in the leaves of *Corylus americana, Populus tremuloides,* and *Quercus ellipsoidalis* the chlorophyll *a/b* ratio falls in autumn.

The different rates of chlorophyll destruction are explained by EGLE (1944) and GOODWIN (1958) by the different sensitivities to acid. According to them chlorophyll *a* decomposes fast er because it is less resistant to the acid environment than chlorophyll *b* is.

Our results partly differ from the findings of the above authors. Chlorophyll *b* can also decompose faster than chlorophyll *a* does. Therefore, the chlorophyll *a/b* ratio may rise, too during the autumn colouration of leaves. (Cf. in Table: *Betula p. var. purpurea* and *Rubus i.*).

Apart from a few exceptions, it seems to be common however that in autumn chlorophyll *a* decomposes faster than chlorophyll *b* does. We cannot accept completely the different sensitivity to acid for an explanation of the different decomposition.

The chlorophyll *a* molecule in solution (ether, alcohol, acetone) decomposes faster under the influence of light than does chlorophyll *b* (MARÓTI, 1970). The autumn colouration of leaves is connected with the destruction of the thylacoid membrane. It follows from the different light sensitivity of the chlorophylls released that in the majority of plants chlorophyll *a* decomposes faster.

In the leaves of *Rubus i.* and *Betula p.* var. *purpurea* in some red pigments, anthocyans, accumulate during the autumn colouration of leaves. The leaves, therefore, do not bacome yellow, but red. It is to be supposed that one of the tasks of the anthocyans is to protect chlorophyll *a* from the light-induced desctruction.

In case of Robinia p. we consider it possible that part of the chlorophyll *a* is morestrongly connected to the thylacoid membrane, slowing its rate of decomposition a while.

We investigated the pigment content of coloured leaves for three autumns (1972, 1973, 1974). We observed two types of chlorophyll decomposition: pheophytinic decomposition, and the decolourization of chlorophylls.

a) Pheophytinic decomposition of chlorophylls

If the temperature reaches even a value of -2 °C in the small hours of the morning, then ice-crystals from in the cells. These destroy the membranes, and permit the cellfluid of low pH to get into the plastids. The fall-ont of Mg from the chlorophyll, and thus the formation of pheophytin, is promoted by the H^{+}-ions. On the other hand, it is known on the basis of the investigations of KRINSKY (1966), SAPOZHNIKOV (1969), MARÓTI and SZAJKÓ (1972) that in the dark (at night) mainly epoxidative reactions take place. These already become slower at 15 °C ,and at about 0 °C they are completely hindered. As the carotenoids are not epoxidized in the cold, they cannot protect the chlorophylls against photodestruction.

b) Autumn decolourization of chlorophylls

In case of a long, mild autumn (with 15 °C to -1 °C temperature in the small hours of the morning (the electrontransport chain between the two photochemical systems (supposedly at the cytochromes) may be blocked. The photolysis of water goes on, the conjugated double-bonds of the chlorophylls are saturated by the „H-atoms" produced, and the oxygen favours the accumulation of epoxi-carotenes.

Pecularities of the decomposition of carotenoids

In the autumn leaves the decomposition of chlorophylls may occur some days earlier, and to a more considerable degree, than that of the carotenoids. In case of the five studied species, carotene is the most sensitive and follows the decomposition of chlorophyll the fastest. According to STRAIN (1959), during the autumn colouration in the leaves, the amount of carotenes falls. As a result of oxidation these are transformed into hydroxi- and epoxi-carotenoids. The high level of lutein in the greenish-yellow leaves could be explained by the facts discussed above.

According to SANGER (1971), neoxanthin and violaxanthin are the most sensitive and in Autumn they decompose the fastest. Our results, however, do not support this conclusion. In the leaves of BETULA P. var. PURPUREA and ROBINIA P. only epoxi-carotenes accumulate. Supposedly the epoxidation of hydroxicarotenes causes the rise of, or the lesser fall in, antheraxanthin, neoxanthin, and violaxanthin.

In the transformation of carotenoids the peculiarities of species and climatic factors are mostly unknown. This may be the cause of the difference between the literary data.

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