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THE EFFECT OF AMITROLE ON THE PIGMENT COMPOSITION AND ULTRASTRUCTURE OF CHROMOPLASTS OF TULIP TREE FLOWERS

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The effects of the bleaching herbicide amitrole on the carotenoid composition and ultrastructure were studied during the transformation of chloroplasts into chromoplasts in the tulip tree *(Liriodendron tulipifera* L.). Amitrole inhibited the accumulation of β-carotene and of an unidentified carotene. The biosynthesis of xanthophylls was similar to that in the control. Amitrole caused the appearance of long closely packed unbranched tubules in some zones of the reticulum. At the last stages of flower development in the chromoplasts treated only plastoglobules with small carotenoid crystaloids remained. The interaction between chromoplast carotenoid composition and ultrastructure is discussed.

Introduction

Amitrole is a bleaching herbicide which has been reported to interfere with carotenoid biosynthesis in higher plants (Wolf 1960, Ashtakala et al. 1989). It inhibits both light and dark carotenoid accumulation (Burns et al. 1971), but according to some authors this inhibition is lower in the dark. On the other hand, amitrole does not inhibit protein biosynthesis (Browen and Carter 1968) in the dark and has a low effect in the light (Feierabend et al. 1979) attributable to the blocking 70S ribosomes assembly (Bartels et al. 1967).

Ultrastructural studies of various plants treated with amitrole reveal that it interferes with chloroplast development by inhibiting the membrane formation (Bartels and Weier 1969, Vrhovec and Wri-

scher 1970, Wrischer et al. 1992). However, the action of amitrole on the ultrastructure and carotenoid composition of chromoplasts has been poorly investigated so far.

Among different types of chromoplasts the rarest and quite inadequately investigated are the reticulo-tubulous ones (Ljubešić 1979). In petals of the tulip tree there are chromoplasts with typical reticulo-tubular structure. The present study investigated the effect of the bleaching herbicide amitrole on the formation of ultrastructures of chromoplasts and on carotenoid biosynthesis, by electron microscopy and pigment analysis.

Materials and Methods

Investigations were carried out on the inner proximal part of the petal green, yellow or orange zone of the tulip tree flower (*Liriodendron tulipifera* L.). The flowers were treated with the bleaching herbicide amitrole (3-amino-1H-1, 2, 4-triazole, Fluka) at the bud stage. A 1 m*M* solution of the herbicide was injected into the central cavity of the bud and 10 to 15 days later the treated material was sampled. Buds injected with pure distilled water (control) did not show any perceivable changes during the experiment.

Small pieces of treated and untreated tulip tree petal tissue were fixed in cold 2% glutaraldehyde in cacodylate buffer (pH 7.2) for 30 minutes, washed several times in buffer and postfixed in 1% buffered osmium tetroxide for 1 hour. The material was dehydrated in graded ethanol series, and embedded in Araldite. It was cut on a Reichert Om U2 ultramicrotome and stained with uranyl acetate and lead citrate. The sections were examined with an Opton EM 10A electron microscope.

For pigment analysis the material was cut into small pieces, mixed with a small amount of $BaCO_3$ and quartz sand and ground in 100% acetone in a mortar to extract the pigments. For quantitative analysis, the carotenoids obtained were separated by thin-layered chromatography on silica gel G plates with a mixture of petrol ether (60—80°C) : ethyl acetate : diethylamine (58 : 30 : 12) as solvent. The bands were eluted with 80% acetone. The absorbance for chlorophylls at 663 nm and 645 nm and for carotenoids at 450 nm was measured with a Specol 10 (Zeiss, Jena) spectrophotometer.

Results

Macroscopic observations

Control. The petals of the tulip tree had a 1—2 cm long intensively coloured zone on its inner side. In the bud this zone was pale green. During the flower development (about 10 days) this zone changed into yellow-green, and later became intensively orange in the control flowers.

Amitrole. The coloured zone of the petals in treated flowers did not show any marked changes in comparison with untreated flowers. Only the orange colour was less intense than in the control. However, the period of flowering was considerably longer — about 15 days.

Pigment analysis

Control. The pale green zone of the petals from the bud contained chlorophylls and carotenoids as in normal photosynthetic tissue (Table I). During the development of the flower the chlorophylls disappeared and the amount of carotenoids rapidly increased, especially the β-carotene and an unidentified carotene (Table I). This unidentified carotene was of a red-orange colour, and on the thin layer chromatography it showed an Rf value of about 0.85.

Amitrole. The chlorophylls disappeared during the development of both the treated flowers and the untreated ones. At the same time the concentration of carotenoids increased, but reached only 33.8% of the amount in the control flowers. The highest inhibition occured in the biosynthesis of an unidentified carotene (the content equaled only 25% of that in the control); in the biosynthesis of the β-carotene it was still somewhat lower (31% of control). At the same time the amount of the xanthophylls remained equal to that of untreated flowers (Table I).

Table 1. Content of carotenoids and chlorophylls in petals during the development of untreated (control) and treated (1 mM amitrole) tulip tree flowers. Values expressed as mg/g fr. wt.

		Control		Amitrole	
	Bud	Young flower	Old flower	Young flower	Old flower
Totai carotenoids	0.0333	0.1777	0.1894	0.0430	0.0640
β-carotene	0.0087	0.1543	0.1337	0.0249	0.0419
Unidentified carotene	—	0.0113	0.0335	0.0063	0.0084
Xanthophylls	0.0246	0.0121	0.0222	0.0116	0.0137
Chlorophylls	0.011	—	—	—	—

Ultrastructure of plastids

Control. The pale green petals in the flower bud contained chloroplasts which were not yet fully differentiated (Fig. 1). These chloroplasts were small and contained a reduced thylakoid system with only few grana. In the stroma few small plastoglobules could be observed. The change in the tissue colour corresponded to the disappearance of grana and stroma thylakoids and in the appearance of numerous branched and curved tubules, which formed a large network (reticulum) (Fig. 2). Plastoglobules increased in number and size and were located uniformly among the tubules of the network. Very large starch grains could be observed in chromoplasts. Parallel with flower development the network of tubules desintegrated and small crystals of carotene (probably β-carotene) grew out of plastoglobules (Fig. 3). In section the shape of these crystals was rhomboidal.

Amitrole. The process of chloroplast transformation into chromoplasts in treated flowers was very similar to the same processes in the control during the first stages. Thylakoids disappeared and numerous tubules were formed. However, in addition to the normal network of branched tubules, areas with long unbranched tubules existed. These tubules were closely packed forming long bundles of more or less parallel and partially straight tubules. In cross sections such bundles often showed a very regular arrangement of tubules (Figs. 4, 5). In such areas with long and unbranched tubules there were no plastoglobules.

When the flowers developed, all tubular structures of chromoplasts desintegrated and only plastoglobules remained. In these plastoglobules the crystallization of carotenes began, but the morphology of these crystaloids is quite different from those in untreated chromoplasts. They grew out from the inside of plastoglobules and they did not have the characteristic rhomboidal shape (Figs. 6, 7). Sometimes they looked like fibriles or crystaloids with sharp ends.

Discussion

The results show that the treatment with amitrole induces considerable changes of carotenoid accumulation and composition in the tulip tree petals, although macroscopically there were no marked visible effects. The amount of total carotenoids in treated tulip tree flowers was 3-times lower than in the control. This observation is in accordance with the study of Wrischer et al. (1991) on chromoplasts of *Calceolaria* flowers. However, in *Calceolaria* chromoplasts the amitrole strongly inhi-

Fig. 1. Chloroplast from the green zone of untreated tulip tree petal. The plastid contains small grana. 30,000 : 1.

Fig. 2. Chromoplasts from the orange zone of untreated tulip tree petal of a fully grown flower. The reticulum is well developed, large starch grains and numerous plastoglobules are present. 30,000 : 1.

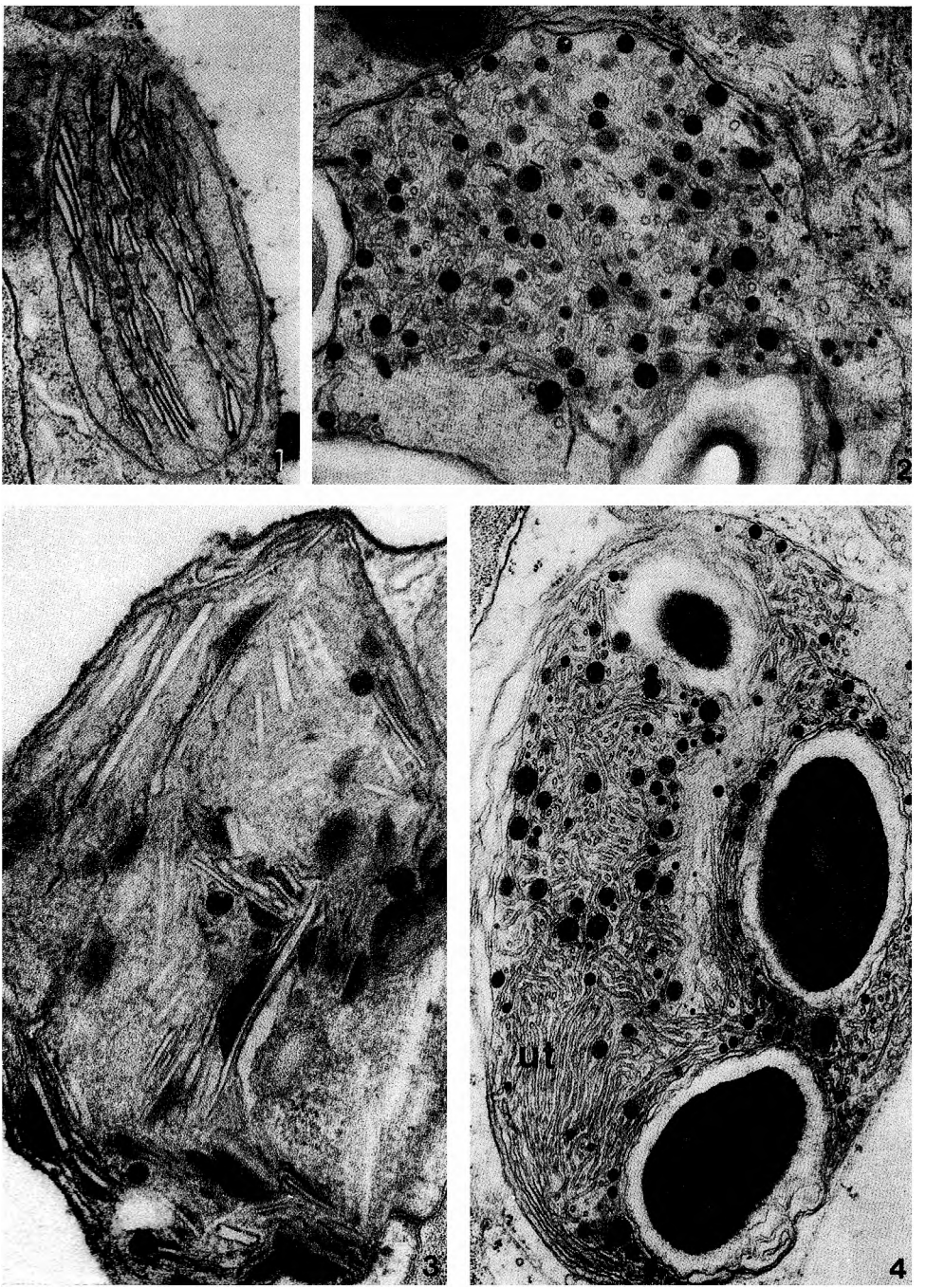
Fig. 3. Chromoplast from an old fully developed untreated petal flower showing numerous carotene crystals in addition to plastoglobules. 72,000 : 1.

Fir. 4. Chromoplast from a fully developed flower treated with amitrole. In the typical reticulum there is a zone with long unbranched tubules (ut). 30,000 : 1.

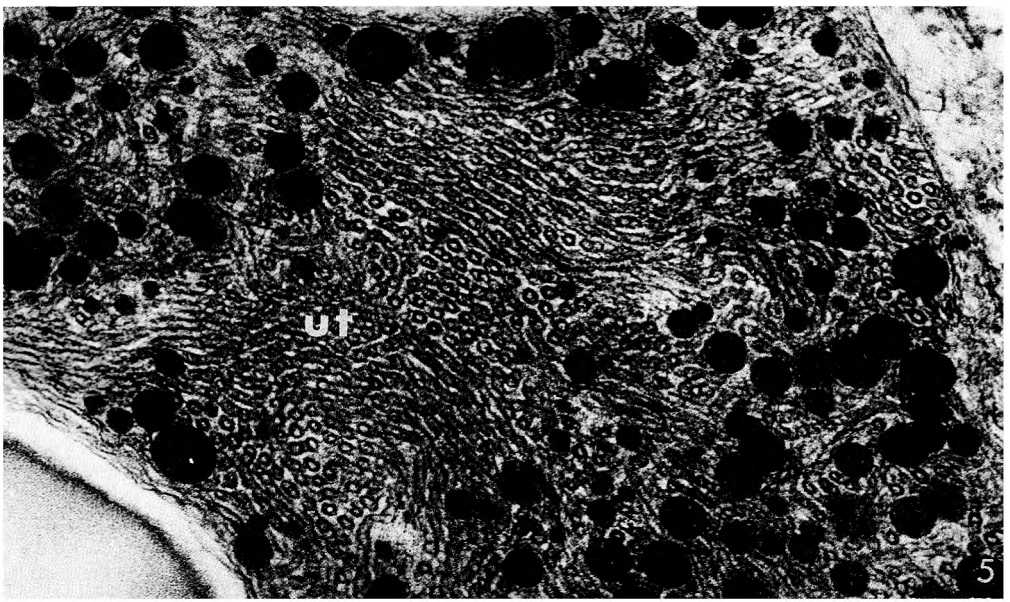
Fig. 5. Chromplast from a fully developed flower treated with amitrole. The bundle of closely packed unbranched tubules (ut) in cross section. 66,000 : 1.

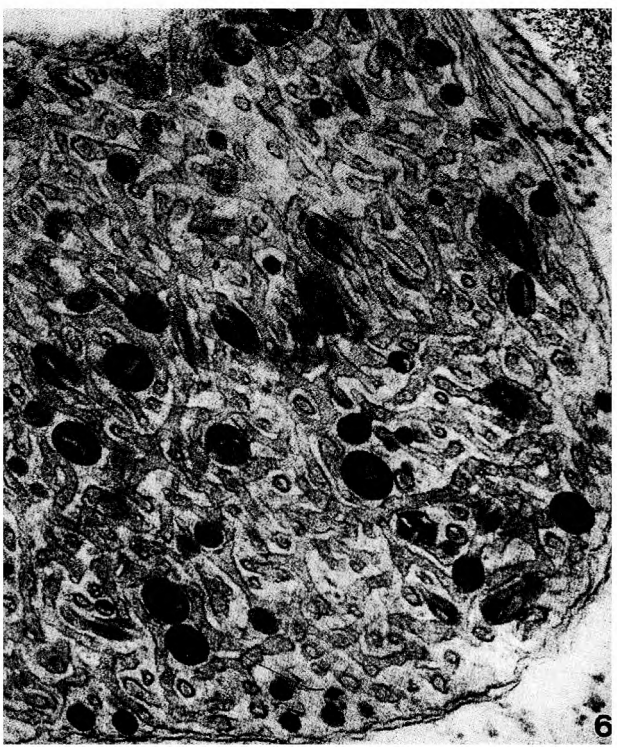
Fig. 6. Chromoplast from a fully developed flower tretted with amitrole. Among the tubules of the reticulum plastoglobules with small crystaloids of carotenoid appear, 64,000 : 1.

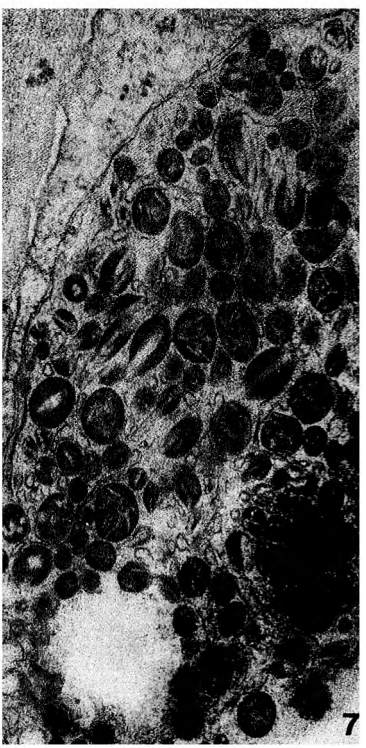
Fig. 7. Part of a chromoplast from an old fully developed flower treated with amitrole. The tubules disappear and numerous (probably carotenoid) crystals are formed inside the plastoglobules, 42,000 : 1.



Figs. 1—4.







Figs. 5—7.

bited the biosynthesis of xanthophylls, while the amount of carotene was unaffected. In tulip tree it is quite the opposite. The carotene biosynthesis is inhibited and the accumulation of xanthophylls is unchanged. In our opinion this disagreement is a consequence of quite different ultrastructures of these chromoplasts. The chromoplasts in *Calceolaria* flowers contain only the so called CIM-s (chromoplast internal membranes, Liedvogel et al. 1976). The chemical structure of the CIM-s and that of the tubules of the reticulum in tulip tree chromoplasts are completely different.

It is interesting that the carotenoid inhibition is remarkably lower in chloroplasts (Buschmann et al. 1980, Feierabend et al. 1982) than in chromoplasts. The sublethal concentration of amitrole (1 m*M*) lowered the accumulation of the total carotenoid in chloroplasts to 10—50%. The stronger inhibition of carotenoid accumulation by amitrole in the light (Wolf 1960, Burns et al. 1971, Buschmann et al. 1980) is well documented. Our exprimenets were carried out in sun light. Especially in the last strages of flower development the petals were exposed to direct sun light. We believe that light intensity has not great influence on the intensity of carotenoid inhibition in amitrole treated chromoplasts of the tulip tree. The rates of inhibition in treated buds or young flowers (in which the observed zone was still in the shade), and in fully developed old flowers (where the investigated zone was in full sun light) are similar.

During the development of amitrole treated tulip tree flowers the process of chlorophyll disappearance was very similar to that of the control. In amitrole treated material photodestruction of chlorophylls (Ashtakala et al. 1989) as a consequence of the lack of the protective role of carotenoids was not present, because during the period of chlorophyll destruction in the closed bud, the petals were protected from strong light.

Our results indicate that the sublethal concentration of the amitrole applied does not drastically affect the morphology of the tubular reticulum. The tubules were only partly unbranched and we suppose that their chemical composition was possibly somewhat changed. In chromoplasts of tulip tree treated with SAN 9789 (Hloušek-Radojčić and Ljubešić 1985) the amount of unidentified carotene was very high and in these chromoplasts there was no tubular reticulum, but there were only large quantities of big plastoglobules. After the amitrole treatment the accumulation of unidentified carotene was strongly inhibited. At the same time in these chromoplasts the number of plastoglobules was reduced, because the regions of the reticulum with closely packed unbranched tubules were practically without plastoglobules. According to these data we conclude that the unidentified carotene is probably directly connected with plastoglobules. In these plastoglobules crystals formed in the last stages of chromoplast development. However, the shape of these crystals in chromoplasts treated with amitrole and SAN 9789 was not identical with that in the control. This fact indicates that changes in the carotenoid composition of plastoglobules occur, and they will be the subject of further investigations.

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SAŽETAK

UTJECAJ AMITROLA NA SASTAV PIGMENATA I ULTRASTRUKTURU CVJETOVA TULIPANOVCA

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Istražen je utjecaj herbicida amitrola na sastav karotenoida i finu građu tijekom pretvorbe kloroplasta u kromoplaste u cvjetovima tulipanovca (*Liriodendron tulipifera* L.). Amitrol srpečava nakupljanje β-karotena i nepoznatog karotena, dok je biosinteza ksantofila jednaka kao u kontroli. Amitrol uzrokuje pojavu dugih gusto poredanih nerazgranjenih tubula u pojedinim područjima retikuluma. U posljednjem stadiju razvitka tretiranih cvjetova preostaju u plastidima samo plastoglobuli sa sitnim kristaloidima u njima. Raspravljena je međusobna povezanost sastava karotenoida i fine građe kromoplasta.

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