

THE EFFECT OF AMITROLE ON THE FINE STRUCTURE OF DEVELOPING CHLOROPLASTS

Mit deutscher und kroatischer Zusammenfassung
Sa sadržajem na njemačkom i hrvatskom jeziku

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Amitrole (3-amino-1, 2, 4-triazole) strongly affects developing plastids (Bartels 1964, Signol 1965, Wrischer and Vrhovec 1969). In leaf tissue it provokes chlorosis (for literature review see Kirk and Tilney-Bassett 1967). The effect of amitrole on the fine structure of leaf chloroplasts has been already described by several authors (Jacobson and Rogers 1961, Signol 1965, Guillot-Salomon 1966, Bartels 1969). During our experimental work several new data concerning this subject were noticed and are described in the present work. Different plants were used in the experiments; the mode of the injury was however always the same.

Material and Methods

The plants were cultivated under light either from the seeds, as *Zea mays* (cv. »DH«) and *Phaseolus vulgaris* (cv. »Butterfisol«), or were kept in aquaria as *Elodea canadensis*, *Elodea densa* and *Wolffia arrhiza*. The plants were treated with a 10^{-4} , 10^{-3} or 10^{-2} M solution of amitrole for 1 or 15 hours.

The plants were used for light and electron microscopic investigations at different time intervals after the chlorosis had appeared in leaves.

Whole leaves — as in *Elodea* — or hand cut sections of unfixed material were used for light microscopic investigations.

For the electron microscopy portions of leaves were fixed in 1% glutaraldehyde, postfixated in 1% OsO_4 and embedded in araldite. Ultrathin sections were cut with a Reichert Ultramikrotom OmU2, stained

with lead citrate (Reynolds 1963) and examined in a Siemens Elmiskop I.

Results

Our experiments have confirmed earlier statements that amitrole affects only the plastids in differentiating tissues.

Light microscopic investigations have shown that the number of plastids in chlorotic tissue is considerably lowered and that the plastids are smaller and less green than in the control. When the leaf chlorosis is not strong, damaged plastids can occur among the normal ones in the same cell. In damaged plastids red, probably carotenoid, crystals are rather conspicuous. They appear in form of needles or rhombs (Fig. 1). The crystals are dissolved during dehydration of the tissue in ethanol or acetone. For this reason they cannot be seen in the electron microscope, their location representing probably empty vacuoles with sharp edges, which are sometimes found in the plastid stroma (Fig. 2).

The fine structure of the plastids in chlorotic leaf tissue is considerably altered. Well developed grana, as are seen in the chloroplasts of untreated leaves (Fig. 3, 4), are very rare. Several very long thylakoids are very often stuck together in such a way that the intra- and inter-thylakoidal spaces can entirely disappear. In such cases the individual membranes could not be distinguished any more (Fig. 6). Only the intrathylakoidal spaces of the outer thylakoids in a granum are sometimes dilated (Fig. 7). Individual vesicles, derived possibly from the disintegrated lamellar system, are also seen in some damaged plastids (Fig. 2). Some of the thylakoids, which are stuck together and have blurred outlines, show a very fine striation in cross sections with a periodicity of about 12—13 nm. The striation is oriented more or less perpendicularly to the length of the thylakoids (Fig. 8, 9). On very thin sections in such structures, when they are cut planely or slightly obliquely, spherical particles (measuring 7—8 nm in diameter) are arranged in a very regular hexagonal pattern. The spaces between the centres of the individual particles measure 12—13 nm (Fig. 10). Oblique sections show clearly the transitions between the hexagonal arrangement of globular particles and the striation (Fig. 10), thus proving that both structures are identical.

Viewed in the light microscope, the plastids in the chlorotic parts of amitrole treated *Elodea* leaves contain, besides red carotenoid crystals (which do not exhibit any fluorescence), greenish lamellar formations which show a red fluorescence. They are doubtlessly identical with the lamellae which are stuck together and were observed in the electron microscope.

The stroma of the damaged plastids contains very few ribosomes. Here and there cloud-like aggregations of somewhat darker material are visible (Fig. 6).

The fine structure of the chloroplasts, which were treated by amitrole when already fully grown, never shows any alterations. In *Elodea* unusually big starch grains were noticed in the chloroplasts.

In the meristematic leaf tissue the proplastids are not affected by amitrole either. This was clearly seen when the plants were examined several weeks after the end of the treatment, so that the parts of the leaf — which during the treatment were in the meristematic stage — could grow out. Such tissues resemble those of the untreated leaves both macroscopically and in their fine structure (Fig. 5).

Amitrole affects only the plastids and even those only during their differentiation. Alterations in other cell organelles have never been observed.

Discussion

Our experiments have confirmed earlier data that amitrole affects only the differentiating plastids (Linser and Kiermayer 1957, Bartels 1964, Signol 1965, Guillot-Salomon et al. 1967, Wrischer and Vrhovec 1969). Chloroplasts from the already green leaves, as well as proplastids of the meristematic tissue, are never affected.

Carotenoid crystals in the plastids of chlorotic, amitrole treated leaves have not been described before. Parallely with the strong reduction of proteins (Bartels 1964), and polar lipids (Guillot-Salomon et al. 1966) the synthesis of chlorophylls and carotenoids is also strongly inhibited by amitrole, although some unusual precursors of carotenoids were detected (Guillot-Salomon et al. 1967). The appearance of the carotenoid crystals could probably be linked with these phenomena.

The disappearance of the true grana, noticed in amitrole treated plastids (Bartels et al. 1969), is accompanied by the appearance of very long thylakoid sheaths, which are stuck together (Guillot-Salomon et al. 1967). Such phenomena are not limited only to the effect of amitrole. They were observed e.g. also in an *Oenothera* hybrid with the disharmony between the genom and the plastom (Schötz and Diers 1968).

Spherical particles arranged in a very regular hexagonal pattern, which have been noticed in some thylakoids stuck together are unusual, although spherical particles were detected in thylakoids of normal chloroplasts in freeze-etched material (Mühlethaler 1966), as well as in isolated, negatively stained thylakoids (Park and Biggins 1964). Bronchart (1967) has shown pictures of isolated thylakoids, where in some places spherical particles — having a diameter of 9 nm — are arranged in a hexagonal pattern. Bronchart interpretes such particles as a part of the so-called quantasome (Park and Biggins 1964). Regular arrangement of spherical particles in the thylakoid membranes of normal leaf chloroplasts has not been observed in the sections before, although some authors claim that the thylakoid membranes are built up of spherical particles (Weier et al. 1965, Hohl and Hepton 1965). According to the findings of our light microscopic studies, the thylakoids which are stuck together still contain some chlorophyll, because they show red fluorescence. As in such damaged plastids the synthesis of many chemical constituents (e.g. the polar lipids, Guillot-Salomon et al. 1966) is drastically inhibited, it seems probable that during the differentiation some constituents — which normally fill up the places between the spherical particles giving smooth appearance to the normal thylakoids — fail to be synthesised.

The blurred outlines of such structures remind us somewhat of the pictures of grana-lysis in ripening fruits of *Capsicum annuum* shown by Spurr and Harris (1968), although these authors could not observe hexagonally arranged particles in such structures. It is sure,

that in our case the structures, which bear the hexagonally arranged particles, represent an advanced stage of chloroplast injury. Whether the regular arrangement of particles in these structures is the direct consequence of the injury caused by amitrole, or whether such particles are only better visible after this treatment because other substances are missing in the membranes, is unknown as yet.

It may be of interest to note that quite similar pictures, showing a fine striation of some stacks of lamellae, were recently observed in ripening chromoplasts of pumpkin fruit (Devidé 1970).

The disappearance of the ribosomes from the injured plastids is in accordance with the findings by Bartels et al. (1967, 1969) that amitrole causes complete disappearance of the chloroplastic ribosomes.

The accumulation of starch observed in green parts of the *Elodea* leaves could perhaps be related to similar results found by Castelfranco and Bisalputra (1965) on *Scenedesmus* cells treated with amitrole.

Summary

Growing plants of *Zea mays*, *Phaseolus vulgaris*, *Elodea canadensis*, *Elodea densa* and *Wolffia arrhiza* were treated with 10^{-4} to 10^{-2} M solutions of amitrole and investigated by light and electron microscopy at different intervals after chlorosis appeared in the leaves.

It has been shown that only those plastids were affected by amitrole which were in the stage of differentiation during the treatment.

In addition to changes already known the light microscope revealed characteristic crystals of carotenoids in damaged plastids.

In the electron microscope considerable changes in the ultrastructure have been found. Instead of normal grana there appear long lamellae stuck together. Some of them show a clear striation (period: 12—13 nm) in cross section. In plane sections this striation has been shown to be identical with hexagonally arranged globular particles (diameter 7—8 nm). The possible relations of this structure to the molecular organisation of the normal thylakoids and other details are discussed.

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ZUSAMMENFASSUNG

DIE WIRKUNG VON AMITROL AUF DEN FEINBAU JUNGER CHLOROPLASTEN

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Junge Pflanzen von *Zea mays*, *Phaseolus vulgaris*, *Elodea canadensis*, *Elodea densa* und *Wolffia arrhiza* wurden mit 10^{-4} bis 10^{-2} M Lösungen von Amitrol behandelt und nach dem Auftreten der Chlorose in verschiedenen Zeitabschnitten lichtmikroskopisch und elektronenmikroskopisch untersucht.

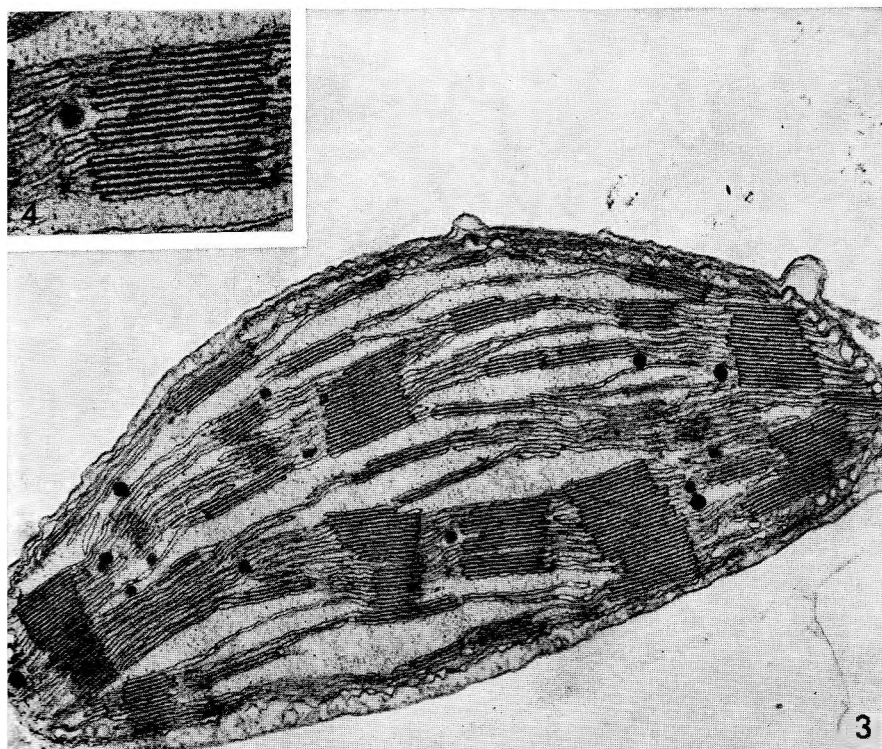
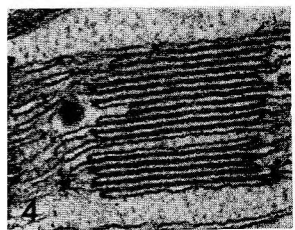
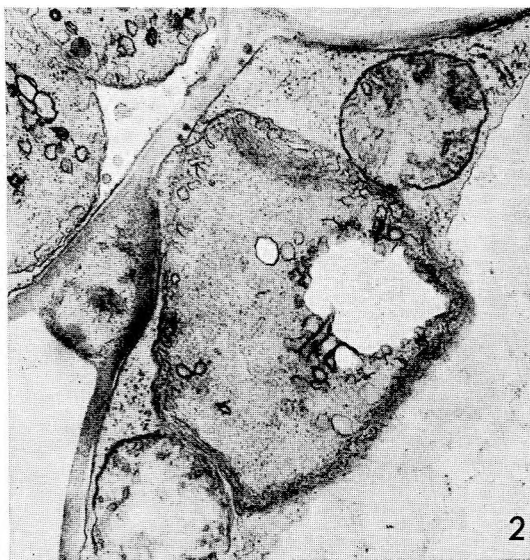
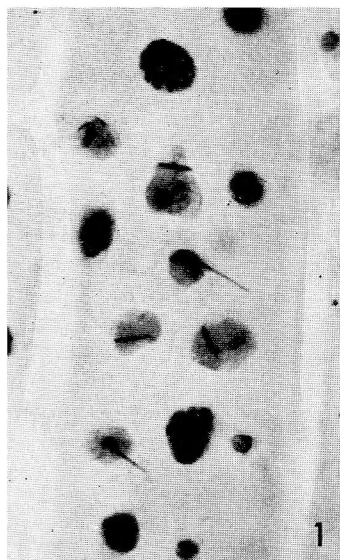
Die vorliegenden Untersuchungen haben gezeigt, dass nur jene Plastiden angegriffen werden, die sich während der Behandlung im Stadium der Differenzierung befinden.

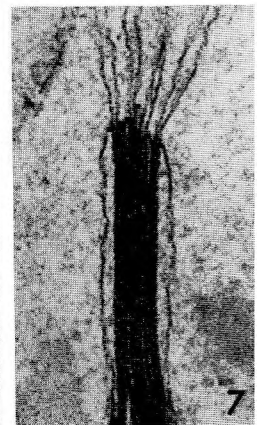
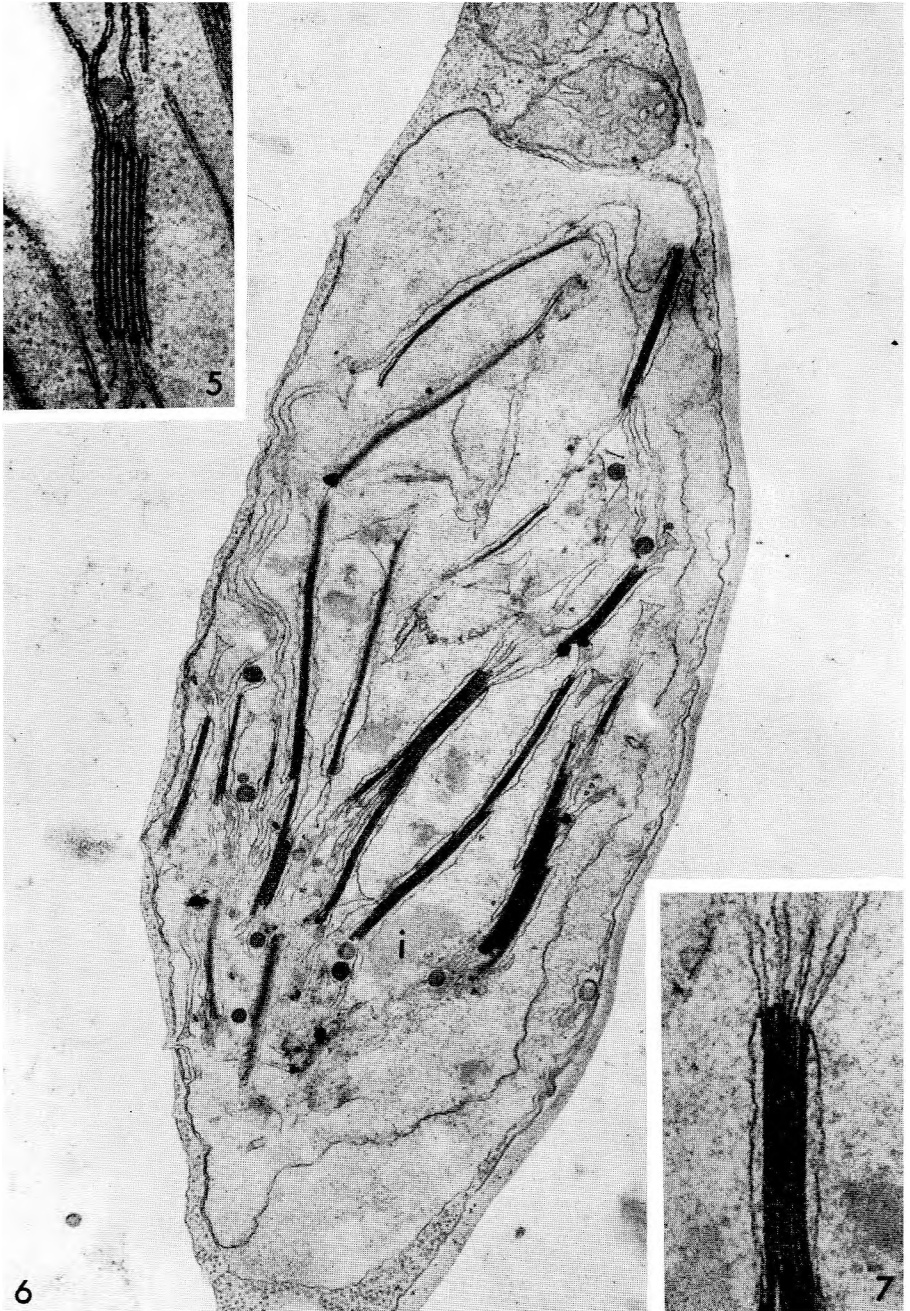
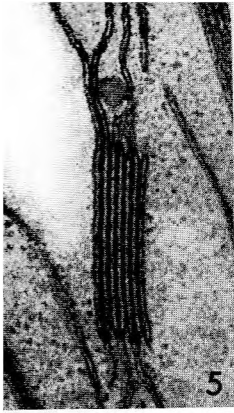
Neben den bereits bekannten Veränderungen konnten im Lichtmikroskop in geschädigten Chloroplasten charakteristische Carotinoid-Kristalle festgestellt werden.

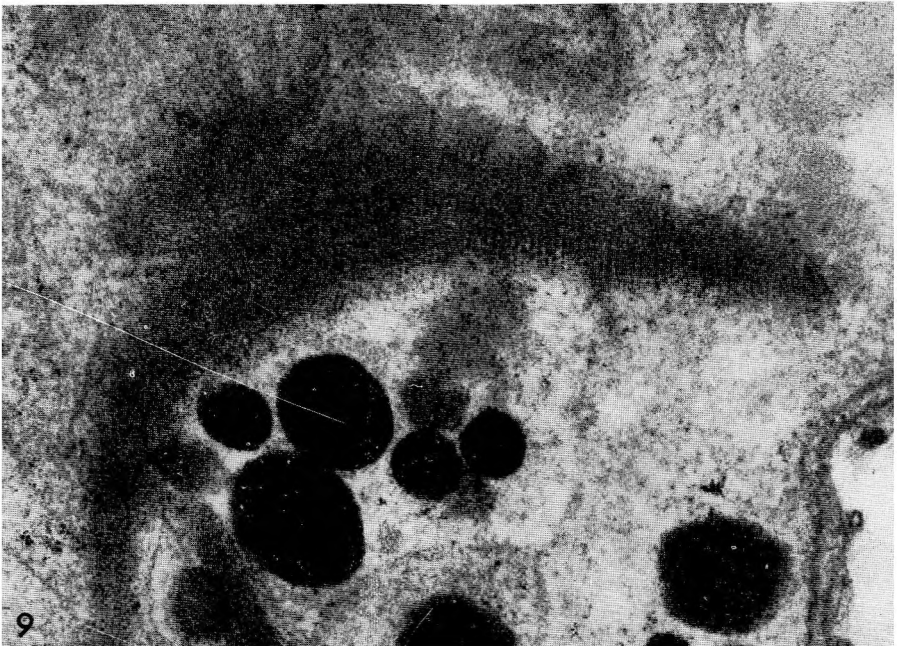
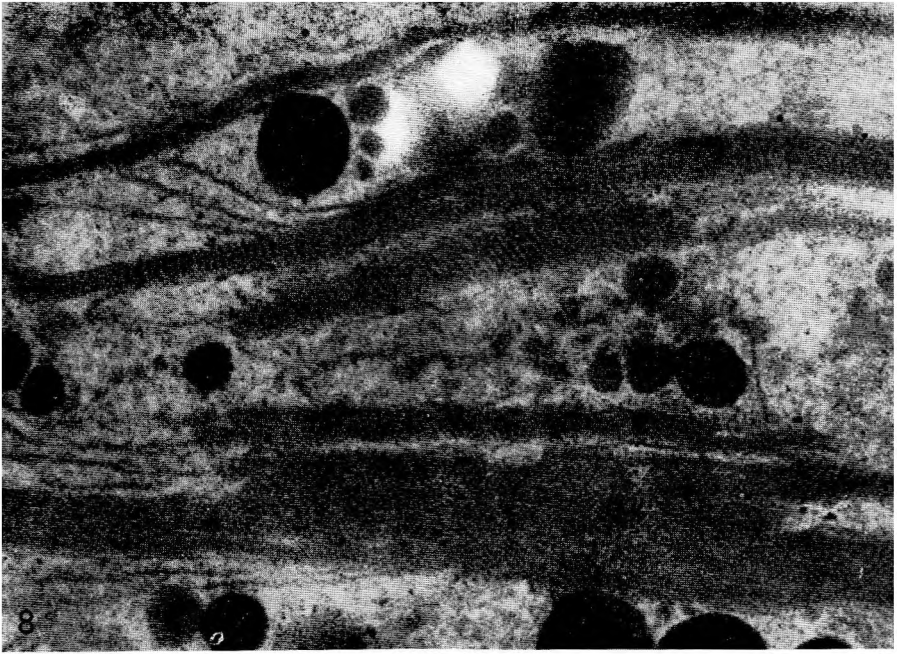
Das Elektronenmikroskop zeigte wesentliche Veränderungen im Feinbau der Plastiden. Statt der normalen Grana erscheinen lange zusammengeklebte Lamellen. Einige dieser Lamellen zeigen im Schnitt eine feine Streifung (Periode 12 — 13 nm). An flachgeschnittenen Lamellen sieht man, dass diese Streifung durch Überlagerung von hexagonal angeordneten kugelförmigen Teilchen (Durchmesser 7 — 8 nm) zustande kommt. Die möglichen Beziehungen dieser Struktur zur makromolekularen Organisation der normalen Thylakoiden und andere Einzelheiten werden diskutiert.

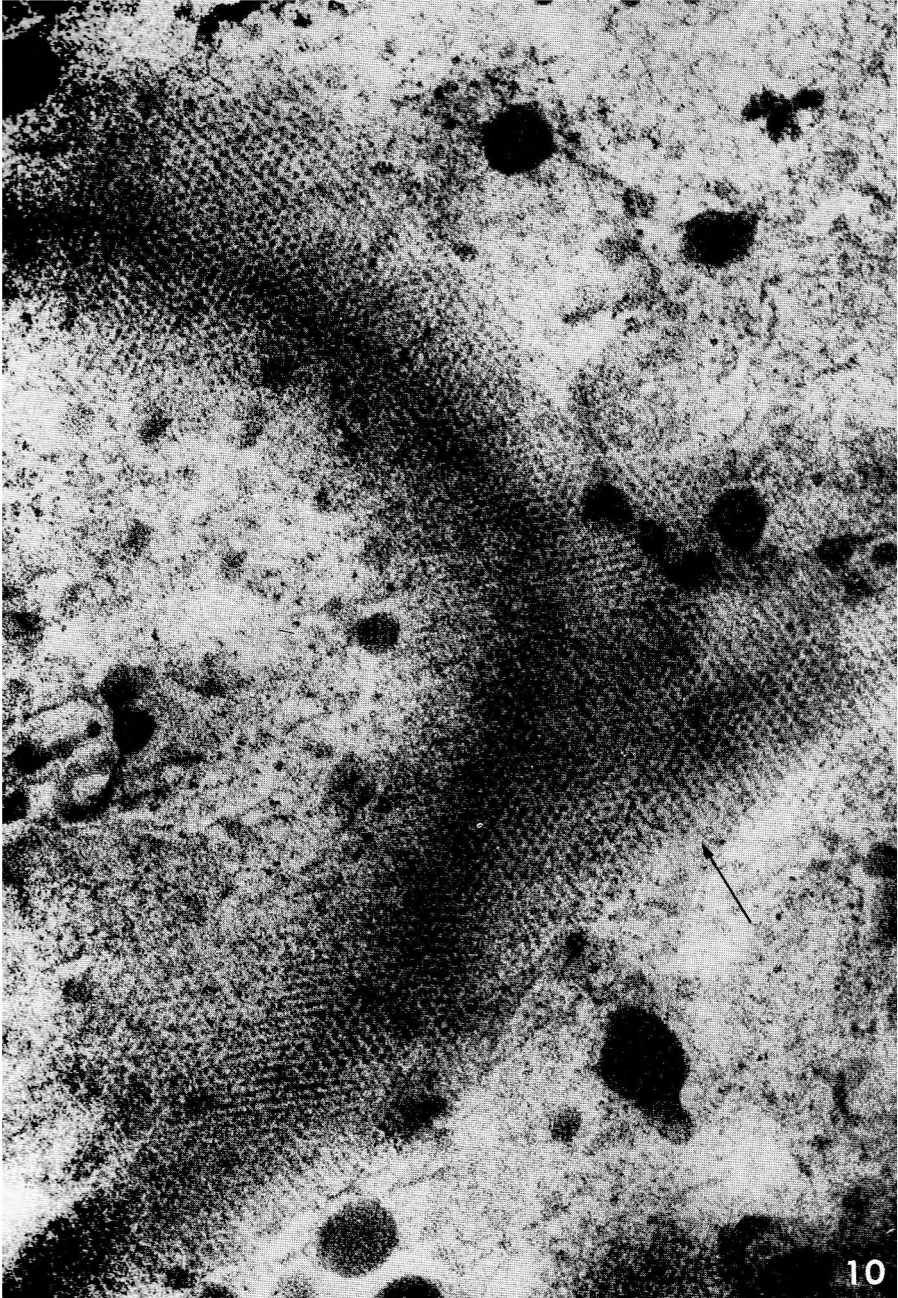
Explanation of figures

- Fig. 1. *Elodea densa*, leaf. 10^{-4} M amitrole 1 hour, photographed 19 days after the end of the treatment (photomicrograph). In some damaged plastids rod-shaped crystals are visible. 1150 : 1.
- Fig. 2. *Zea mays*, leaf. 10^{-3} M amitrole 15 hours, fixed 30 days after the end of the treatment. In the plastid a vacuole with sharp edges is visible. 19 000 : 1.
- Fig. 3. *Zea mays*, chloroplast from an untreated leaf (control). 24 000 : 1.
- Fig. 4. Portion of the chloroplast from Fig. 3 showing a granum. 48 000 : 1.
- Fig. 5. *Phaseolus vulgaris*, green (secondary) leaf. 10^{-3} M amitrole 15 hours, fixed 12 days after the end of the treatment. A granum is visible. 60 000 : 1.
- Fig. 6. *Phaseolus vulgaris*, primary (chlorotic) leaf. 10^{-3} M amitrole 15 hours, fixed 7 days after the end of the treatment. The thylakoids are stuck together. In the stroma cloudy inclusions (↑) are visible. 25 000 : 1.
- Fig. 7. Portion of the plastid from Fig. 6 with thylakoids which are stuck together. 60 000 : 1.
- Fig. 8. *Elodea canadensis*, leaf 10^{-4} M amitrole 1 hour, fixed 21 days after the end of the treatment. A fine striation in the thylakoids, which are stuck together, is visible. 100 000 : 1.
- Fig. 9. The same material as in Fig. 8. 100 000 : 1. (Note the globular structure!)
- Fig. 10. *Elodea canadensis*, leaf. 10^{-4} M amitrole 1 hour, fixed 14 days after the end of the treatment. A regular, hexagonal arrangement of particles is visible in plane section through the thylakoids which are stuck together. The transition to the striation pattern is visible in regions where membranes are bent, so that they lie obliquely to the plane of section (↑). 125 000 : 1.









SADRŽAJ

DJELOVANJE AMITROLA NA FINU GRAĐU KLOORPLASTA U RAZVITKU

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Mlade biljke (*Zea mays*, *Phaseolus vulgaris*, *Elodea canadensis*, *Elodea densa* i *Wolffia arrhiza*) tretirane su 10^{-4} do 10^{-2} M otopinama amitrola te istražene svjetlosnim i elektronskim mikroskopom u različitim vremenskim razmacima nakon što se pojavila kloroza.

Pokazalo se da su djelovanjem amitrola promijenjeni samo oni kloroplasti koji su se u vrijeme tretmana nalazili u stadiju diferencijacije.

Pored već poznatih promjena svjetlosnim mikroskopom zapaženi su u oštećenim kloroplastima karakteristični kristali karotenoida.

U elektronskom mikroskopu primijećene su u kloroplastima znatne promjene. Umjesto normalnih grana pojavljuju se duge slijepljene lamele, od kojih neke pokazuju u presjeku jasno pruganje (perioda: 12—13 nm). U plošno prerezanim takvim lamelama to se pruganje pokazalo identičnim s heksagonalnim rasporedom globularnih čestica promjera 7—8 nm. Diskutirane su moguće veze te strukture s makromolekularnom organizacijom normalnih tilakoida i druge pojedinosti.

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