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THE EFFECT OF SAN 9789 ON TULIP TREE CHROMOPLASTS

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The herbicide SAN 9789 has been used to study the synthesis of carotenoids and ultrastructural changes in chromoplasts of tulip tree (*Liriodendron tulipifera* L.) flowers. SAN 9789 inhibits the synthesis of β-carotene and interferes with the formation of chromoplast lipids. It prevents the formation of crystals and reticulum, but on the other hand it increases the accumulation of plastoglobules in chromoplasts of the flowers treated.

Introduction

Herbicides offer useful tools for the investigation of the synthesis of pigments, lipids and proteins. The most interesting group of them are pyridazinone herbicides which specifically interfere with different, well defined, biosynthetic steps.

SAN 9789 blocks desaturation reactions in carotenoid synthesis causing the accumulation of phytoene (Bartels and McCullough 1972, Frosch et al. 1979). It also interferes with the membrane lipid formation (Hilton et al. 1971) and can cause aberrant ultrastructural changes in the plastid structure (Bartels and Hyde 1970, Ridley and Ridley 1979, Khan et al. 1979).

The present study was initiated to obtain more detail about the effects of herbicide SAN 9789 on the chromoplasts from the orange zone of the petals of tulip tree, *Liriodendron tulipifera* L. These chromoplasts, when fully developed, contain a network of branched tubules and numerous plastoglobules (Ljubešić 1978). According to Sitte (1974) they belong to the reticulo-tubulous type.

Materials and Methods

Developmental studies were carried out on the inner side of the petal yelow or orange zone of tulip tree (*Liriodendron tulipifera* L.). Flowers were treated with herbicide at the bud stage. A 2×10^{-4} mol solution of the herbicide SAN 9789 (4-chloro-5-(methylamino)-2-(α,α,α-trifluoro-m-tolyl)-3(2H) pyridazinone) was injected into the central cavity of the bud and ten days after the injection the material was sampled. Buds injected with pure distilled water did not show any perceivable changes.

Pieces of treated and untreated tulip tree petal tissue were fixed for 1 hour in cold 2% glutaraldehyde-cacodylate buffer (pH 7,2) solution, washed three times with buffer, and postfixed for 2 hours in 2% buffered osmium tetroxide. The material was then washed with distilled water, 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 a Siemens Elmiskop I.

Carotenoids were extracted in 100% acetone, separated by thin-layer chromatography on silica gel G plates in petrolether : ethylacetate : diethylamine (14,5 : 7,5 : 3) and determined spectrophotometrically with average extinction coefficients at 450 nm according to Davies (1976).

Results and Discussion

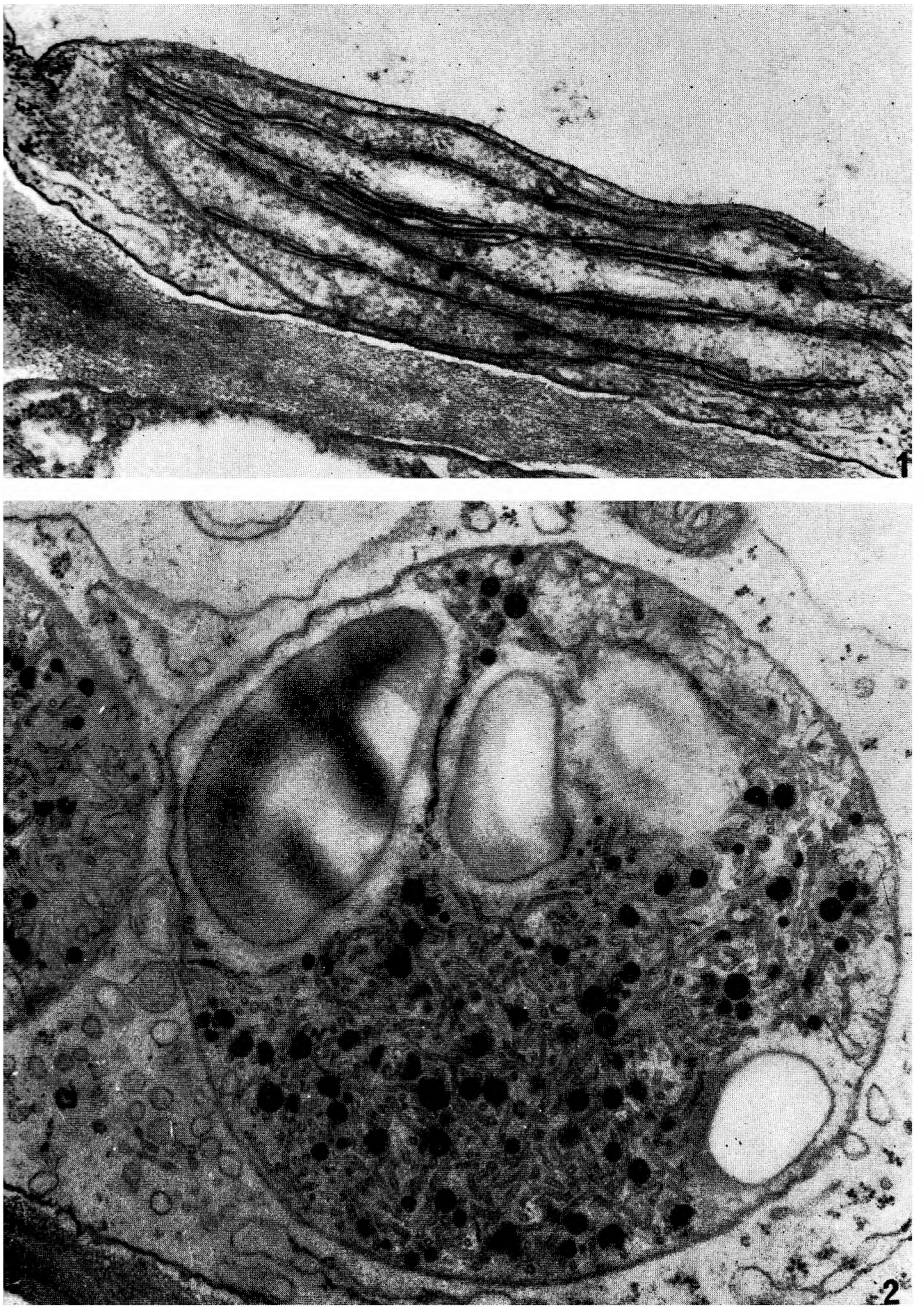
Petals of the tulip tree in the bud-stage have, on their inner side, a pale-green, 1 cm wide zone, which turns to orange during the ripening. In SAN 9789-treated flowers this zone becomes yellow.

The plastids of the immature untreated flowers from pale-green zone contain a reduced thylakoid system with only a few grana (Fig. 1). In the stroma few small plastoglobules can be observed. The change in the tissue colour corresponds to the appearance of numerous branched and curved tubules, which form a large network (reticulum). Parallel with the increase of the chromoplast volume, the number and size of the plastoglobules also increase. Small, disorganized thylakoid membranes and large starch grains can be observed in chromoplasts (Fig. 2) (Ljubešić 1978). As the flower develops, the network-structure of the chromoplasts disintegrates and small crystals of β-carotene grow out of plastoglobules (Fig. 3).

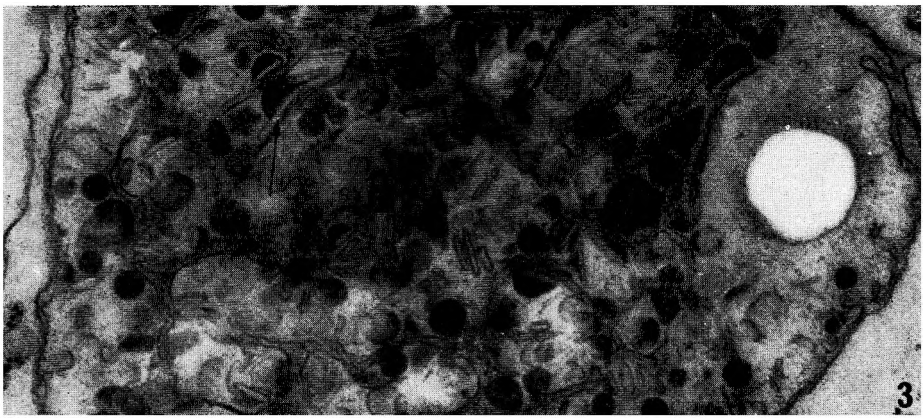
In contrast, the plastids of SAN 9789-treated flowers contain only poorly developed reticulum, but numerous large plastoglobules (Fig. 4). Reduced formation of the reticulum is probably the result of the inhibition of synthesis of some lipids. The data obtained in this field report that in treated plants SAN reduces the content of the linolenic acid (18 : 3) in galactolipids and the content of the trans-Δ^3—hexadecenoic acid(16:1) in phosphatidylglycerol (Hilton et al. 1971, Khan et al. 1979). Our ultrastructural observations suggest that the synthesis of

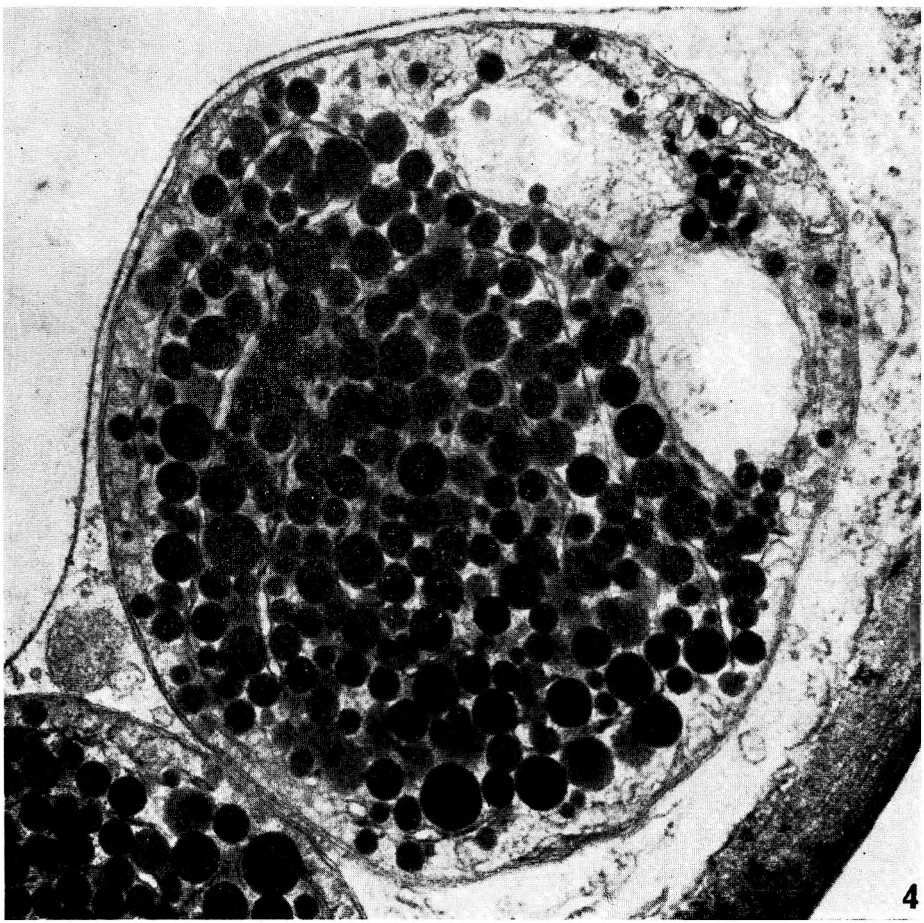
Fig. 1. Chloroplast from the pale-green zone of the untreated tulip tree petal. Plastid contains few plastoglobules and a small thylakoid system. 34,000 : 1.

Fig. 2. Chromoplast from the orange zone of the untreated tulip tree petal, showing a well-developed reticulum, large starch grains and numerous small plastoglobules. 28,000 : 1.



Figs. 1—2.





Figs. 3—4.

Table 1. Carotenoid content of untreated and treated (2×10^{-4} mol SAN 9789) petals. Values expressed as a percentage of total pigments.

	β-carotene	unidentified carotene	lutein	violaxanthin	neoxanthin
Control	72,0	6,6	6,9	7,2	5,9
SAN 9789 2×10^{-4} mol	27,4	20,8	25,0	14,2	12,5

some classes of lipids is inhibited. The lipids whose synthesis is not inhibited, form large plastoglobules.

There are considerable quantitative differences between the carotenoid contents of the treated and untreated flowers of tulip tree. SAN 9789 almost completely inhibits the synthesis of β-carotene (Table 1). Pigment analysis and ultrastructural investigations confirm these findings, because in treated petals there are no crystals either in contact with plastoglobules or in any other part of the chromoplasts (Fig. 4). There are many other similar reports about the action of SAN 9789 on carotenoid biosynthesis (Bartels and McCullough 1972), but it is still disputable whether SAN 9789 directly inhibits enzymes in carotenoid synthesis, or primarily affects the lipid composition of the chromoplasts (Feierabend and Schubert 1978). It is possible that the inhibition of lipid synthesis causes the modified structure which might alter the carotenoid synthesis.

In contrast to the findings of Kleudgen (1979), we found no inhibition on xanthophyll synthesis. The results of our experiments show that only the carotene synthesis is blocked, while the xanthophyll synthesis remains unaffected. But they do not support the present scheme for the carotenoid synthesis (Britton 1976). It is possible that an alternative pathway might exist in the synthesis of carotenoids. This aspect and the inhibition of lipid compounds will be investigated later.

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Fig. 3. Part of a chromoplast from the orange zone of the untreated tulip tree petal at the end of the flowering. Note the typical reticulum and numerous small crystals (arrow), which grow out from plastoglobules. 38,000 : 1.

Fig. 4. Chromoplast from the yellow zone of the tulip tree petal (2×10^{-4} mol SAN 9789). Chromoplasts are full of large and osmiophilic plastoglobules and with only sparcely organized reticulum. Remnants of thylakoids can be observed. 48,000 : 1.

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Sažetak

UTJECAJ HERBICIDA SAN 9789 NA KROMOPLASTE TULIPANOVCA

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U proučavanju ultrastrukturnih promjena i sinteze karotenoida u kromoplastima cvjetova tulipanovca (*Liriodendron tulipiera* L.) koristili smo se herbicidom SAN 9789. On inhibira sintezu β-karotena i djeluje na stvaranje kromoplastnih lipida. Sprečava stvaranje kristala i retikuluma, ali povećava broj plastoglobula u kromoplastima obrađivanih cvjetova.

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