ACTA BOT. CROAT. VOL. 38, 29—34, 1979.

UDC 58
CODEN: ABCRA2
YU ISSN 0365—0588

UDC 576.31 : 581.174.2 : 582.931.4

CHROMOPLASTS OF *FORSYTHIA SUSPENSA* (THUNB.) VAHL.

II. THE EFFECT OF ISOPROPYL N-PHENYLCARBAMATE

NIKOLA LJUBEŠIĆ and MELITA RADIĆ

(Laboratory of Electron Microscopy, Ruđer Bošković Institute, Zagreb)

Received January 26, 1979

Introduction

Isopropyl N-phenylcarbamate (IPC) has been used in agriculture as a herbicide for a number of years. In plant materials it has blocked the mitosis and produced multinucleate cells and giant polyploid nuclei (Ennis 1948, Doxey 1949). Ennis (1948) concluded that IPC, like colchicine produces some effects on the mitotic spindle. Colchicine destroys the fibrous birefringent component of the spindle apparatus, i. e. the spindle microtubules. IPC, in contrast to colchicine, does not destroy the spindle microtubules, but influences their reorientation and formation of the multipolar spindle apparatus (Hepler and Jackson 1969, Coss and Pickett-Heaps 1974). Brown and Bouck (1974) have found that IPC is also effective in the formation of cytoplasmic and flagellar microtubules.

The investigations of the effects of IPC on plastids have shown that it inhibited photosynthetic activity of chloroplasts (Trebst et. al. 1968, Dodge 1975, Wrischer and Botka 1978). In comparison with other herbicides, IPC is a weak inhibitor of photosynthesis. IPC is more effective on ultrastructure of plastids (Herichova 1972, 1973, Wrischer and Botka 1978) especially if they were treated during the earlier stages of development.

The presence of chromoplasts, containing tubules, originating from the chloroplasts in petals of *Forsythia suspensa*, were suitable for some experiments in which the effect of IPC on the disintegration of photosynthetic apparatus and development of tubules has been studied.

Material and Methods

One year old branches of *Forsythia suspensa* (Thunb.) Vahl. with buds were collected from the bushes growing in the gardens of Ruđer Bošković Institute in the course of January, February and March. The branches were immersed in tap water (control), or in the solution (10^{-3} M) of isopropyl N-phenylcarbamate (IPC) (Pliva, Zagreb, Yugoslavia) in tap water, and kept in artificial light at about 15° C. The samples were taken at various stages of flower development and prepared for electron microscopy and pigment analysis as described previously (Ljubešić 1979).

Results

The buds treated with IPC opened more slowly then the control ones. The process of flower opening in untreated (control) material lasted about 10 days. However, 5% of treated buds never opened and consequently withered. The petals of treated flowers were shorter in comparison with the control ones. Their length scarcely reached 70% of normal dimension. Their colour was yellow-greenish. The treated flowers began to wither after 12 to 15 days of treatment and two or three days later all of them died.

Light microscopy

Light microscope observations did not show any marked changes in comparison with control, except for the intensification of green colour of the plastids.

Electron microscopy

In spite of the yellowish colour in the treated flowers, about 70% of all plastids were chloroplasts (Figs. 1, 2). It seems that the transformation of chloroplasts to chromoplasts was strongly inhibited. Unfolded petals contained young chloroplasts (Fig. 1). Their stroma was dense with numerous ribosomes. Grana were small. Plastoglobules were small and scarce. The chloroplasts of fully developed petals were normal and

Figs. 1—5.

Plastids from the petals of *Forsythia suspensa* treated with IPC during the process of flower development.

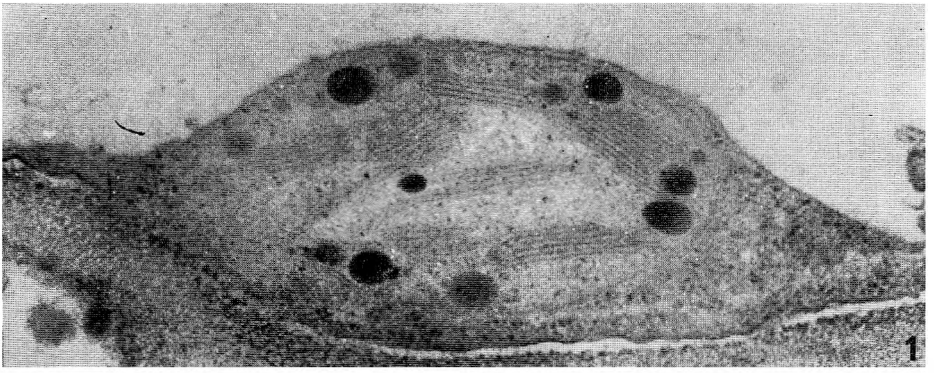
Fig. 1. Chloroplast from unfolded petal (7 days on IPC). 46,000 : 1.

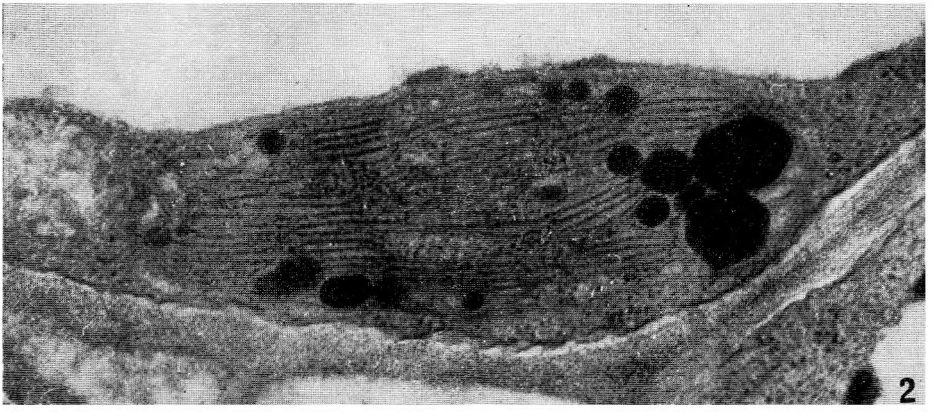
Fig. 2. Chloroplast from open flower (14 days on IPC). Plastoglobules are big and numerous. 46,000 : 1.

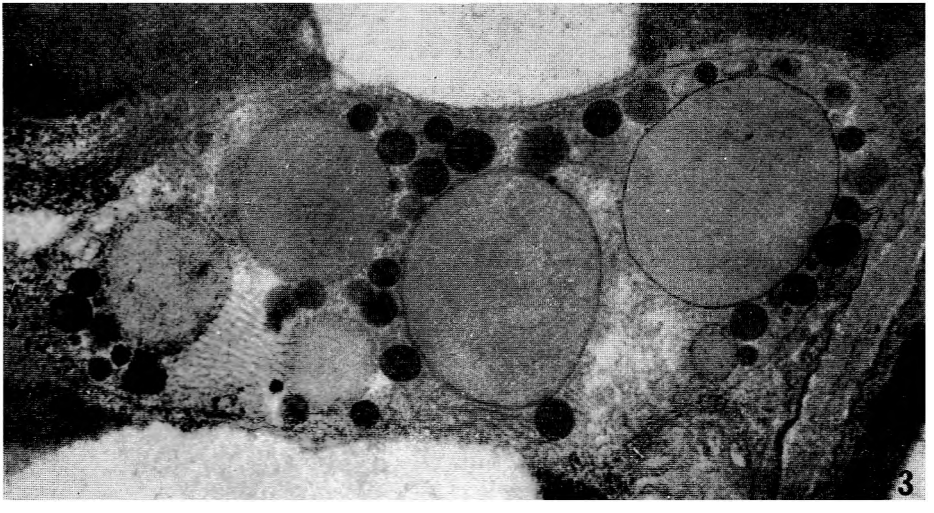
Fig. 3. Chromoplast from open flower (14 days on IPC). Tubules, plastoglobules and "vacuole-like" inclusions are present, but not in their typical arrangement. 40,000 : 1.

Fig. 4. Chromoplasts from open flower (14 days on IPC) slightly different from the typical form. 44,000 : 1.

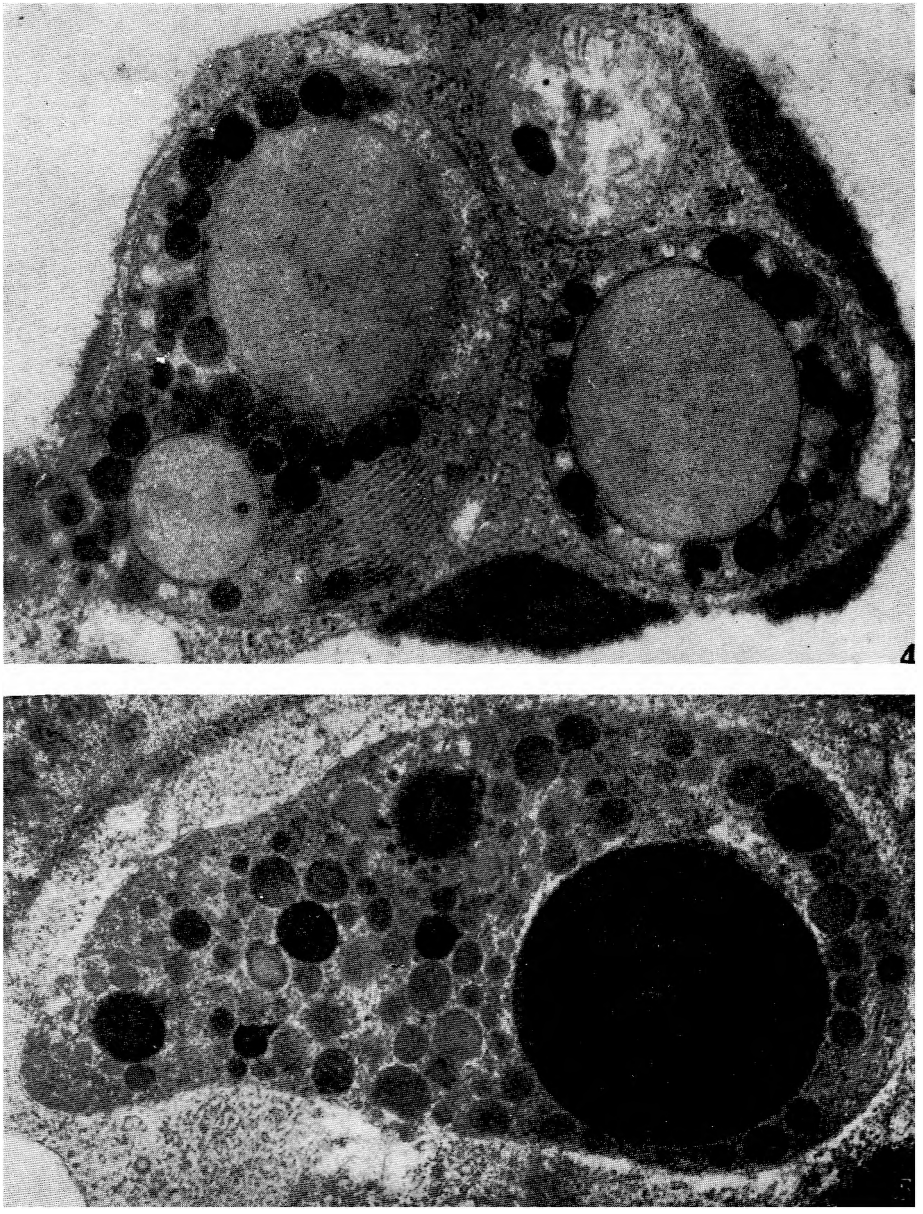
Fig. 5. Chromoplast from a flower at the beginning of withering (15 days on IPC). Only plastoglobules and "vacuole-like" inclusions occur. 34,000 : 1.







Figs. 1—3.



Figs. 4—5.

contained plastoglobules which were bigger than in normal chloroplasts (Fig. 2). The process of flower withering followed the normal senescence and disintegration of chloroplasts (Dodge 1970).

About 30% of plastids belonged to the so-called transitory stages between chloroplasts and chromoplasts. Only a small number of them (1—2%) were chromoplasts typical of *Forsythia suspensa* (Fig. 4). The development and disintegration of these chromoplasts were identical with the same stages in the control (Ljubešić 1979). Other chromoplasts were various in structure (Figs. 3, 5). In the majority of them grana did not exist. Plastoglobules were present but they did not encircle "vacuole-like" inclusions. They were individually dispersed or arranged in small groups in chromoplasts (Fig. 3). The "vacuole-like" inclusions contained more dense material than the same inclusions in control chromoplasts (Fig. 5). The tubules, numerous and arranged in long bundles in normal chromoplasts, were rather scarce, short and very flexibile in treated chromoplasts (Figs. 3, 4). Sometimes they formed short bundles. On the cross section they were similar to the normal. During the process of flower withering all tubules disappeared and the chromoplasts were filled only with plastoglobules and "vacuole-like" inclusions (Fig. 5). The ribosomes disappeared. After a short time all plastids and other organelles disintegrated.

Pigment analysis

IPC changed the ratio of chlorophyll and carotenoids to a greater extent. In control flowers the amount of chlorophyll dropped and that of carotenoids grew rapidly simultaneously with the process of flower opening (Ljubešić 1979). This phenomenon revealed an entirely different fact in treated flowers. The amount of chlorophyll grew and that of carotenoids rapidly dropped (Table I). In treated flowers a relatively small increase in the concentration of all pigments caused by the withering of petals (e. g. dimishing of fresh weight) was also evident (Radić 1978, Ljubešić 1979). However, the qualitative composition of pigments was the same in the control and the treated flowers.

Table I. Effect of IPC (10^{-3} M) on the chlorophyll and total carotenoids (mg/gr fresh wt.) during the development of flowers after 0 (bud), 7 and 14 days of treatment.

	0 day	7 days	14 days
Chlorophyll a+b	0.4189	0.2628	0.3875
Total carotenoids	0.2863	0.4570	0.3166

Discussion

Despite some effective characteristics of IPC as a herbicide (Dodge 1975) the concentration of 10^{-3} M had practically no letal effect on the development of *Forsythia suspensa* flowers. The data obtained by

Keitt (1967), namely that IPC inhibited extension growth of cells, could not be proved. The opening and development of flowers of *Forsythia suspensa* were not inhibited by IPC although this process was caused by extension growth only. However, the treated flowers were smaller than the normal ones. Some light microscopic observations showed that the dimensions of cells in the treated flowers were similar to those in the control, i. e. their extension growth did not diminish. We suggest that during the flower development some mitotic activity occurred (most evident on the petal basis). The treatment with IPC inhibited the normal arrangement of microtubules of the spindle apparatus resulting in a complete blockade of mitosis (Hepler and Jackson 1969). The absence of mitosis during the flower development might be the cause of the smaller dimension of petals.

The effect of IPC on the ultrastructure of plastids is fairly evident. The transformation of chloroplasts into chromoplasts was inhibited in a high percentage. We suppose that IPC inhibited this transformation completely. However, the treated petals contained a certain number (about 30%) of chromoplasts in various stages of differentiation. It seems that the transformation of chloroplasts to chromoplasts began primarily in the petals of buds, i. s. before IPC treatment. Before the treatment the majority of plastids were chloroplasts, a very small number of them chromoplasts and a certain number of them in various stages of differentiation. It is possible that IPC could not prevent the process of transformation of plastids where it had already started. These plastids carried on the differentiation but only to a certain chromoplast stage. We believe that the rearrangement of thylakoids into tubules was a process which had already started and could not be inhibited by IPC. That was one of the reasons why we could not find any stages in which the thylakoids were only partially transformed into tubules, i. e. the plastids with evident thylakoids and tubules. The treated petals contained plastids either having only thylakoids or tubules only.

The mode of action of IPC on plastids remained unknown. Mann et al. (1967) have found that IPC inhibited protein synthesis but preserved chlorophyll. Our investigations support these data, especially in connection with the preservation of chlorophyll.

The most precisely studied effect of IPC was performed on the cytoplasmic microtubules (for review see Hepler and Jackson 1969, Brown and Buck 1974). IPC was exclusively efficient on cytoplasmic microtubules which contained protein only. The treatment of IPC on the petals of *Forsythia suspensa* had no effect on the chromoplast tubules — neither on the process of their formation, nor on the existing tubules. These facts support the opinion that the chromoplasts contained the so-called lipoprotein tubules (Ljubešić 1979). In spite of absence of any *de novo* protein synthesis in treated flowers (Mann et al. 1967), the formation of the lipoprotein tubules was not inhibited. We believe that the material utilized for tubule formation in chromoplasts originated from disintegrated thylakoids (Ljubešić 1979).

Although the effect of IPC on plastids was rather weak, these investigations partially solved the problem of the formation of some chromoplast structures. It seems possible that further experiments concerning the influence of IPC on the pigment composition could give data about the carotenoid synthesis and localization of pigments in chromoplasts.

Summary

The effect of isopropyl N-phenylcarbamate (IPC) on the process of transformation of chloroplasts into chromoplasts in the petals of *Forsythia suspensa* flowers has been studied by the application of light and electron microscopy and pigment analysis.

In treated petals only about 30% of plastids have been transformed into chromoplasts. They contain tubules, "vacuole-like" inclusions and plastoglobules but very rarely in their typical form. A great number of plastids (70%) has not been transformed and preserved the structure of chloroplasts until the withering of the flowers.

The pigment analysis has shown that IPC has inhibited the disappearance of chlorophyll and retarded the synthesis of carotenoids duing the process of flower development.

*

The authors are indebted to Dr. Mercedes Wrischer for reading the manuscript and helpful discussion.

References

Brown, D. L. and *G. B. Bouck*, 1974: Microtubule biogenesis and cell shape in *Ochromonas*. III. Effects of the herbicidal mitotic inhibitor isopropyl N-phenylcarbamate on shape and flagellum regeneration. J. Cell Biol. *61*, 514—536.

Coss, R. A. and *J. D. Pickett-Heaps*, 1974: The effects of isopropyl N-phenyl carbamate on the green alga *Oedogonium cardiacum*. I. Cell division. J. Cell Biol. *63*, 84—98.

Dodge, A. D., 1975. Some mechanisms of herbicide action. Sci. Prog. Oxf. *62*, 447—536.

Dodge, J. D., 1970: Changes in chloroplast fine structure during the autumnal senescence of *Betula* leaves. Ann. Bot. *34*, 817—824.

Doxey, D., 1949: The effect of isopropyl phenyl carbamate on mitosis in rye (*Secale cereale*) and onion (*Allium cepa*) Ann. Bot. *13*, 329—335.

Ennis, W. B., 1948: Some cytological effects of O-isopropyl N-phenyl-carbamate upon *Avena*. Amer. J. Bot. *35*, 15—21.

Hepler, P. K. and *W. T. Jackson*, 1969: Isopropyl N-phenylcarbamate affects spindle microtubule orientation in dividing endosperm cells of *Haemanthus katherinae* Baker. J. Cell Sci. *5*, 727—743.

Herichova, A., 1972: Elektronmikroskopisches Studium des Einflusses von Isopropyl-N-phenylkarbamat und Isopropyl-N-(3-chlorphenyl)- karbamat auf die Zellorganellen der Meristemzellen von Pferdebohnenwurzelspitzen (*Vicia faba* L.). Acta Fac. Rerum Nat. Univ. Comenianae Physiol. Plant. *5*, 41—56.

Herichova, A., 1973: Cytological study on the effect of isopropyl-N-(3-chlorophenyl) carbamate on the division and ultrastructure of meristematic cells of barley (*Hordeum sativum* L.) Acta Fac. Rerum Nat. Univ. Comenianae Physiol. Plant. *6*, 85—107.

Keitt, G. W., 1967: On the mode of action of carbamate herbicides. Physiol. Plant. *20*, 1076—1082.

Ljubešić, N., 1979: Chromoplasts of *Forsythia suspensa* (Thunb.) Vahl. I. Ultrastructure and pigment composition. Acta Bot. Croat. *38*, 23—28.

Mann, J. D., E. Cota-Robles, Kung-Hing Yung, Minn Pu and *H. Haid,* 1967: Phenylurenthane herbicides: Inhibitors of changes in metabolic state. I. Botanical aspects. Biochim. Biophys. Acta *138,* 133—139.

Radić, M., 1978: Djelovanje N-fenil-izopropil-karbamata na razvoj cvijeta vrste *Forsythia suspensa* (Thunb.) Vahl. Diplomski rad, Prirodoslovno-matematički fakultet, Sveučilište u Zagrebu.

Trebst, A., E. Pistorius, G. Boroschiewski und *H. Schulz,* 1968: Die Hemmung photosynthetischer Reaktionen durch Herbicide des Biscarbamat-Typs. Z. Naturforsch. *23 b,* 342—348.

Wrischer, M. and *K. Botka,* 1978: The effect os isopropyl N-phenylcarbamate on the structure and photosynthetic activity of etiochloroplasts. Acta Bot. Croat. *37,* 53—60.

SAŽETAK

KROMOPLASTI U VRSTE *FORSYTHIA SUSPENSA* (THUNB.) VAHL. II. DJELOVANJE N-FENIL-IZOPROPIL-KARBAMATA

Nikola Ljubešić i *Melita Radić*

(Laboratorij za elektronsku mikroskopiju, Institut »Ruđer Bošković«, Zagreb)

Istražen je utjecaj N-fenil-izopropil-karbamata (IPC) na tijek pretvorbe kloroplasta u kromoplaste u latica cvijeta vrste *Forsythia suspensa* uz pomoć svjetlosnog, elektronskog mikroskopa i analize pigmenata.

U tretiranim laticama samo 30% plastida pretvori se u kromoplaste. Ti kromoplasti sadržavaju tubule, vakuolama slične uklopine i plastoglobule, ali vrlo rijetko u tipičnom rasporedu. Veći se broj plastida (70%) ne preobrazi i sačuva građu kloroplasta do početka uvenuća cvijeta.

Analiza pigmenata pokazuje da IPC sprečava nestanak klorofila, a usporava sintezu karotenoida za vrijeme razvoja cvijeta.

Dr Nikola Ljubešić
Institut »Ruđer Bošković«
Bijenička 54
YU-41000 Zagreb (Jugoslavija)

Melita Radić, dipl. inž. biol.
Kosorova 7
YU-41000 Zagreb (Jugoslavija)