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EFFECT OF FLURIDONE ON PLANT DEVELOPMENT, LEAF ANATOMY AND PLASTID ULTRASTRUCTURE OF BARLEY PLANTS

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Summary. The effect of fluridone on leaf anatomy and plastid ultrastructure was studied in barley plants grown under light and dark conditions. The appearance of defective mesophyll cells and chloroplasts with a destroyed thylakoid membrane system was found in fluridone-treated plants grown at a photosynthetic photon flux (PPF) of 600 μmol.m^{-2}.s^{-1}. Treatment of barley plants with fluridone in the absence of light led to alterations in leaf anatomy and to ultrastructural disorders.

***Key words*:** chloroplasts, fluridone, guard cells, *Hordeum vulgare* L.

Introduction

Fluridone and norflurazon are selective inhibitors of carotenoid synthesis in cells. They interfere with desaturation steps of carotenoid biogenesis and thus block the accumulation of carotenoids (Bartels and Watson, 1978; Fong and Schiff, 1979; Eder,1979).

Treatment of plants, algae or cyanobacteria with these herbicides leads to decreases in photosynthesis (Lem and Williams, 1981), chlorophyll per leaf (Vaisberg and Schiff, 1976), and ribosome number per plastid and plastid rRNA synthesis (Bartels and Wothson, 1978; Reib et al., 1983). Lipid composition is also affected (Lem and Williams, 1981).

Carotenoids and chlorophylls are essential constituents of photosynthetic membranes. Lack of carotenoids results in a block in membrane formation and the concurrent bleaching of the organism. Carotenoid-deficient mutants produce photobleached

seedlings owing to the photooxidation of chlorophyll in the absence of carotenoids. Non-mutant seeds treated with fluridone or norflurazon also produce albino seedlings due to photooxidation of chlorophyll (Henson, 1984). In all these treatments plastidogenesis is destroyed and plastid ribosomes are completely absent.

The degree of destruction of the internal structure of chloroplasts varies widely depending on the intensity of irradiance under which the plants are grown, the herbicide concentration used, and the mode of treatment given and species used. In strong white light the cotyledones of norflurazon-treated mustard seedlings did not contain normal chloroplasts, but only small chlorophyll-free rudiments with completely destroyed internal structure (Reib et al., 1983). Treatment of etiolated bean leaves with norflurazon had little effect on the formation of normal prothylakoids and prolamellar bodies (Pardo and Schiff, 1980). This is in marked contrast to *Euglena* where inhibition of carotenoid synthesis with norflurazon resulted in proplastids with undeveloped thylakoid system (Vaisberg and Schiff, 1976).

By growing barley seedlings in darkness, Gamble and Mullet (1986) showed that fluridone inhibited carotenoid accumulation but did not alter plastid biogenesis and protein composition. However, data on the ultrastructure of proplastids were not reported. In addition, it was found that carotenoid-deficient plants are ABA-deficient (Quarrie and Lister, 1984; Henson, 1984), thus leading investigators to suggest a link between chloroplast function and ABA synthesis.

Abscisic acid is considered to be synthesized by the indirect pathway via conversion of carotenoids (Zeevaart and Creelman, 1988). The use of fluridone results in a decrease in ABA accumulation in plants. Although there is a report to show that fluridone fails to inhibit ABA accumulation in *Cercospora rosicola* (Norman, 1991), this herbicide has been found to be effective in many plant tissues (Le Page-Degivry et al., 1990; Hoffman-Benning and Kende, 1992; Xu and Bewley, 1995).

In this study we used fluridone as a tool to study the herbicide's effect on plant development, leaf anatomy and plastid ultrastructure. An attempt was also made to separate the photobleaching effect of fluridone on plastid development from the same effect under non-photooxidative conditions.

Materials and Methods

Plant growth conditions and fluridone treatment

Seeds of *Hordeum vulgare* L.,var. Alfa were imbibed for 24 h in water containing 0 or 10 µmoles fluridone (Eli Lilly Research Laboratories). The hydrated seeds were planted in a Fafard M2 potting mix in a Percival growth cabinet, day/night temperatures 25°C/20°C, RH 60%, and light intensity 600 $\mu mol.m^{-2}.s^{-1}$. Young, fully expanded second leaves of 10-day-old plants were used in all experiments.

For dark treatment experiments, plants were grown for 10 days under the standard growth conditions and in continuous darkness.

Microscopic observations

For scanning electron microscopy (SEM) , freeze-dried samples were sputter coated with Au/Pd and examined with a JEOL JSM-840 operated at 10 kV.

For transmission electron microscopy (EM), leaves were picked, sectioned and fixed with 2.5% glutaraldehyde in 0.05 M phosphate-buffer containing 0.08 M sucrose, pH 7.2. After rinsing in buffer, the samples were postfixed in buffered 1% OsO_4, again rinsed in phosphate buffer, through a graded acetone series and embedded in spurr. Thin sections were cut on a Ultracut E ultramicrotome, stained with 2.5% uranyl acetate followed by Reynold's lead citrate (Reynold's, 1963), and examined at 80 kV using a JEOL 1200 EX transmission electron microscope. One microtome thick sections from the same material were used for the light microscopy (LM) and viewed in a Nikon Microphot-FX-Light Microscope.

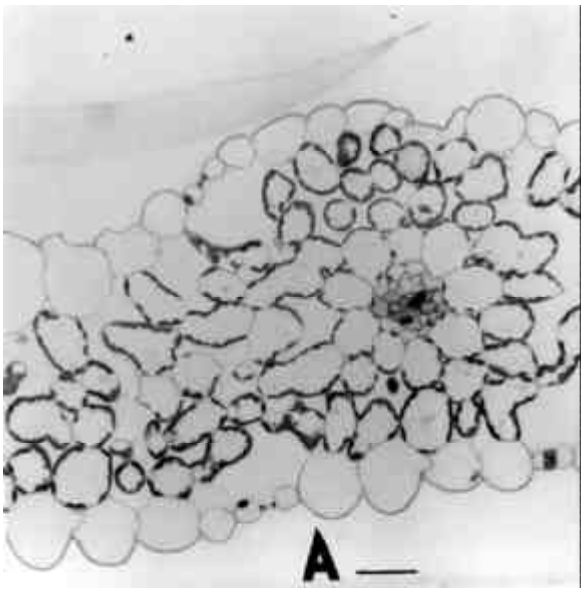
Results

Growth characteristics of barley plants in the presence of fluridone. Leaf anatomy

Treatment of barley plants grown at a PPF of 600 µmol.m^{-2}.s^{-1} with 10 µmoles fluridone caused a strong reduction in the rate of growth. The leaves of treated plants were small, fully developed and completely photobleached. Herbicide treatment led to some stimulation of growth in darkness. Leaves of treated plants were more elongated and less expanded than those of untreated plants. Despite non-photooxidative conditions in darkness, fluridone treatment caused albino appearance of foliar tissue (data not shown).

Control plants had a normal leaf anatomy, with chloroplasts appressed to the plasmalemma of mesophyll cells (Fig. 1A) Fluridone-treated plants differed in their leaf anatomy. Most of the mesophyll cells either did not have well developed chloroplasts or had none. In the latter case the intracellular species were filled with granullar substance. Furthermore, in fluridone-treated plants most of the mesophyll cells appeared to be somewhat bulbous and the intercellular species decreased compared to the controls (Fig. 1B).

Etiolated, dark grown plants showed a typical leaf anatomy with clear differentiation between cell types and well arranged etioplastids against the cell walls (Fig. 2A). Dark grown and fluridone-treated barley plants showed similar leaf anatomy to that of controls. Some of the mesophyll cells contained only a few etioplasts or completely lacked them and their intracellular species were granullar (Fig. 2B).



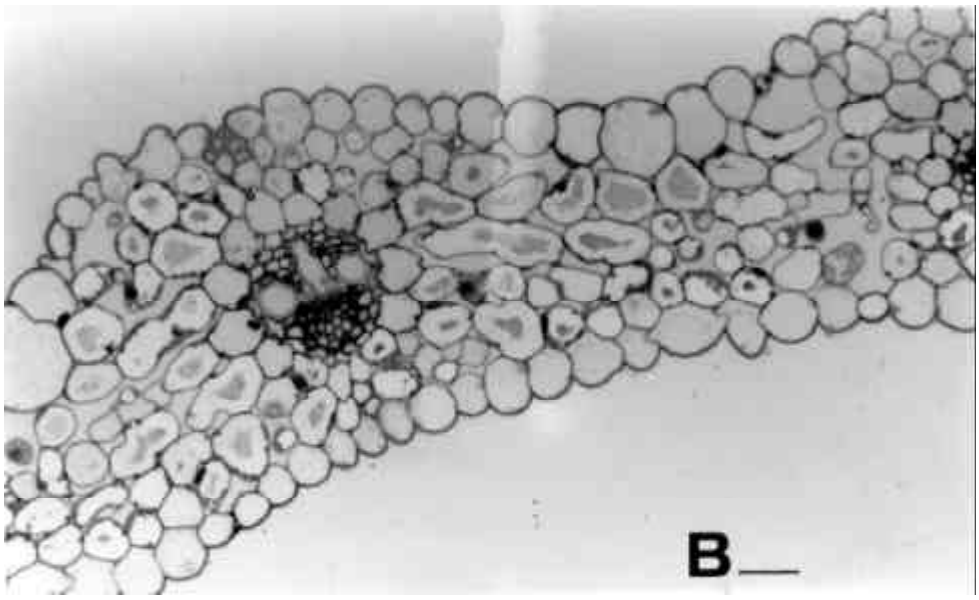


Fig. 1. Effect of fluridone (10 µmoles) on leaf anatomy of barley plants grown at a PPF of 600 µmol.m^{-2}.s^{-1}. A and B, LM micrographs of cross-sections of barley leaves of untreated control (A) and fluridone-treated (B) plants. Bar = 100 µm

Some differences could be distinguished between the shape and size of guard cells. Guard cells of control leaves were arranged in parallel rows as typical for Gramineae (Fig. 3A). Guard cells of treated plants were smaller in size and some of them were sunken relative to the other epidermal cells (Fig. 3B).

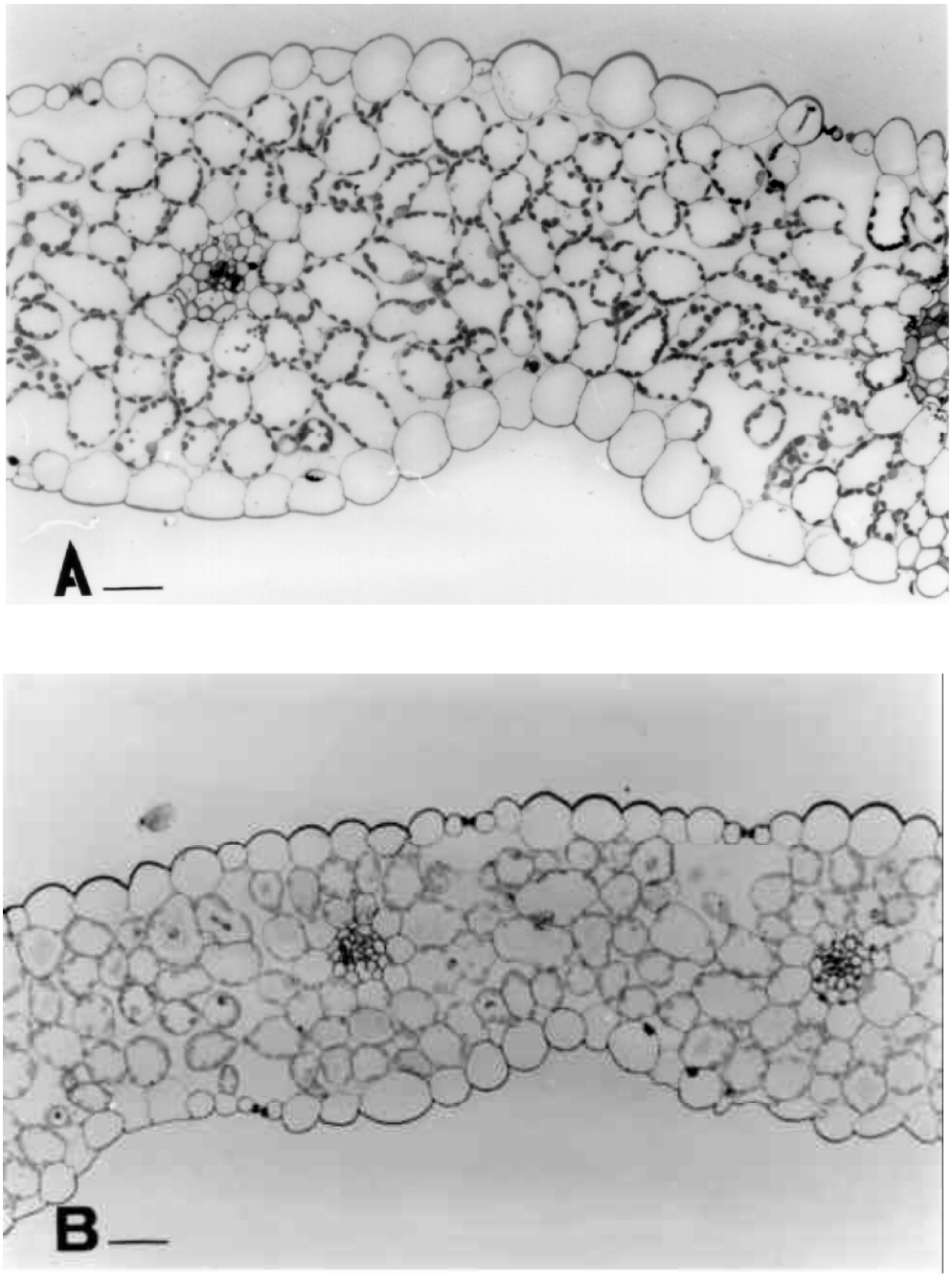


Fig. 2. LM micrographs of cross-sections of etiolated barley leaves. A, untreated control; B, fluridone-treated plants. Bar = 100 µm

Ultrastructure of plastids

The electron microscopic observation revealed distinct differences in the structure of chloroplasts. Chloroplasts of green leaves grown under 600 μmol.m^{-2}.s^{-1} PPF were elongated and contained grana consisting of several normaly arranged thylakoids (Fig. 4A). Treatment of plants with fluridone led to internal destruction of the plastids. The

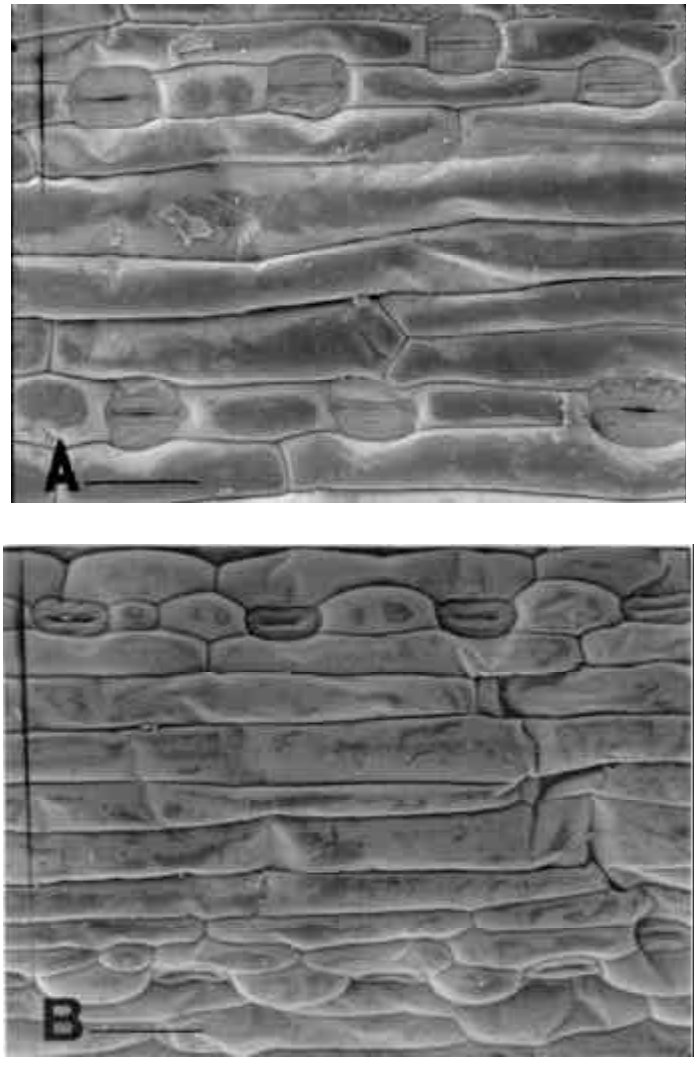


Fig. 3. SEM micrographs of freeze-dried leaf surface (lower epidermis) of barley leaves of untreated (A) and fluridone-treated plants (B). Bar = 10 μm

thylakoids had disappeared and mostly vesicles were present. The changes were exclusively restricted to the internal structure with the outer chloroplast membrane seemingly unimpaired. Starch was always absent from these plastids (Fig. 4B).

Plants that had been grown in darkness (etiolated plants) contained well developed etioplasts showing thylakoids and big crystalline prolamellar body area. Darkly stained plastoglobules were numerous and usually arranged into groups (Fig. 4C). The etioplasts from fluridone-treated leaves were smaller in size, with less prolamellar body area and thylakoid length. The degree of grana staking was lower and many vesicles were present (Fig. 4D).

Discussion

The results of this study show that the inhibitor of carotenoid synthesis fluridone caused albino appearance of foliar tissue. The degree of bleaching did not depend on the intensity of irradiance under which the plants were grown and the herbicide concentration used. The bleaching effect of fluridone was established not only for 10 μmoles fluridone but even after application of 10-fold lower concentration. Treatment of barley plants with fluridone in darkness led to appearance of albino seedlings (data not shown).

In our experiments with dicot *Vicia faba* treatment with 0.1 mM fluridone prevented seed germination, and even after application of 10-fold lower concentration the rate of growth was severely restricted. Our attempts to grow *Vicia* plants in darkness were not successful because the plants did not develop normal well expanded etiolated leaves. Treatment of *Vicia* plants with 10 μmoles fluridone in darkness resulted in a very low rate of growth and also leaves became completely white (Popova, 1995). In contrast, treatment of barley plants with 10 μmoles fluridone in darkness did not restrict the rate of growth.

It is difficult to extrapolate an effect from one plant to another, especially since plants vary in their sensitivity to fluridone, and large variation of concentrations have been used. Although photobleaching is a common characteristic for fluridone action there is a lot of evidence showing that this effect depends on the herbicide concentration used, the mode of treatment given and species used (St John, 1976; Stewart and Voetberg, 1987).

The effect of fluridone was not limited only on plastid ultrastructure, but the leaf's anatomy was also affected (Fig. 1) .The morphogenesis of different cell types was damaged, lack of clear differentiation between them was obvious. The leaves of light-grown and fluridone-treated plants did not contain chloroplasts but only small chlorophyll-free rudiments whose internal structure had almost disappeared. These observations are in agreement with our data reported for *Vicia* plants treated with fluridone (Popova, 1995) and also with some other data (Pardo and Schiff, 1980).

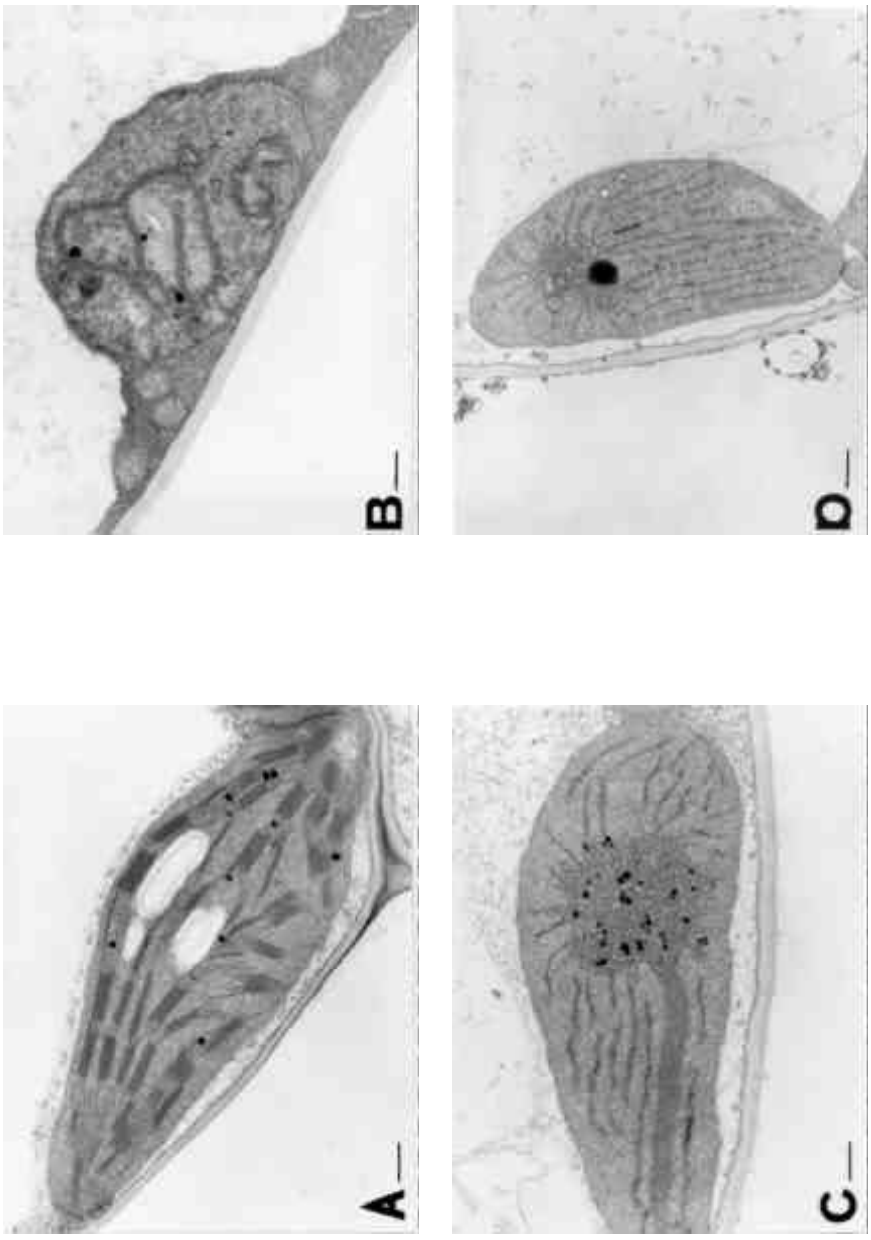


Fig. 4. Electron micrographs of chloroplasts and etioplastids of barley plants that were grown at different light regimes. A and B, chloroplasts from untreated control (A) and grown in the presence of 10 µmoles fluridone (B), and a PPF of 600 µmol.m^{-2}.s^{-1}; C and D, ethioplasts from untreated control (C) and fluridone-treated (D) plants grown in darkness. Scale bar = 500 nm

Our explanation is based on the facts that lack of carotenoids (after fluridone treatment) results in a block of membrane formation, of membrane constituents and assembly.

Treatment with fluridone in darkness produced effects on etioplast structure similar to those of light-treated seedlings (Fig 4). The development of etioplasts, prolamellar bodies and thylakoid membranes showed appearance of ultrastructural disorders. We could hypothesize that fluridone blocks carotenoid synthesis in darkness and/or leads to destruction of polyunsaturated fatty acids of the galactolipids and accelerates unfolding of thylakoid membranes.

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