

# Herbicides which Interfere with the Biosynthesis of Carotenoids and Their Effect on Pigment Excitation, Chlorophyll Fluorescence and Pigment Composition of the Thylakoid Membrane

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Bleaching Herbicides, Carotenoids, Chlorophylls, Fluorescence, Pigment Excitation

Plants grown in the presence of the herbicides assayed synthesized chlorophylls during growth at low fluence rates. Subsequent irradiation with higher fluence rates of red light induced a strong chlorosis with SAN 6706 being a much stronger herbicide than J 852 or amino-triazole. All herbicides assayed also changed the content and composition of chlorophylls, carotenoids and pigment-protein-complexes of the thylakoid membrane and therefore the pigment excitation and chlorophyll fluorescence emission spectra of the plastid. With increasing herbicide toxicity the main characteristic emission bands at 690 and 730 nm disappeared and new emission bands at 715 (J 852) and 700 nm (SAN 6706) appeared. Such "artificial" membranes with a changed pigment composition were very susceptible to light. Presented data may be taken as evidence, that the lack of photoprotective cyclic carotenoids caused by the specific action of a bleaching herbicide is the primary event that may lead to a disturbed formation of the thylakoid membrane and its destruction by light and oxygen.

## Introduction

In the presence of certain herbicides that interfere with the biosynthesis of carotenoids leading to an accumulation of acyclic biosynthetic intermediates chloroplast development is inhibited [1–5]. Plants grown at low fluence rates in the presence of these herbicides develop photosynthetically inactive plastids with altered biochemical and biophysical properties of their internal membranes [6, 7]. Such plastids are very useful tools to gain more insight into the role of carotenoids as accessory light-harvesting pigments in photosynthesis and also in chloroplast development [8].

## Materials

Radish plants were grown on muslin in continuous white light (100 lux, 0.33 W/m<sup>2</sup>, 21 ± 2 °C, 60 ± 5% relative humidity). As bleaching herbicides aminotriazole (amitrole, 3-amino-1,2,4-triazole, 0.5 mM), J 852 (2-isopropylamino-4-methyl-6-isobutyloxy-pyrimidin, 0.05 mM) and SAN 6706 (4-chloro-5-(dimethylamino)-2- $\alpha,\alpha,\alpha$ -trifluoro-*m*-tolyl)-3(2H)-pyridazinone, 0.05 mM) were assayed [9]. After 6 days growth in dim light the untreated

controls and the herbicide-treated plants were irradiated with continuous red light ( $\lambda_{\max}$  660 nm, 1500 lux, 2.1 W/m<sup>2</sup>). Before the beginning and at different times during the red light irradiation cotyledons were harvested and their chlorophyll a fluorescence transients (Kautsky effect) and low temperature fluorescence excitation and emission spectra recorded. During the measurement of the fluorescence excitation spectra all spectra were automatically corrected for changes in the intensity of the excitation light. Carotenoids were extracted from the isolated thylakoid fractions and assayed using standard techniques [10, 11]. Chromatography of acyclic and cyclic carotenoids was carried out as described in [11].

All presented data are representatives of at least 5 independent experiments.

## Results and Discussion

After 6 days growth at low fluence rates (0.33 W/m<sup>2</sup>) the untreated controls and the herbicide-treated plants were green, even though their total amount of pigments was different. The amitrole-treated plants like the controls were also photosynthetically active (Fig. 1) and exhibited the normal characteristics in their low temperature fluorescence excitation and emission spectra (Figs. 2 and 3; [12–17]). Plants treated with J 852 or

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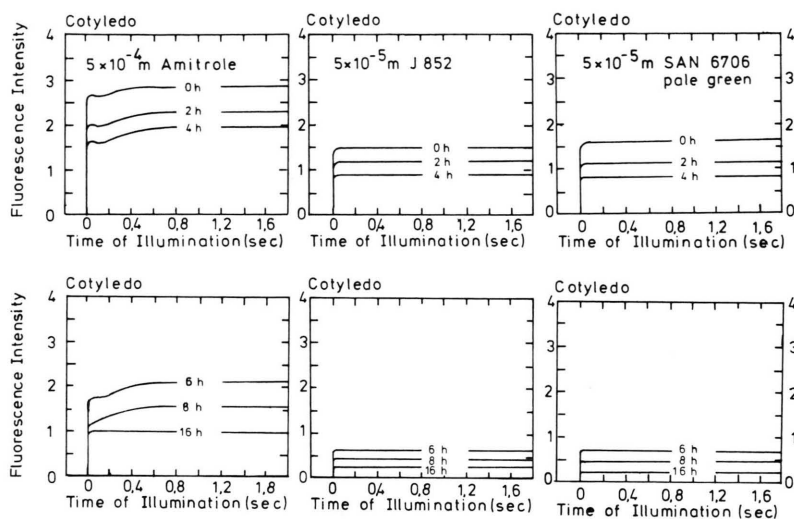


Fig. 1. Chlorophyll *a* fluorescence transients of radish cotyledons after 6 d growth in dim light in the presence of bleaching herbicides and after irradiation with red light.

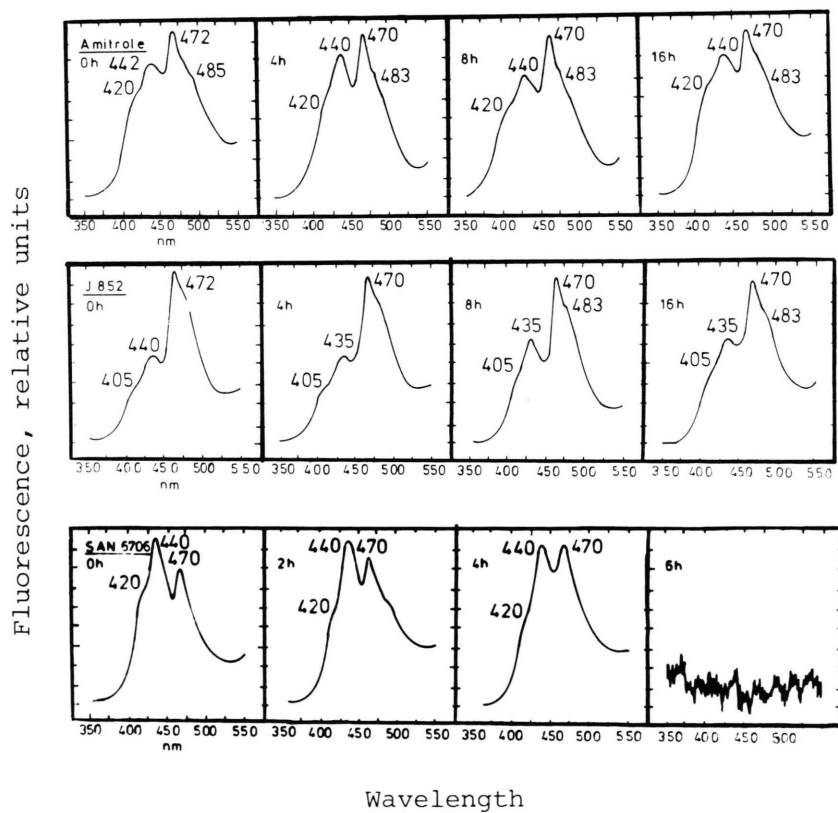


Fig. 2. Low temperature fluorescence excitation spectra of radish cotyledons after 6 d growth in dim light in the presence of bleaching herbicides and after irradiation with red light [7].

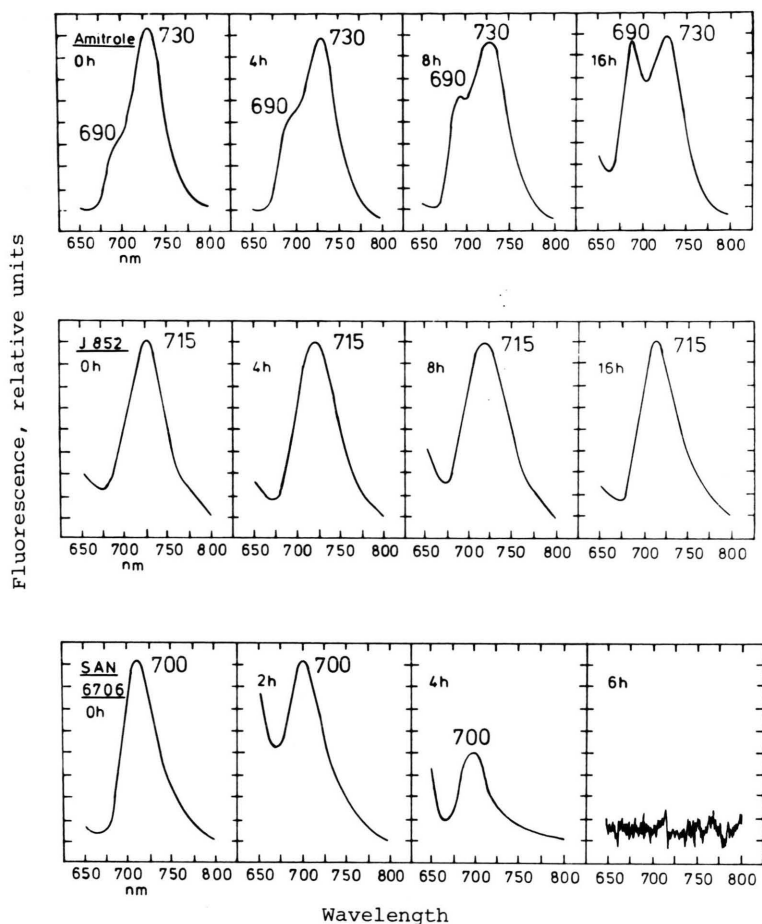


Fig. 3. Low temperature fluorescence emission spectra of radish cotyledons after 6 d growth in dim light in the presence of bleaching herbicides and after irradiation with red light [7].

SAN 6706 were photosynthetically inactive and their pigment excitation and chlorophyll fluorescence emission spectra were completely different from those of untreated controls [7]. The main and sole fluorescence emission maximum obtained was at 700 nm in the SAN-treated and at 715 nm in the J 852-treated plants suggesting that in these plants transfer of excitation energy between the two photosystems is hindered and that certain pigment-protein-complexes of the thylakoid are missing. Evidence for this has already presented for the SAN-treated plants [4].

After transfer to high fluence rates of red light ( $2.1 \text{ W/m}^2$ ) the untreated controls synthesized chlorophylls and carotenoids and photosynthesis was maintained during adaptation to the new light condition. In all herbicide-treated plants a rapid degradation of chlorophylls and cyclic carotenoids

was obtained. Even in the plants grown in the presence of 0.5 mM amitrole pigments and photosynthesis disappeared within 16 h of irradiation with red light (Figs. 1–3). The J 852- and SAN-treated plants were very susceptible to light and virtually no chlorophylls were present in their cotyledons after 16 h of irradiation.

All herbicides assayed inhibited the biosynthesis of cyclic carotenoids normally contained in a chloroplast and biosynthetic intermediates such as phytoene, phytofluene, zeta-carotene, neurosporene and lycopene accumulated (Fig. 4; [9]). If such carotenoid depleted but still green plants were irradiated with red light, their chlorophyll and carotenoid content decreased very rapidly. The susceptibility of all herbicide-treated plants towards light was depending on the capability of their plastids to synthesize cyclic carotenoids. This

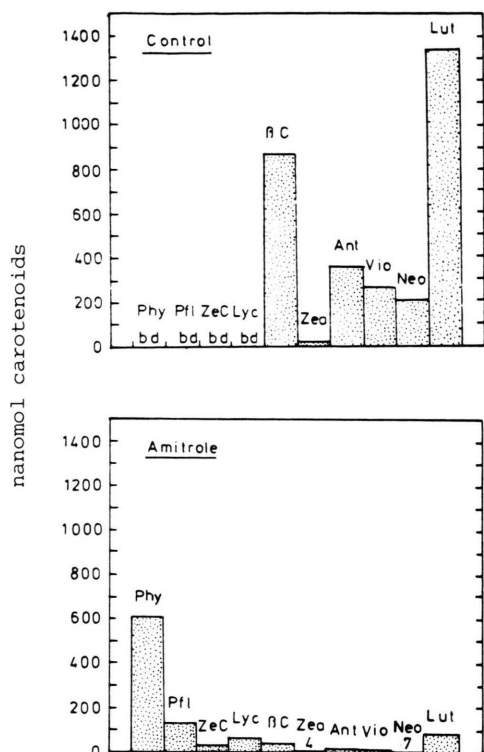


Fig. 4. Carotenoid content and composition of radish thylakoids after 6 d growth in dim light on water (control) or in the presence of 0.05 mM amitrole. Phy=phytoene, Pfl=phytofluene, ZeC=Zeta-carotene, Lyc=lycopene, βC=β-carotene, Zea=Zeaxanthin, Ant=Antheraxanthin, Vio=Violaxanthin, Neo=Neoxanthin, Lut=Lutein.

observation most likely suggests that carotenoids are essential constituents of the individual pigment-protein complexes in the thylakoid membrane and that the lack of photoprotective carotenoids is the primary event that finally leads to the destruction of the thylakoid membrane by light and oxygen [18].

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