# The Effect of Phytochrome and Proteinsynthesis-Inhibitors on the Formation of Chlorophylls and Carotenoids in Radish Seedlings Treated with Photosystem II and Bleaching Herbicides

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Photosystem II Herbicides, Chlorotic Herbicides, Chlorophylls, Carotenoids, Phytochrome, Inhibitors

Etioplasts of radish seedlings treated with photosystem II (DCMU, bentazon) and chlorotic herbicides (amitrole, SAN 6706) were tested on their ability to perform the phytochrome mediated chlorophyll and carotenoid biosynthesis. The cytoplasmic influence on the chloroplastic action of herbicides was also investigated by inhibition of protein synthesis either in the chloroplast with chloramphenicol or in the cytoplasm with actidion.

In all herbicide treated radish seedlings a phytochrome mediated chlorophyll and carotenoid biosynthesis was obtained as found in control plants. In plants treated with DCMU the biosynthesis of carotenoids is enhanced compared to the control plants, while SAN 6706 significantly suppresses the carotenoid formation.

It is concluded, that photosystem II and chlorotic herbicides do not interfere with the primary action of phytochrome but rather do develope their effects on the terpenoid metabolism through phytochrome.

Chloramphenicol applied at the time of sowing very strongly suppresses the formation of chlorophylls and carotenoids in control plants. The sensitivity towards actidion is, however, very much lower. If chloramphenicol and actidion treated plants were also supplied with herbicides, the pigment pattern is completely different. In SAN 6706-treated plants chloramphenicol acts synergistic, resulting in an even lower chlorophyll and carotenoid content than in plants supplied only with chloramphenicol. On the other hand SAN 6706 in combination with actidion enhances the formation of pigments, leading to a much higher chlorophyll and carotenoid content as in plants treated only with actidion. A stimulatory effect on the formation of chlorophylls and carotenoids was also found for DCMU-treated plants in combination with actidion.

The observation, that in plants treated with SAN 6706 together with actidion, which inhibits protein synthesis in the cytoplasm, the herbicidal response in the chloroplast is completely abolished, gives evidence that the photooxidative action of SAN 6706 in the chloroplast is developed by the cytoplasm.

## Introduction

It is generally believed that the herbicidal response is manifestated in the chloroplast. Photosystem II herbicides like DCMU and bentazon develop their toxicity via inhibition of the photosynthetic electron transport [1], while bleaching herbicides like amitrole and particularly SAN 6706 cause a photooxidative destruction of chlorophylls and carotenoids [2–5]. The primary action especially of bleaching herbicides is still unknown. It was assumed that chlorosis develops by the photooxidative destruction of chlorophylls induced by a carotenoid deficiency [2]. But it was also proposed that in addition the inactivation of peroxisomal enzymes [2] and changes in the ratio of saturated to unsaturated fatty acids of the glycolipids [6] contribute to the photooxidative damage of the chloroplast.

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In this communication the influence of active phytochrome on the development of the herbicidal activity was investigated. It is known that chlorophyll and carotenoid biosynthesis are controlled by phytochrome [7–9], but whether the action of herbicides interferes with phytochrome is unknown. By using protein synthesis inhibitors it was also investigated, whether the chloroplastic action of the investigated herbicides is developed or influenced by the cytoplasm.

## Materials and Methods

Radish seedlings were grown for 6 d on $10^{-4}$ m DCMU and bentazon (Photosystem II herbicides) as well as $10^{-4}$ m amitrole and $10^{-5}$ m SAN 6706 (chlorotic herbicides) in total darkness. The structure of the used herbicides is shown in Fig. 1.

Investigations concerning the influence of active phytochrome were performed by irradiating the her-

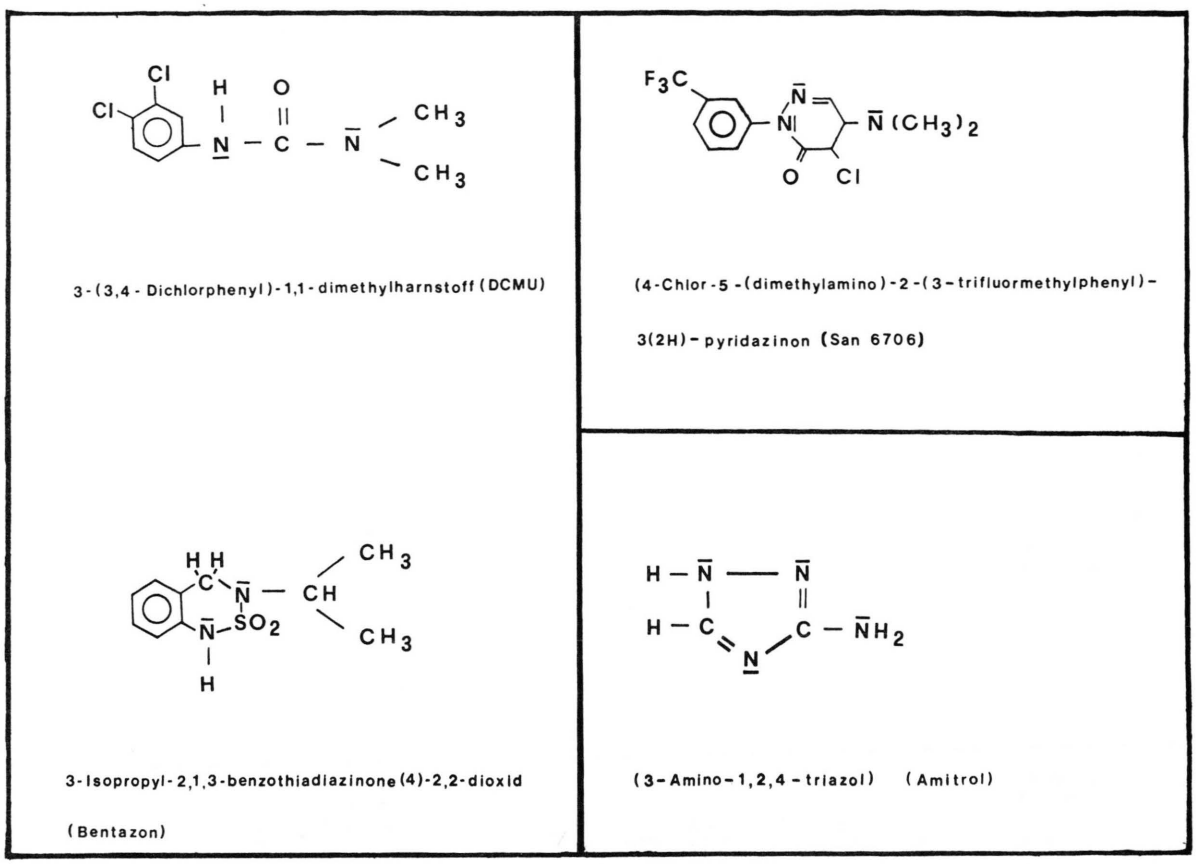


Fig. 1. Structure of herbicides assayed: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Bentazon, 3-isopropyl-1H-2,1,3-benzothiadiazin-4-(3H-one-2,2-dioxide); Amitrole, 3-amino-1,2,4-triazole; SAN 6706, 4-chloro-5(dimethyl-amino)-2-α,α,α-trifluoro-*m*-tolyl-3-pyridazinone.

bicide treated plants with 5 min red light, 5 min far red light and 5 min red followed by 5 min far red light for 6 days every 24 h.

For the investigation of the cytoplasmic influence on the chloroplastic action of herbicides control and herbicide treated plants were also supplied with $10^{-4}$ m chloramphenicol (for 6 days) and $10^{-4}$ m actidion (from the 3rd to the 6th day) in total darkness and thereafter illuminated for 24 h with fluorescent white light (0.8 W/cm²).

Chlorophylls were extracted and determined according to Ziegler and Egle [10]. Carotenoids were extracted into cold acetone, the crude extract saponified with KOH, chromatographied and quantitatively determined according to Hager and Meyer-Bertenrath [11] using an absorbance coefficient of $E$ (1%/1 cm) = 2500.

## Results

### *Etiolement*

Besides protochlorophyll(ide), all carotenoids contained in etioplasts of control plants, were also found in plastids of etiolated radish seedlings supplied with DCMU, bentazon, amitrole and SAN 6706. While DCMU-, bentazon- and amitrole-treated seedlings exhibit nearly the same carotenoid content as the control plants, SAN 6706 reduces the formation of xanthophylls (Fig. 4).

### *Phytochrome*

Chlorophyll and carotenoid biosynthesis are controlled by active phytochrome. It was, however, not yet investigated, whether herbicides paticularly bleaching herbicides like amitrole and SAN 6706 in-

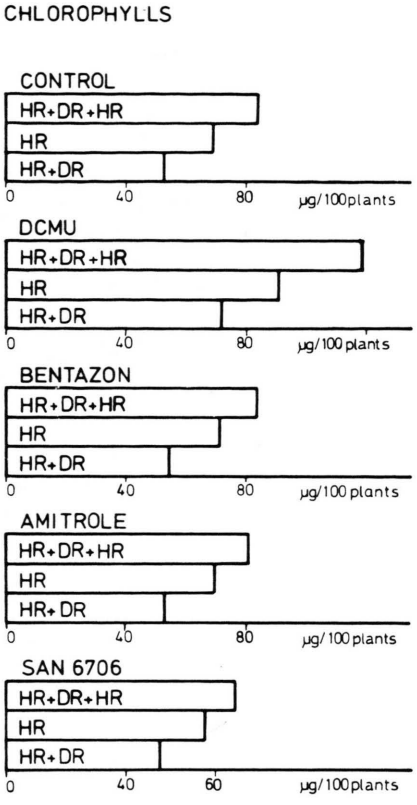


Fig. 2. The effect of active phytochrome on the chlorophyll content of etiolated radish seedlings supplied with photosystem II and bleaching herbicides.

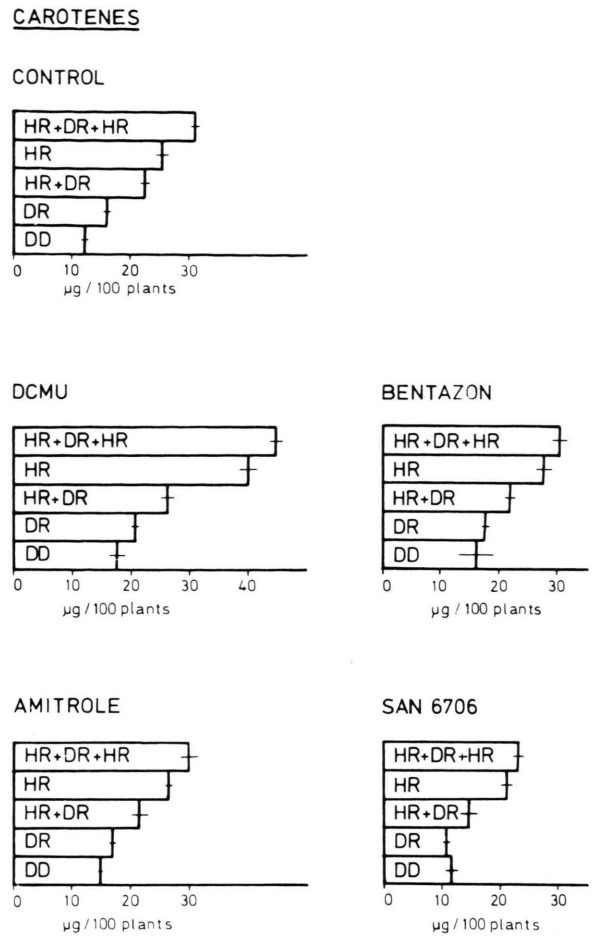


Fig. 3. The effect of phytochrome on the carotene content of etiolated radish seedlings supplied with photosystem II and bleaching herbicides.

terfere with the primary action of phytochrome. As shown in Figs 2–4 in all herbicide-treated plants chlorophyll and carotenoid formation is controlled by active phytochrome like in untreated radish seedlings, but the control is quantitative rather than qualitative. There are no differences in the accumulation of chlorophyll between control and herbicide-treated plants except for DCMU which shows an enhancement effect.

The phytochrome mediated biosynthesis of chlorophylls and carotenoids is enhanced in radish treated with DCMU, while in SAN 6706-treated plants the accumulation of carotenoids is significantly suppressed (Figs 3, 4). Bentazon- and amitrole-treated plants exhibit nearly the same carotenoid content as control plants. It is of particular interest, that in radish seedlings supplied with the photosystem II herbicides DCMU and bentazon the $\beta$-ionon xanthophylls zeaxanthin, antheraxanthin, violaxanthin and neoxanthin are enhanced in their biosynthesis by phytochrome, while the $\alpha$-ionon xanthophyll lutein is formed to the same extent as in untreated plants.

*Inhibitors*

It is known that actidion (cycloheximide) inhibits protein synthesis in the cytoplasm, while chloramphenicol inhibits protein synthesis in the chloroplasts and mitochondria. The application of inhibitors of translation should help to understand the action of herbicides on the chlorophyll and carotenoid accumulation particularly for the bleaching herbicide SAN 6706.

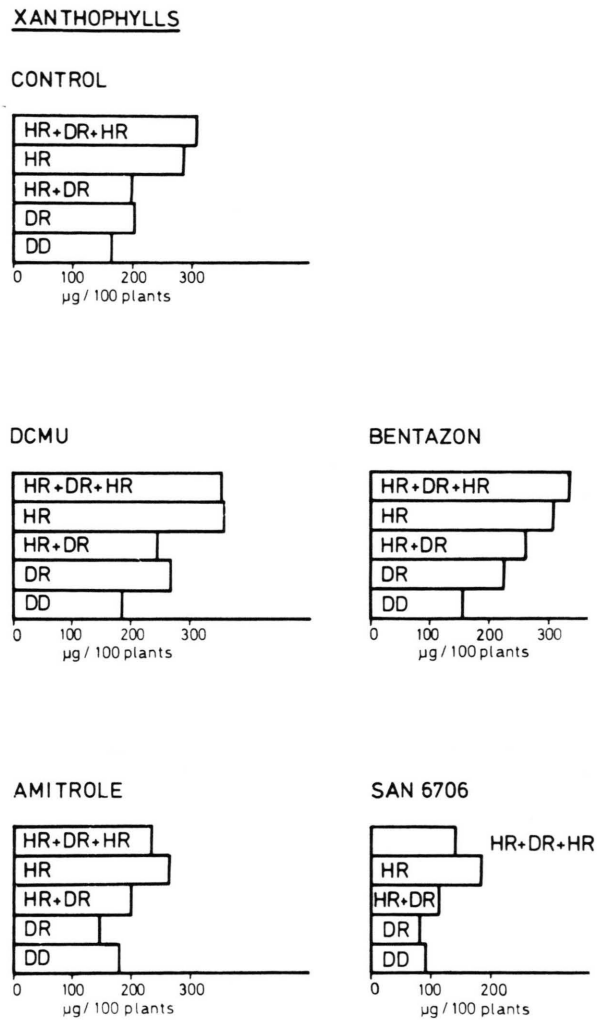


Fig. 4. The effect of phytochrome on the xanthophyll content of etiolated radish seedlings supplied with photosystem II and bleaching herbicides.

Chloramphenicol applied at the time of sowing very strongly suppresses the formation of chlorophylls and carotenoids in radish seedlings [12, 13]. The sensitivity towards actidion (applied after 3 d darkness) is, however, very much lower (Figs 5, 6). If plants treated with chloramphenicol and actidion were also supplied with a photosystem II or bleaching herbicide, the pigment pattern is completely different from that of control plants. If plants grown for 6 days in total darkness were also supplied with $10^{-5}$ m SAN 6706 and thereafter irradiated for 24 h with fluorescent white light (0.8 W/m²) the chlorophyll and carotenoid content is lower than in radish supplied only with chloramphenicol (Figs 5, 6). This shows that chloramphenicol in SAN 6706-treated plants enhances the SAN-induced bleaching effect. On the other hand, if in SAN-treated radish seedlings protein synthesis in the cytoplasm is inhibited after 3 days by $10^{-4}$ m actidion and plants are irradiated after 6 days for 24 h with white light, then the bleaching effect of SAN is completely abolished and the chlorophyll and carotenoid content is even higher in these plants than in radish treated only with actidion (Figs 5, 6).

This result shows that protein synthesis in the cytoplasm is required for the development of the herbicidal response. A stimulatory effect on the chlorophyll and carotenoid formation was also obtained for DCMU-treated plants in combination with actidion.

## Discussion

Degradation of herbicide treated plants results from changes in a large number of physiological

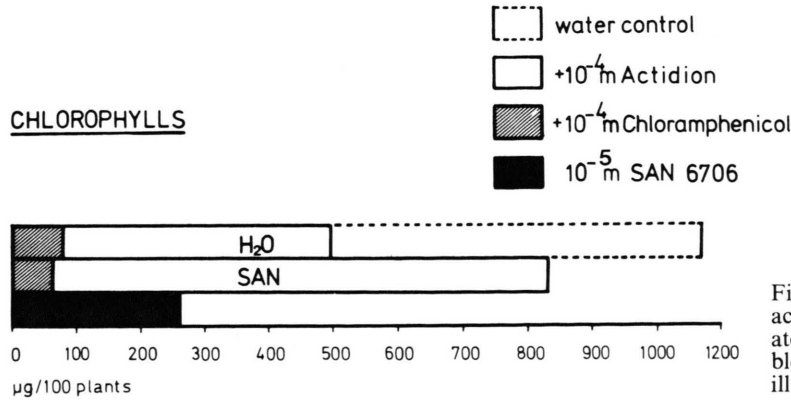


Fig. 5. The effect of chloramphenicol and actidion on the chlorophyll content of etiolated radish seedlings supplied with the bleaching herbicide SAN 6706 after 24 h illumination with fluorescent white light.

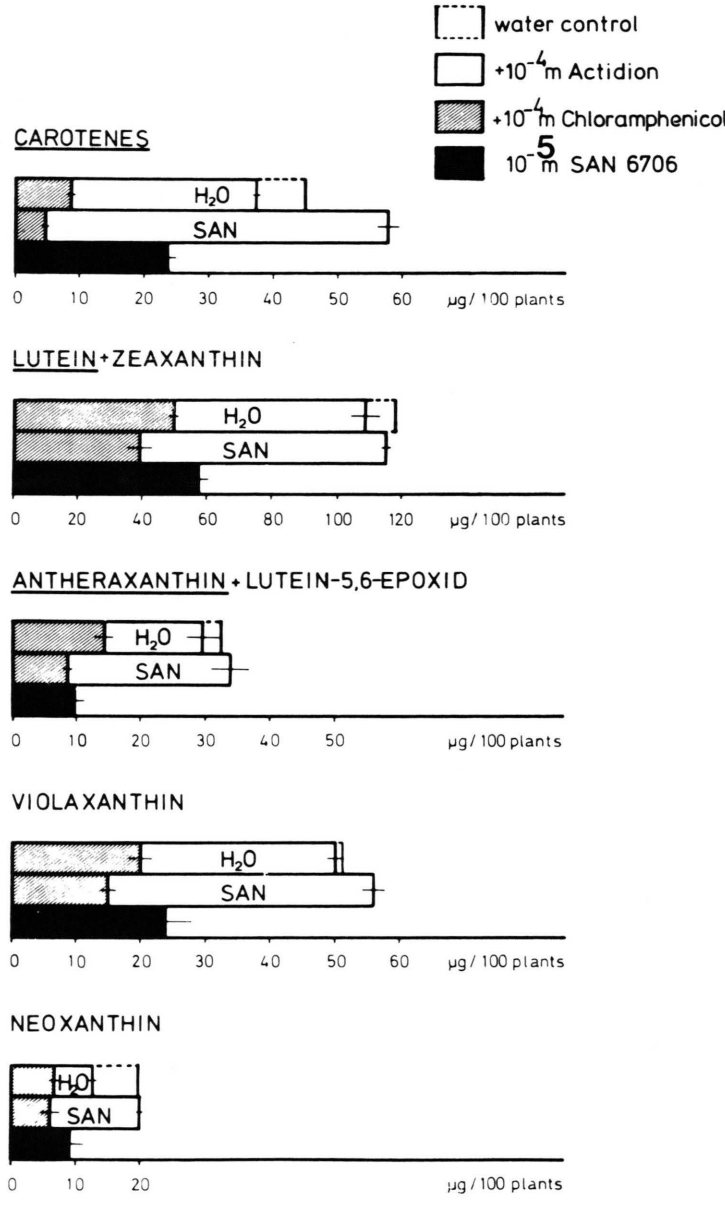


Fig. 6. The effect of chloramphenicol and actidion on the carotenoid content of etiolated radish seedlings supplied with $10^{-5}$ m SAN 6706 after 24 h illumination with fluorescent white light.

reactions and mainly by blocking chloroplastic ones [14]. Ureas and benzothiadiazinones are known to inhibit the photosystem II dependent Hill reaction while triazoles and pyridazinones block the accumulation of chlorophylls and carotenoids and disturbe the assembly of the thylakoid membrane [2].

Chlorophylls and carotenoids are essential constituents of the thylakoid membrane. Their biosynthesis is controlled by active phytochrome and proceeds exclusively in the chloroplast. Therefore we investigated the action of phytochrome on the biosynthesis of chlorophylls and carotenoids in radish treated with photosystem II and bleaching herbicides. The aim was, to obtain whether phytochrome is involved in the development of the herbicidal toxicity.

As shown in Figs 2–4 in all herbicide treated seedlings a phytochrome mediated chlorophyll and

carotenoid biosynthesis takes place like in untreated control plants. Only SAN 6706 reduces the accumulation of carotenoids up to 50%. From this result we conclude that photosystem II and bleaching herbicides do not interfere with the primary action of phytochrome but do develop their toxicity under the influence of phytochrome. This assumption is supported by the observation that the herbicidal response on the terpenoid metabolism of the chloroplast is established predominantly in continuous red light [15].

Besides specific reactions in the chloroplast many investigations reveal a herbicide effect in the cytoplasm and propose that the extrachloroplastic effects contribute to the development of the herbicidal toxicity in the chloroplast [2]. To analyse this subject further we inhibited protein synthesis of the herbicide treated plants either in the chloroplast or in the cytoplasm. As shown in Figs 5 and 6 inhibition of protein synthesis in the chloroplast enhances the SAN 6706 induced bleaching effect. On the other hand application of actidion which inhibits protein synthesis in the cytoplasm completely abolishes the bleaching effect of SAN leading to an accumulation of chlorophylls and carotenoids with an even higher concentration than in plants treated only with actidion. From this it is concluded that the effect of SAN 6706, which causes a photooxidative destruction of pigments is enhanced by the simultaneous inhibition of protein synthesis in the chloroplast. The observation, that in plants treated with SAN 6706 in combination with actidion no photooxidation of pigments occurs gives strong evidence that the photooxidative damage of the chloroplast under the influence of the bleaching herbicide SAN 6706 is developed by the cytoplasm. This means that SAN 6706 either is metabolized into a toxic form in the cytoplasm and thereafter transferred into the chloroplast or that SAN 6706 can only develop its herbicidal response in combination with a cytoplasmic factor. Further investigations concerning this problem are in progress.

*Acknowledgements*

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