Herbicides which Inhibit Electron Transport or Produce Chlorosis and Their Effect on Chloroplast Development in Radish Seedlings.
III. Plastid Pigment and Quinone Composition

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The effect of DCMU, bentazon, amitrole and SAN 6706 on the formation of chloroplast pigments and quinones was investigated using plants that were grown in total darkness or continuous white, red or far-red light.

All herbicides assayed affected the formation of chlorophylls, carotenoids and quinones but DCMU had only minor effects. Like for chlorophylls and carotenoids the formation of quinones was most suppressed in plants grown in the presence of the herbicide in continuous white or red light, but the effect on the formation of quinones was much lower as compared to the pigments. The observation that the biosynthesis of quinones is still maintained in SAN 6706 treated bleached plastids which are lacking chlorophylls and carotenoids indicates that quinones are synthesized at the plastitd envelope and stored in the osmiophilic plastoglobuli.

Amitrole and SAN 6706 induced a strong chlorosis. It was of particular interest that chlorosis was also induced by the photosystem II inhibitor bentazon. DCMU was not effective. The inhibitor concentration for 50% inhibition in the chlorophyll and carotenoid content was 5×10^{-6} M for SAN 6706,3×10^{-4} M for amitrole and 10^{-3} M for bentazon.

As already reported by others SAN 6706 treated plants accumulated phytoene in large amounts. The highest phytoene content was observed in plants that were grown in the dark. Amitrole treated plants accumulated lycopene. But in addition other carotenoid precursors like phytoene and phytofluene were also accumulated. In contrast to phytoene lycopene was only accumulated in plants that were grown in the light.

Particularly for SAN 6706 and amitrole the expression of the bleaching effect was depending on the light intensity and light quality that was used during plant growth. The herbicide effect ewas predominantly expressed at higher light intensities and after irradiation with red light. The observation that the induction of chlorosis is very sensitive to red light as compared to white or blue light is suggesting that phytochrome is involved in the development of the herbicide toxicity. It also supports that in SAN treated plants chlorophylls are photodecomposed directly by light because of the lack of photoprotecting carotenoids but mainly β-carotene in these plastids. Further support for this was given by the demonstration that SAN treated plants which were grown at very low light intensities turned green and were photosynthetically active.

Introduction

Herbicides which affect photosynthesis have been used successfully for the investigation of electron transport mechanisms and the assembly of the thylakoid membrane during chloroplast development [1 – 7]. In this process pigment protein complexes which are involved in the absorption of sun light and the transfer of the absorbed energy to the photosynthetic reaction centers like the associated electron carriers are inserted into the thylakoid membrane in a highly specific orientation in order to guarantee photosynthesis under optimum conditions [6, 8 – 11].

Particularly photosystem II inhibitors have given much insight into the organization of the photosystem II complex [1, 6, 12]. Only recently bleaching herbicides which interfere with the biosynthesis of carotenoids and the accumulation of chlorophylls have been used extensively for the investigation of the light harvesting systems and the photosynthetic reaction centers [13 – 15].

In previous contributions we have reported that the photosystem II herbicides DCMU and bentazon and the bleaching herbicides amitrole and SAN 6706 have many sites of action within the chloroplast [14 – 16]. Photosystem II herbicides inhibit electron transport at the donor side of photosystem II but in addition alter the long wavelength chlorophyll fluorescence emission and interfere with the formation of subthylakoid particles [15]. The bleaching pyridazinones and aminotriazoles changed the pigment protein complex composition of the thylakoid membrane drastically leading to a disturbed transfer of

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light energy from the light harvesting systems to the photosynthetic reaction centers and finally to the photooxidative damage of the chloroplast [15].

How far these herbicide induced alterations in the transfer of light energy are caused by the disorientation of the pigment protein complexes or by the changed pigment composition of the thylakoid membrane should be worthwhile for an investigation.

In order to approach this problem further the chlorophyll, carotenoid and quinone content of radish seedlings that were grown in the presence of the photosystem II inhibitors DCMU and bentazon and the chlorosis inducers SAN 6706 and amitrole was investigated. The effect of light intensity and light quality on the expression of the herbicide toxicity was also investigated. The consequences of the herbicide induced changes in plastid pigment and quinone composition for the absorption and transfer of light energy the stability of the thylakoid membrane and photosynthesis will be discussed.

Materials and Methods

Cultivation of plants

Application of photosystem II herbicides (DCMU, bentazon) and bleaching herbicides (SAN 6706, Amitrole) as well as cultivation of plants was carried out as described previously [17]. Irradiation of plants with fuorescent white light was performed using Osram Fuora lamps (55 W; 8 W/m^2). Irradiation with red light was carried out using Philips lamps (TL 40 W/15; 1.6 W/m^2), a red filter (Plexiglas 555, Fa. Röhm Darmstadt) and an infrared filter. The far-red light source consisted of Osram Linestra lamps (120 W; 1.0 W/m^2) a blue filter (Plexiglas 627, Fa. Röhm Darmstadt) and an infrared filter (KG1, Fa. Schott Mainz).

Extraction, chromatography and quantitative determination of plastid pigments and quinones

After 6 days growth in total darkness or continuous red, far-red or white light plants were harvested and their pigments and quinones extrated [17]. Chlorophylls were quantitatively estimated according to Ziegler and Egle [18]. Quinones were separated and quantitatively estimated according to Lichtenthaler *et al.* [19] and Grumbach [17]. Separation and quantitative determination of carotenoids was carried out according to Egger [20], Hager and Meyer-Bertenrath [21] and Britton and Goodwin [22].

For the rapid and convenient separation of chlorophylls and carotenoids a new separation system consisting of silicagel ready plates or neutralized silica gel and diethylether as solvent system was developed. All presented data are means of six independent experiments ± standard error.

Results

Formation of plastid pigments and quinones

Etiolated radish seedlings are devoid of chlorophylls but synthesize carotenoids and quinones [17]. In the dark bentazon, DCMU and amitrole had no inhibitory effect on the formation of carotenoids and quinones but their biosynthesis was rather pronounced (Table I – III). Only in SAN 6706 treated seedlings the carotenoid and quinone content was drastically reduced. Similar results were also obtained from plants that were grown in the presence of the herbicides in continuous far-red light although the total carotenoid and quinone content was much higher due to phytochrome action [23]. The small amounts of chlorophylls that were obtained in these plants may be explained by the trace amounts of light (< 730 nm) that was still passing through the filter system of the far red light source.

As already reported in previous investigations the main differences in the accumulation of plastid pigments and quinones became visible only in the light and after longer illumination times (Table I – III) [24].

In radish that was grown in the presence of DCMU, or bentazon the carotenoid content was reduced as compared to the untreated plants but even more drastically in plants that were grown in the presence of amitrole, or SAN 6706 (Fig. 2). The observation that the photosystem II inhibitor bentazon was also inducing chlorosis like the bleaching herbicides SAN 6706 and amitrole was very surprising (Figs 1 and 2).

As compared to the carotenoids the quinone content of the plastid was only slightly reduced by all herbicides assayed. However, it was of particular interest that the expression of the herbicide effect in continuous white light was exactly the same or even pronounced when the plants were grown in continuous red light (Table I – III).

Table I. Chlorophyll content (µg/400 plants) of radish seedlings treated with photosystem-II or bleaching herbicides after 6 d growth in the dark or continuous far-red, red or white light.

	Control	10^{-5} M SAN 6706	10^{-4} M Amitrole	10^{-4} M DCMU	10^{-4} M Bentazon
Chlorophylls					
dark	–	–	–	–	–
cont.far-red light	738.8	892.8	777.2	705.2	983.6
cont.red light	16192.0	208.8	7814.8	15280.4	14725.6
cont.white light	24462.2	213.8	6374.4	19383.3	17235.0

Table II. Carotenoid content (µg/400 plants) of radish seedlings treated with photosystem-II or bleaching herbicides after 6 d growth in the dark or continuous far-red, red or white light.

	Control	10^{-5} M SAN 6706	10^{-4} M Amitrole	10^{-4} M DCMU	10^{-4} M Bentazon
Carotenoids					
dark	665.8	413.6	783.2	807.8	687.2
cont.far-red light	949.8	1169.6	1116.0	1111.6	1166.0
cont.red light	3280.8	266.4	1954.8	2493.2	2004.4
cont.white light	4545.6	215.2	1796.0	2705.6	2474.4

Table III. Prenylquinone content (µg/400 plants) of radish seedlings treated with photosystem-II or bleaching herbicides after 6 d growth in the dark or continuous far-red, red or white light.

	Control	10^{-5} M SAN 6706	10^{-4} M Amitrole	10^{-4} M DCMU	10^{-4} M Bentazon
Prenylquinones					
dark	715.1	447.8	612.7	659.9	690.0
cont.far-red light	1410.8	989.9	1096.1	1024.5	996.7
cont.red light	1291.6	942.8	1061.2	994.9	1054.7
cont.white light	1362.4	1007.4	973.8	1002.9	846.9

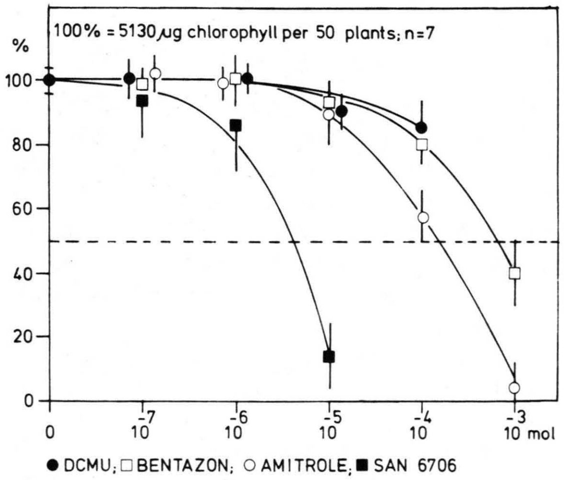

Fig. 1. Inhibition of chlorophyll formation by photosystem II or bleaching herbicides. Plants were grown for 6 days in continuous white light.

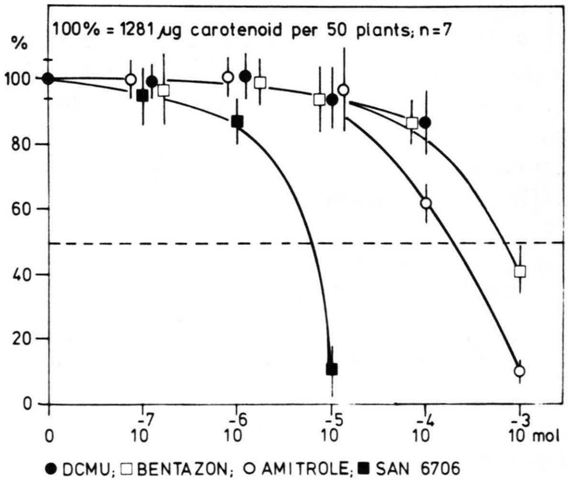

Fig. 2. Inhibition of carotenoid formation by photosystem II or bleaching herbicides. Plants were grown for 6 days in continuous white light.

Formation of carotenoids

Light acting through phytochrome enhances the formation of chlorophylls, carotenoids and quinones (Table I – IV, Table VI) [25 – 27]. In etiolated radish seedlings the lowest carotenoid content was observed while plants grown in continuous far red light accumulated carotenoids in higher amounts (Table IV). The highest carotenoid content was found in plants that were grown in continuous white or red light.

As shown in Table II in the used concentrations amitrole and SAN 6706 did not suppress the formation of carotenes and xanthophylls in the dark or continuous far red light while in plants that were grown in the presence of DCMU or bentazon carotenoid formation was even enhanced. It should be mentioned, however, that under unphysiological high concentrations of SAN 6706 (10^{-4} M) or amitrole (10^{-3} M) the carotenoid content was also drastically reduced in etiolated plants. Only in the light (white or red) the herbicides assayed suppressed the formation of carotenoids. Plastids from plants grown in the presence of DCMU or bentazon in the light contained less β-carotene and less xanthophylls than untreated plants. A similar reduction but no inhibition in the carotene and xanthophyll content was also obtained in amitrole treated plants (Table IV).

As compared to amitrole SAN 6706 induced a strong chlorosis and the β-carotene and xanthophyll content was reduced to 5% of that from untreated plants.

Formation of the carotenoid precursors phytoene and lycopene

Amitrole and SAN 6706 interfere with the biosynthesis of carotenoids [28 – 32]. Amitrole specifically inhibits the cyclization of lycopene to α- and β-carotene [16]. SAN 6706 inhibits the desaturation of phytoene [29 – 31]. How far the inhibitory effect of both herbicides is due to light intensity and light quality is shown in Table V. Plants grown in the presence of DCMU, bentazon or untreated plants contained no phytoene and lycopene only in very small amounts (Table V). Irrespective of the light in-

Table IV. Carotenoid content (µg/400 plants ± SE) of radish seedlings treated with photosystem-II or bleaching herbicides after 6 d growth in the dark or continuous far-red, red or white light.

	Control	10^{-5} M SAN 6706	10^{-4} M Amitrole	10^{-4} M DCMU	10^{-4} M Bentazon
β-Carotene					
dark	49.2 ± 4.1	47.6 ± 5.7	59.2 ± 3.9	70.4 ± 7.2	64.8 ± 5.9
cont.far-red light	80.0 ± 3.9	123.2 ± 4.7	330.0 ± 3.0	88.0 ± 2.5	112.0 ± 4.9
cont.red light	679.2 ± 12.4	34.8 ± 2.4	628.0 ± 25.0	443.6 ± 9.4	401.2 ± 14.0
cont.white light	1122.4 ± 9.5	82.4 ± 0.3	480.8 ± 5.5	576.0 ± 1.3	566.4 ± 2.8
Lutein + Zeaxanthin					
dark	363.6 ± 1.9	202.0 ± 3.1	381.2 ± 1.6	406.4 ± 1.7	333.6 ± 1.4
cont.far-red light	681.2 ± 40.1	622.8 ± 17.2	429.2 ± 4.4	582.4 ± 20.0	620.0 ± 17.4
cont.red light	1474.8 ± 18.0	102.8 ± 3.3	677.6 ± 10.5	1274.4 ± 30.4	814.4 ± 14.5
cont.white light	1861.6 ± 31.2	99.2 ± 2.0	560.0 ± 16.0	1236.0 ± 21.2	1047.2 ± 10.8
Antheraxanthin					
dark	90.0 ± 1.3	49.6 ± 0.3	120.8 ± 1.6	114.4 ± 1.5	114.0 ± 0.9
cont.far-red light	68.8 ± 1.2	171.2 ± 8.1	95.2 ± 5.7	108.0 ± 7.5	180.8 ± 6.7
cont.red light	458.8 ± 24.0	42.0 ± 1.3	162.0 ± 7.1	208.8 ± 13.4	236.8 ± 4.0
cont.white light	396.8 ± 12.8	16.0 ± 2.4	249.6 ± 16.0	198.4 ± 12.4	198.4 ± 10.0
Violaxanthin					
dark	132.2 ± 1.4	88.0 ± 1.5	184.0 ± 2.0	185.6 ± 1.4	154.0 ± 1.4
cont.far-red light	155.0 ± 3.3	172.8 ± 10.0	184.8 ± 5.7	221.6 ± 11.2	253.2 ± 7.4
cont.red light	342.8 ± 16.0	53.2 ± 3.1	304.0 ± 10.1	279.2 ± 11.8	316.8 ± 4.1
cont.white light	562.4 ± 16.4	11.2 ± 1.2	281.6 ± 14.4	336.8 ± 8.0	342.4 ± 11.6
Neoxanthin					
dark	30.8 ± 1.4	26.4 ± 2.8	38.0 ± 1.7	31.0 ± 0.4	20.8 ± 0.5
cont.far-red light	44.8 ± 1.0	79.6 ± 5.8	76.9 ± 5.0	111.6 ± 7.2	74.0 ± 1.9
cont.red light	325.2 ± 17.8	33.6 ± 1.7	183.2 ± 8.1	287.2 ± 12.0	235.2 ± 3.0
cont.white light	582.4 ± 12.0	6.4 ± 0.4	224.0 ± 9.6	358.4 ± 10.4	320.0 ± 7.2

Table V. Formation of lycopene and phytoene (μg/400 plants) in radish seedlings treated with photosystem-II or bleaching herbicides after 6 d growth in the dark or continuous far-red, red or white light.

	Control	10^{-5} M SAN 6706	10^{-4} M Amitrole	10^{-4} M DCMU	10^{-4} M Bentazon
Phytoene					
dark	n.d.	1138.0 ± 58.7	60.1 ± 4.7	n.d.	n.d.
cont.far-red light	n.d.	578.2 ± 10.5	632.4 ± 28.7	n.d.	n.d.
cont.red light	n.d.	894.8 ± 40.1	98.0 ± 18.7	n.d.	n.d.
cont.white light	n.d.	712.3 ± 21.7	200.0 ± 11.4	n.d.	n.d.
Lycopene					
dark	n.d.	1.0 ± 0.1	6.8 ± 1.3	n.d.	n.d.
cont.far-red light	n.d.	3.8 ± 0.3	61.0 ± 2.5	n.d.	n.d.
cont.red light	trace	3.2 ± 0.2	148.0 ± 3.7	trace	trace
cont.whithe light	trace	3.4 ± 0.4	136.0 ± 2.8	trace	trace

tensity or light quality that they received SAN 6706 treated plants accumulated phytoene in large amounts. Lycopene was present in these plants only in trace amounts. The highest accumulation of phytoene occurred in SAN treated plants that were grown in the dark. Slightly lower concentrations were obtained during growth in continuous far red, red or white light.

Amitrole treated plants accumulated lycopene. In contrast to the biosynthesis of phytoene in SAN treated plants lycopene biosynthesis was enhanced by light (Table V). While etiolated amitrole treated plants contained lycopene only in minute amounts much more carotenoid precursor was synthesized in continuous far red light. The highest lycopene content was detected in plants that were grown in red or white light.

Besides in lycopene plastids from amitrole treated plants were also enriched in phytoene. Particularly growth in continuous far red light stimulated in formation of phytoene. How far these differences obtained in the phytoene and lycopene content are controlled by phytochrome is under investigation. However, as already shown for carotenoids the effect of SAN 6706 or amitrole on the biosynthesis and accumulation of lycopene and phytoene was also very similar in plants grown in continuous white or red light.

Formation of quinones

Phytochrome enhances the formation of quinones (Table VI) [27]. While in etiolated seedlings the lowest quinone content was detected growth in continuous far red, red or white light pronounced the formation of all quinones determined. In contrast to the differences obtained in the carotenoid content between untreated plants that were grown in far red and white (or red) light the quinone content was more or less the same (Table VI).

All herbicides assayed suppressed the formation of plastoquinone-9, plastohydroquinone-9, α-tocopherol and α-tocoquinone but the inhibitory effect was much less as compared to the carotenoids. Again similar as for carotenoids the main inhibitory effect on the formation of quinones was expressed only during growth in continuous white or red light.

Among all herbicides assayed SAN 6706 exhibited the strongest effect. In SAN treated plants the concentration of the oxidized quinones plastoquinone-9 and α-tocoquinone was drastically reduced while the formation of α-tocopherol and plastohydroquinone-9 was unaffected. This observation most likely suggests that quinone biosynthesis can still take place in SAN bleached plastids and that the newly synthesized quinones are probably stored in the osmiophilic plastoglobuli. An enrichment in plastoglobuli of SAN bleached plastids has been demonstrated by electron microscopical studies [33].

The influence of light intensity on the herbicide effect

That herbicides which inhibit electron transport or produce chlorosis express their toxicity only in the light and after longer illumination times has been reported by several independent groups [24, 34–36]. This is simply explained by the fact that the electron transport chain the primary target for the photosystem II inhibitors is only formed in the light during chloroplast development. On the other hand bleaching herbicides like SAN 6706 and amitrole interfere with the existing carotenoid biosynthesis al-

Table VI. Prenylquinone content (µg/400 plants ±SE) of radish seedlings treated with photosystem-II or bleaching after 6 d growth in the dark or continuous far-red, red or white light.

	Control	10^{-5} M SAN 6706	10^{-4} M Amitrole	10^{-4} M DCMU	10^{-4} M Bentazon
Plastoquinone-9					
dark	270.3±24.9	122.0±13.5	181.0±18.0	170.5±18.1	192.5±20.8
cont.far-red light	370.5±25.0	211.8±13.5	343.0±55.0	265.5±18.0	264.8±17.0
cont.red light	442.6±42.5	84.9±21.4	208.0±26.0	282.6±23.0	274.1±33.0
cont.white light	428.3±15.8	96.4±10.0	217.4±28.0	340.8±25.0	223.3±28.5
Plastohydroquinone-9					
dark	201.5±21.3	65.9±12.5	224.0±17.4	217.5±25.0	238.8±25.7
cont.far-red light	382.6±30.0	228.6±35.0	261.3±25.4	303.7±30.1	235.2±30.4
cont.red light	288.3±25.1	283.4±21.7	336.1±22.0	270.0±19.0	269.6±20.0
cont.white light	349.1±18.4	305.5±33.7	281.0±14.1	193.2± 9.6	160.4±17.2
α-Tocopherol					
dark	211.3±14.6	231.9±36.0	179.9±10.0	241.2±35.0	228.8±25.5
cont.far-red light	618.3±35.0	512.5±55.1	460.5±50.2	418.0±38.4	463.2±40.0
cont.red light	520.2±48.0	551.2±17.2	488.2±38.0	413.8±48.0	490.2±26.1
cont.white light	544.0±35.1	572.9±27.8	453.8±16.4	442.2±58.9	435.7±34.7
α-Tocoquinone					
dark	32.0± 5.4	28.0± 6.6	27.8± 7.4	30.7± 5.8	29.9± 4.3
cont.far-red light	39.4± 8.6	37.0± 1.5	31.3± 5.0	37.3± 2.1	33.5± 1.0
cont.red light	40.5± 8.0	23.3± 2.0	28.9± 4.0	28.5± 2.1	20.8± 3.3
cont.white light	41.0± 7.1	32.6± 5.2	21.6± 6.4	26.7± 3.7	27.5± 4.0

ready in the etioplast. However, SAN treated plants which are grown at low light intensities turn green and contain fully developed chloroplasts which are photosynthetically active while at higher light intensities the leaves are lacking chlorophylls and carotenoids. Plastids from these leaves are photosynthetically inactive. From this it is suggested that not only the primar action of the bleaching herbicide on carotenoid biosynthesis but rather the combined action of strong light and oxygen is leading to the photooxidative destruction of the chlorophylls which are no more protected by the carotenoids.

The influence of light quality on the herbicide effect

In previous experiments we have shown that all herbicides assayed do not interfere with the action of

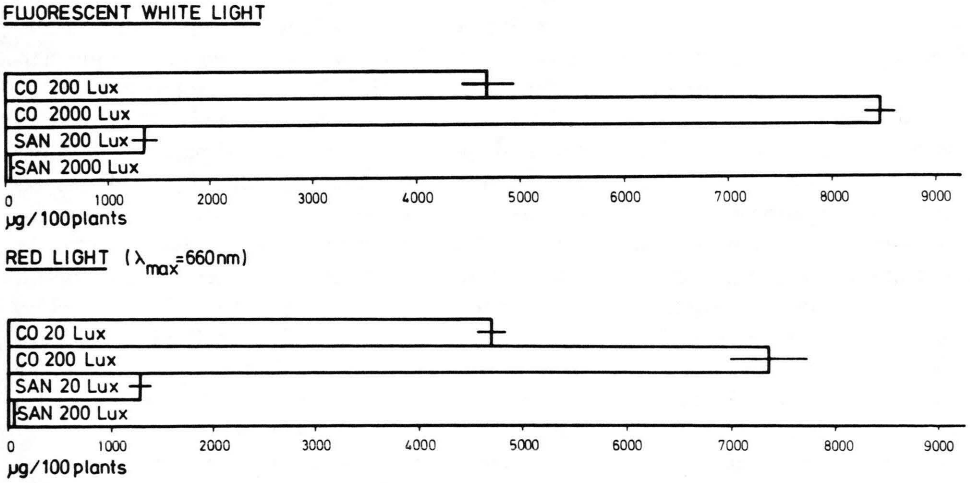

Fig. 3. Effect of different intensities of white or red light on the formation of chlorophylls in radish seedlings that were grown in the presence of the bleaching herbicide SAN 6706.

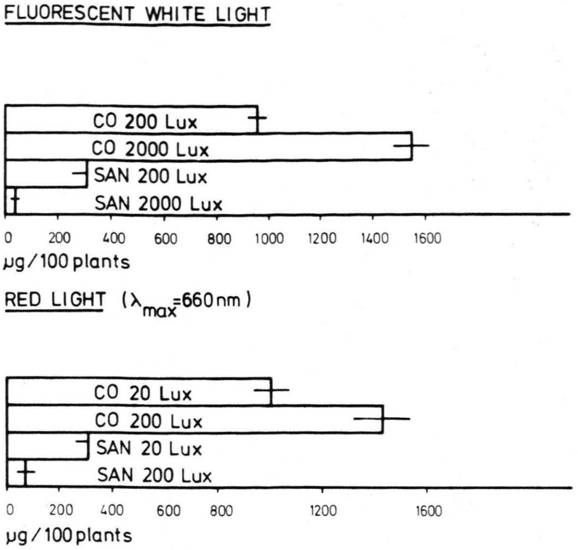

Fig. 4. Effect of different intensities of white or red light on the formation of carotenoids in radish seedlings that were grown in the presence of the bleaching herbicide SAN 6706.

phytochrome [23]. Phytochrome is rather pronouncing the herbicide effect. This is supported by the observation that the herbicide effect is predominently expressed during growth in red light. That the induction of chlorosis by bleaching herbicides is very sensitive to red light was also reported by others [34, 35].

Discussion

The application of the photosystem II inhibitor DCMU did not change the pigment and quinone composition of the plastid drastically although photosynthesis was inhibited (Tables I – VI). Like DCMU, bentazon was also inhibiting photosynthesis but in addition exhibited chlorotic properties (Figs 1 and 2). This may be the reason why bentazon besides inhibiting electron transport at the donor side of photosystem II was also affecting photosystem I while DCMU was mainly affecting photosystem II.

As compared to the electron inhibitors the pyridazinone SAN 6706 and aminotriazole induced a strong chlorosis leading to the photooxidative destruction of the chloroplast. SAN 6706 which specifically inhibits the desaturation of phytoene was a much stronger chlorosis inducer than amitrole which inhibits the cyclization of lycopene (Figs 1 and 2, Table V). This observation most likely suggests that lycopene in part is capable of replacing the protective function of β-carotene or carotenoids. Further support for the photoprotective function of carotenoids was given by the fact that the bleaching effect of SAN 6706 and amitrole was only expressed in strong light and was nearly abolished in plants that were grown under low light intensities.

An analysis of the influence of different light qualities on the herbicide effect revealed that the herbicide effect was very sensitive to red light suggesting that phytochrome is involved in the expression of the herbicide effect. It also shows that the chlorophylls themselves are photodecomposed directly in red light in SAN treated carotenoid deficient plastids

As compared to the photosynthetic pigments the formation of plastid quinones is not a direct target of herbicide action neither for photosystem II nor for bleaching herbicides. Quinone biosynthesis may be suppressed in the plastids by the lack of ATP or reducing aequivalents caused by the inhibition of photosynthesis but is still maintained and the synthesized quinones are stored in the osmiophilic plastoglobuli if they can not be incorporated into the thylakoid membrane. This has also been detected by the observation of high amounts of plastoglobuli in SAN bleached or bentazon inhibited plastids [33, 37].

Chlorophylls and carotenoids are bound to protein [38] and contained in the thylakoid membrane in highly specific orientated integral protein complexes [2, 8, 39]. Nearly half of the chlorophylls and probably all xanthophylls are contained in the light harvesting complex [39]. Purified photosystem I particles are enriched in β-carotene and contain no xanthophylls [40 – 42]. Photosystem II particles also contain β-carotene as major carotenoid [43, 44]. As accessory pigments carotenoids absorb light and transfer the absorbed energy to the photosynthetic reaction centers [45]. They also protect the chloroplast from photooxidative destruction which is caused by singlet oxygen or triplett chlorophylls [46]. This protection is predominently fulfilled by β-carotene which is very close associated to the chlorophyll a molecules in photosystem I [47, 48].

Particularly in greening leaves chlorophyll tripletts have been detected [49, 50] and it is known that during the first hours of greening, similar as it is the case in plants that are grown in the presence of a bleaching herbicide, when the thylakoid membrane is not yet fully developed the newly formed chlorophylls are very photolabile. Therefore under conditions where chlorophylls and carotenoids are not yet integrated into the thylakoid membrane carotenoids must be present for the protection of chlorophylls and developing photosynthesis. Any herbicide that is inhibiting carotenoid biosynthesis may therefore disturbe this protection mechanism leading to a photooxidative damage of the reaction centers.

On the other hand pure preparations of photosystem I and II particles are enriched in β-carotene and the removal of β-carotene from chloroplasts by organic solvents inhibits photosynthetic activity [51, 52]. This may explain the observation that in plastids from radish seedlings that were grown in the presence of SAN 6706 under low light intensity photosynthesis is maintained. Under these conditions there is enough β-carotene present and chlorophylls like photosynthesis are not destroyed by photooxidative processes. In chloroplasts from these cotyledons only photosystem I is slightly affected as was demonstrated previously by the decrease in the long wavelength chlorophyll fluorescence emission at 740 nm and PAGE-studies as well as the lower carotenoid content [15]. As compared to SAN, in plastids from amitrole treated plants that were greened under low light intensities, photosytem I was much less affected supporting that the protective function of β-carotene can be partly replaced by lycopene which is present in these plastids in large amounts.

As compared to the photosystem II inhibitors DCMU and bentazon which affect photosynthesis by inhibiting electron transport the bleaching herbicides SAN 6706 and amitrole interact with the photoprotection mechanism of the chloroplast by inhibiting carotenoid biosynthesis. During photodecomposition of the chloroplast the effect on photosystem I seems to be the initial response to the herbicide attack which induces a carotene deficiency. Only later a photodestruction of preexisting chlorophylls and carotenoids in the thylakoid membrane takes place. This photodestruction is probably caused by the inhibition of the cyclic electron transport around photosystem I as demonstrated by Ridley [35]. Photosystem I and cyclic electron transport seem to be very important for the dissipation of excess light energy and any herbicides that affects or destroyes this protection mechanism either by inhibiting electron transport or by inhibiting carotenoid biosynthesis and changing the β-carotene to chlorophyll ratio in photosystem I may finally induce chloroplast degradation.

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