Mechanism of Bleaching in Leaves Treated with Chlorosis-Inducing Herbicides

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Bleaching of chlorophyll was studied in the leaves of rye seedlings (*Secale cereale* L.) treated with four chlorosis-inducing herbicides of different potency (weak photodestructions, group 1: aminotriazole, haloxidine; strong photodestructions, group 2: San 6706, difunone). Chlorophyll deficiency and particularly the inactivation of a chloroplast marker enzyme, NADP-dependent glyceraldehyde-3-P dehydrogenase, that occurred in the presence of group 2 herbicides were stronger in red, than in blue, light.

When grown in white light of low intensity (10 lx) herbicide-treated leaves contained chlorophyll, 70 S ribosomes and unimpaired activities of NADP-dependent glyceraldehyde-3-P dehydrogenase. At 10 lx only the leaves treated with SAN 6706 and difunone were strongly carotenoid-deficient but not those treated with group 1 herbicides. After all herbicide treatments 10 lx-grown leaf tissue was, however, not capable of photosynthetic O_2-evolution indicating some disorder of photosynthetic electron transport. Leaf segments grown at 10 lx were exposed to a high light intensity of 30000 lx at either 0 °C or 30 °C. In treatments with group 1 herbicides chlorophyll accumulation was stopped in bright light at 30 °C but breakdown was not apparent. Only at 0 °C and in the presence of high, growth-reducing, herbicide concentrations chlorophyll was slightly degraded. The RNAs of the 70 S ribosomes were, however, clearly destroyed at 30000 lx and 30 °C in aminotriazole-treated leaves. In leaves treated with group 2 herbicides chlorophyll was rapidly degraded at 30000 lx both at 0 °C and 30 °C, however, only in the presence of O_2, indicating a true photooxidative and mainly photochemical nature of the reactions involved. This chlorophyll breakdown was accompanied by the photodestruction of 70 S ribosomes and the inactivation of NADP-glyceraldehyde-3-P dehydrogenase. In treatments with group 1 herbicides photoinactivation of the latter enzyme did not occur, although it was clearly localized in the bleached plastids, as demonstrated by gradient separation of organelles.

In the presence of group 2 herbicides the chlorosis was originating from a direct photooxidation of chlorophyll, accompanied by a massive destruction of other plastid constituents and functions. In treatments with group 1 herbicides photodestructions appeared to be much weaker and insufficient to affect chlorophyll directly. Mediated through some photodestructive interference with obviously more sensitive plastid components, such as their ribosomes, further chlorophyll accumulation was, however, prevented.

Introduction

The action of various herbicides that induce chlorosis when applied to growing plants or cultures [1–5] is not attributed to any primary interference with steps of chlorophyll biosynthesis itself since synthesis and phototransformation of protochlorophyll(ide) were not blocked [1] and observations on the inhibition of single enzymes of chlorophyll synthesis [6] were not confirmed as general symptoms of the herbicide action in other systems [7]. That, finally, in the mature bleached leaf the capacity for chlorophyll synthesis may well be limited by a rather low capacity for ALA and protochlorophyll(ide) synthesis seems to be a secondary consequence in the syndrom of events connected with herbicide-induced bleaching [8]. The primary action of the chlorosis-inducing herbicides is at present mostly seen in a strong inhibition of carotenoid synthesis [1, 5, 9]. Chlorophyll-sensitized photodynamic reactions occurring in the absence of protecting carotenoids [10] are assumed to lead to the photodestruction of chlorophyll as well as of other chloroplast constituents, such as lipids, nucleic acids and proteins [1, 8, 9, 11, 12]. San 9789 and difunon have been shown to act as inhibitors of β-carotene formation also in the fungus *Phycomyces* [4] and recently a direct *in vitro* inhibition of an individual enzyme of carotenoid biosynthesis, phytoene synthetase, by the pyridazinone herbicide San 9789 has been demonstrated in a cell-free system from *Phycomyces* [13].

Abbreviations: ALA, δ-aminolevulinic acid, San, Sandoz.

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Several observations question, however, whether bleaching originated in all instances solely from a herbicide-induced carotene-deficiency. In *Scenedesmus* carotenoids were themselves photooxidized only in light and the amount photooxidized did not correlate with the disappearance of chlorophyll [3]. Photosynthetic oxygen evolution was also strongly inhibited [3]; the pyridazinone herbicides are to some extent capable of interfering directly with the photosynthetic electron transport [9]. In addition, these herbicides lead to an inactivation of catalase and some also suppressed the appearance of other peroxisomal enzymes [2, 8]. Such herbicide effects could also give rise to photooxidative damage since disorders of the photosynthetic electron transport chain can lead to the generation of several activated oxygen forms and also the function of peroxisomal photorespiration is regarded as a "safety valve" necessary for protection against photodestructive damage (for reviews see ref. [14]). The photodestruction of chloroplast ribosomes occurring after treatments with chlorosis-inducing herbicides [2, 12] is certainly a secondary effect, can, however, contribute to the origin of the chlorosis since ribosome-deficient plastids are unable to accumulate chlorophyll [15, 16].

In the present investigation we have used leaves grown at a low light intensity to discriminate immediate herbicide effects from those resulting secondarily from photodestructive events. The latter were studied after transfer of low-light-grown leaf sections to a high light intensity. This gave the possibility to compare whether chlorophyll was in all treatments with chlorosis-inducing herbicides directly photodegraded. We have further characterized the mode of photodestruction of chlorophyll and other chloroplast constituents with regard to an involvement of photodynamic or metabolic reactions, the need of O_2, and the efficiency of different light qualities. The approach of exposing dim-light-grown leaf segments to bright light has been shortly and in preliminary form introduced in a previous summarizing report [8].

Materials and Methods

Plant material and growing conditions

Experiments were performed with seedlings of winter rye (*Secale cereale* L. cv. Petkus "Kustro"). The seeds were surface-sterilized by a 5 min vacuum infiltration and about 30 min soaking in a freshly prepared, filtered solution of 3% calcium hypochlorite, thoroughly washed with demineralized H_2O and placed in plastic boxes on filter paper (Schleicher & Schüll, No. 598) moistened with either distilled H_2O, or a herbicide solution, as indicated. The seedlings were grown for 6 days at 22 °C.

Continuous irradiation with white light was provided by fluorescent tubes (Astra, 40 W, Tageslicht and Warmton de Luxe in alternating sequence) giving an incident intensity of either 5000 ± 500 lx or 10 lx (Warmton de Luxe only). Continuous red ($5.5-6.0 \times 10^{-5}$ W cm^{-2}) and blue light ($8.5-9.0 \times 10^{-5}$ W cm^{-2}) were obtained from combinations of Philips fluorescent tubes TL 40 W/15 with Röhm & Haas (Darmstadt, Germany) Plexiglas filter Red 501, or Philips TL 40 W/18 in combinations with Röhm & Haas filter Blue 627, respectively, as described [17].

The following herbicides and concentrations were used: 0.25 and 0.3 mM 3-amino-1,2,4-triazole (amitrole), 0.05 and 0.15 mM 3,5-dichloro-2,6-difluoro-4-hydroxypyridine (haloxidine), 0.01 and 0.02 mM 4-chloro-5-(dimethylamino)-2,α,α,α,(trifluoro-m-tolyl)-3(2H)-pyridazinone (Sandoz 6706, metflurazon), 0.05 and 0.1 mM 5-dimethylamino-methylene-2-oxo-4-phenyl-2,5-dihydrofuranecarbonitrile-(3) (difunone, EMD-IT-5914).

Bleaching experiments at high light intensity

From 6-day-old rye seedlings grown at 10 lx the upper and lower quarters of each leaf were excised in dim green safety light. The resulting middle sections were divided into two halves and arranged in parallel order in petri dishes (5.0 cm diameter) containing 7.0 ml 0.1 M K-phosphate buffer, pH 6.0. The petri dishes were placed on two layers of moist filter paper in glass-covered plastic boxes. These plastic boxes were immersed either in a water bath of 30 °C or in an ice bath of 0 °C, and the latter were covered with another glass-plate. After preincubation in darkness until the appropriate temperature was reached (at least 30 min) the leaf sections were exposed to an incident light intensity of 30000 ± 1000 lx provided by high pressure mercury lamps (Südlicht, HSL-S 250 W TT V3).

For experiments under N_2-atmosphere the ice bath was made air-tight by covering it with a plexi-

glass plate, streamed with N_2 gas for 30 min before the onset of the 30000 lx illumination, and kept tightly closed during the illumination period.

Photosynthetic O_2-evolution

Respiratory O_2-consumption and photosynthetic O_2-evolution were assayed with an YSI model 53 oxygen monitor, as described [18], using segments of ca. 1 mm length cut from the middle sections (see above) of the first leaves of 6-day-old seedlings grown at 10 lx.

Extraction and separation of nucleic acids

Nucleic acids were extracted by a modification of the method of Parish and Kirby [19] and separated by electrophoresis on 2.5% polyacrylamide gels, as described [16].

Isolation and gradient centrifugation of plastids

Homogenates were prepared from the leaves of 6-day-old seedlings grown at 5000 lx, as previously described [18] and centrifuged for 15 min at $39000 \times g$ (Spinco 25.2 rotor) on a discontinuous sucrose gradient (modification of gradient B of ref. [18]). The gradients consisted for aminotriazole-grown leaves of 9 ml 53% (w/w) and 18 ml 44% sucrose, and for San 6706-grown leaves of 7 ml 53%, 11 ml 42.5% and 11 ml 35.5% sucrose. The sucrose solutions were contained in 0.05 M tricine-KOH and 1 mM EDTA, pH 7.5. The gradients were fractionated by collecting successive 1-ml samples from an ISCO density gradient fractionator, model 183, connected to a fraction collector. Absorbance at 254 nm was monitored by an ISCO UA-2 absorbance monitor.

Preparation of cell-free extracts

For the preparation of cell-free extracts for the estimation of enzyme activities the first leaves of 20 seedlings were washed and ground with a pestle in ice-cold mortar with 50 mM Tris-HCl buffer, pH 7.5, containing 4 mM dithioerythritol. The extracts were adjusted to a final volume of 10 ml, filtered through a sintered glass funnel (Schott & Gen., No. 3D1), and centrifuged for 5 min at $250 \times g$.

Analytical methods

Pigments were extracted with 90% acetone. Chlorophyll was determined according to Arnon [20]. Total carotenoids were estimated from acetone extracts according to Metzner *et al.* [21].

Enzymes were assayed spectrophotometrically at 25 °C. Catalase (EC 1.11.1.6), fructose-1,6-bisphosphate aldolase (EC 4.1.2.13), NADP-dependent glyceraldehyde-3-P dehydrogenase (EC 1.2.1.13), NAD-specific malate dehydrogenase (EC 1.1.1.37), shikimate dehydrogenase (EC 1.1.1.25), and triosephosphate isomerase (EC 5.3.1.1) were assayed as previously described [18].

Sucrose concentrations were determined with a Zeiss Abbé-refractometer, Modell A.

The experiments were performed four to six times. Figs. 3, 4, and 7 show the results of representative experiments. All other data are averages of the measurements. Standard deviations of the mean are indicated.

Results

Comparison of herbicide-induced bleaching under light of different spectral quality

When rye seedlings were continuously grown in either red or blue light of equal quantum flux density at appropriate herbicide concentrations to evoke a complete chlorosis while allowing maximal growth in white light of 5000 lx [2], the leaves became chlorotic under both light qualities. In red light the leaves were, however, more completely bleached than in blue light where the leaves always accumulated some, and clearly more, chlorophyll, than in red light, mainly in the leaf tips (Fig. 1).

The differences of herbicide-induced photodestructions in red or blue light were still more pronounced for the activity of the chloroplast stroma enzyme NADP-glyceraldehyde-3-P dehydrogenase (Fig. 1). Its activity was under all conditions, also in untreated controls, higher in blue, than in red, light. Inactivation of NADP-glyceraldehyde-3-P dehydrogenase occurred only in treatments with San 6706 or difunon (group 2 herbicides) and was virtually as complete in red as in white light [2] but only slight in blue light (Fig. 1). A similar behavior was observed for peroxisomal enzymes (Feierabend and Kemmerich, in preparation).

Development in dim light (10 lx)

The seedlings were grown in dim light of 10 lx in order to weaken photooxidative events. At 10 lx

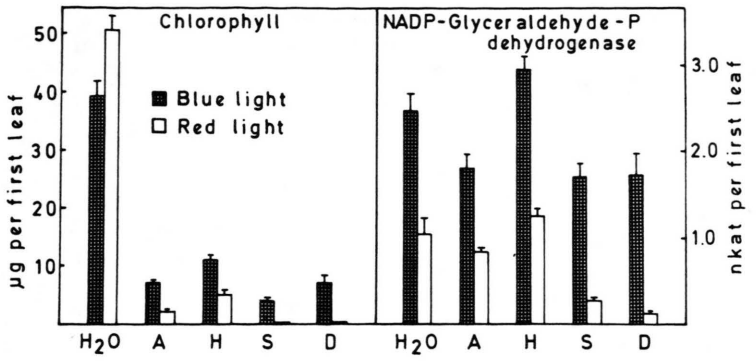

Fig. 1. Total chlorophyll contents and activities of NADP-glyceraldehyde-3-P-dehydrogenase in the first leaves of 6-day-old rye seedlings grown in either blue, or red, light of equal quantum flux density on H_2O or on solutions of the following herbicides: A, 0.25 mM aminotriazole; H, 0.05 mM haloxidine; S, 0.02 mM San 6706; D, 0.05 mM difunon.

leaves of untreated control seedlings formed only 37% of the chlorophyll found at 5000 lx. However, all herbicide-treated leaves accumulated more chlorophyll at 10 lx than at 5000 lx, reaching 40 to 100% of the chlorophyll content in untreated control leaves grown at 10 lx (Fig. 2). At 10 lx the total carotenoid contents of the herbicide-treated leaves were also higher than at 5000 lx. After treatment with aminotriazole and haloxidine the ratio of carotenoids to chlorophyll was similar to that of control leaves. Though in the presence of San 6706 and difunon this ratio was considerably lower, more carotenoids were formed at 10 lx than at 5000 lx. This was not only indicated by measurements from total pigment extracts but confirmed by ether extraction of the carotenoids after saponification of the chlorophyll with 10% methanolic KOH. Absorbance spectra of the total carotenoids formed in treated plants at 10 lx seemed to indicate that at least part of the carotenoids consisted of less unsaturated forms than in the controls (data not shown). A major part of the carotenoids in herbicide-treated leaves from 10 lx was presumably localized in the leaf tips, since their relative proportion appeared to be smaller in the middle sections (Fig. 5), as compared to the complete leaves (Fig. 2).

In 10 lx-grown leaves the NADP-glyceraldehyde-3-P dehydrogenase was not inactivated in the presence of San 6706 and difunon but reached almost as high activities after all herbicide treatments as in untreated controls (Fig. 2). Analysis of the ribosomal RNAs revealed that at 10 lx after all herbicide treatments 16 S and 23 S rRNAs, and thus presumably plastid 70 S ribosomes, were present (Fig. 3, 0 h), while they had been missing at 5000 lx [2]. Though the herbicide-treated leaves appeared to be, in several respects, only little impaired at 10 lx, photosynthetic O_2-evolution was not observed with leaf segments when assayed at 25000 lx light (Fig. 4). In aminotriazole- and difunon-treated leaves the respiration rate was somewhat slowed after illumination, conceivably indicating some photosynthetic O_2-production that was, however, in no instance sufficient to compensate respiratory O_2-consumption.

Photodestructions after transfer from low (10 lx) to high light intensity (30000 lx)

Segments of leaves grown at 10 lx were exposed for up to 12 h to a high light intensity of 30000 lx. In order to have a tissue of fairly uniform behavior the tips and basal parts of the leaves were dis-

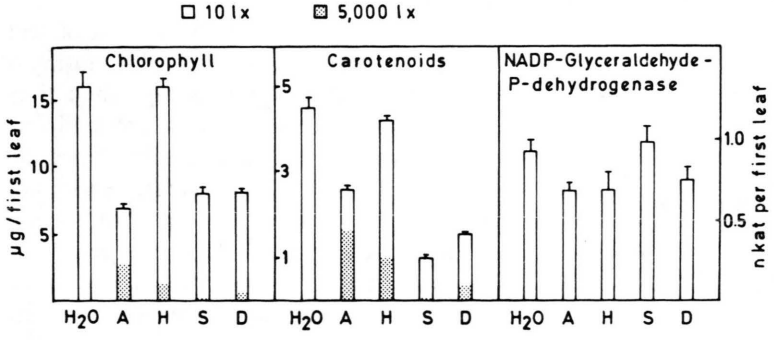

Fig. 2. Total contents of chlorophyll and carotenoids and activities of NADP-glyceraldehyde-3-P dehydrogenase in the first leaves of 6-day-old rye seedlings grown in continuous white light of 10 lx or 5000 lx on H_2O or on different herbicide solutions. The chlorophyll and carotenoid contents of control leaves grown on H_2O at 5000 lx were 44.0 ± 2.5 and 10.2 ± 0.4 µg per first leaf. Abbreviations and concentrations as in Fig. 1.

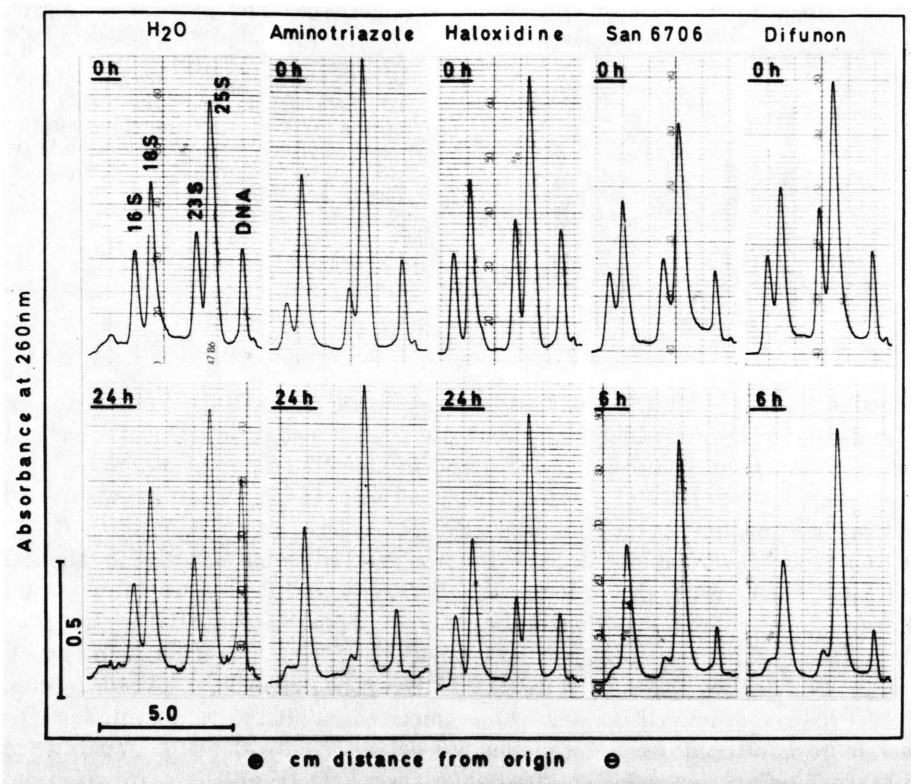

Fig. 3. Electrophoretic separation of cytoplasmic (18S and 25S) and plastidic (16S and 23S) rRNA from the middle sections of first leaves of 6-day-old rye seedlings grown in continuous white light of 10 lx on H_2O or on different herbicide solutions before (upper row: 0 h) and after an exposure to 30000 lx white light at 30 °C for 6 h or 24 h, as indicated (lower row). 0.25 mM aminotriazole, 0.05 mM haloxidine, 0.02 mM San 6706, 0.25 mM difunon.

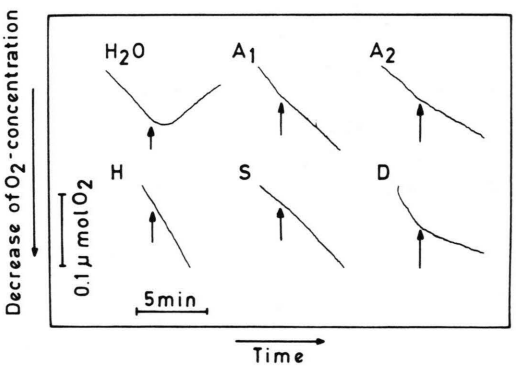

Fig. 4. Representative tracings of respiratory oxygen consumption of photosynthetic oxygen evolution by small segments of first leaves of 6-day-old rye seedlings grown in continuous white light of 10 lx on H_2O or on different herbicide solutions. Arrows indicate start of illumination with 25000 lx white light. A_1, 0.25 mM, A_2, 0.3 mM aminotriazole; H, 0.05 mM haloxidine; S, 0.02 mM San 6706; D, 0.1 mM difunon.

carded and only the middle sections used because the tips had a tendency to form more chlorophyll than the rest of the leaf and the basal leaf parts were still in an incompletely differentiated state. In order to discriminate purely photochemical reactions from those involving metabolic and enzyme-catalyzed reactions the exposures to 30000 lx were performed both at 0 °C and at 30 °C.

At 30 °C the chlorophyll content of control leaf sections from 10 lx increased considerably during a 6 h illumination at 30 °C, particularly when they were floated on water. Though the chlorophyll formation was lower on a phosphate buffer of pH 6.0, the latter was used for all further experiments since a low pH is favorable for the uptake of several substances that were applied in further experiments [22]. Since the behavior of chlorophyll under the high light intensity was not influenced by the

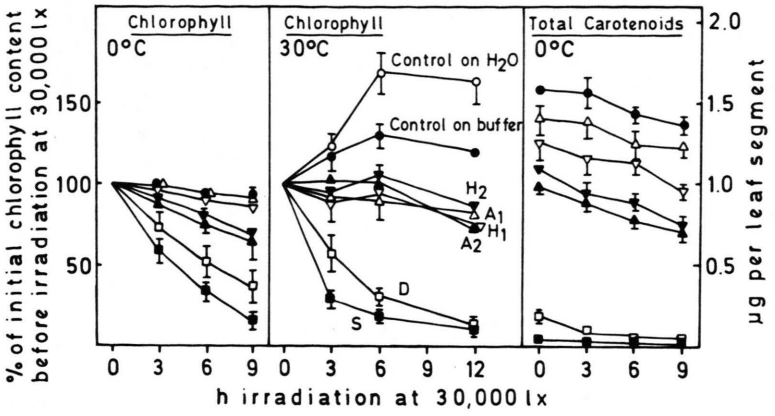

Fig. 5. Changes of the contents of total chlorophyll and carotenoids in middle sections of the first leaves on 6-day-old rye seedlings grown in continuous white light of 10 lx on H_2O or on different herbicide solutions after exposure to 30000 lx at either 0 °C or 30 °C. A_1, 0.25 mM, A_2, 0.3 mM aminotriazole; H_1, 0.05 mM, H_2, 0.15 mM haloxidine; S, 0.02 mM San 6706; D, 0.1 mM difunon.

presence or absence of the herbicides in the incubation medium the latter were generally omitted from the buffer.

In leaf segments grown at even high concentrations of aminotriazole or haloxidine (group 1 herbicides) at 10 lx the chlorophyll content remained constant or decreased only slightly after exposure to 30000 lx at 30 °C. By contrast, in treatments with San 6706 or difunon chlorophyll decreased quite strikingly at 30 °C (Fig. 5). At 0 °C where no chlorophyll synthesis occurred photodegradation of chlorophyll was not significantly greater in treatments with group 1 herbicides than in untreated controls when usual herbicide concentrations were applied. The slight decrease of the chlorophyll content was in all instances accompanied by a slow decline of the total carotenoids (Fig. 5). Only when high, growth-reducing, concentrations were applied during growth at 10 lx chlorophyll degradation in light of 30000 lx was significant at 0 °C in treatments with group 1 herbicides (Fig. 5). In treatments with group 2 herbicides a rapid bleaching of chlorophyll was seen after exposure to 30000 at 0 °C. Carotenoid contents were extremely low and further declined under the high light intensity (Fig. 5). Initial rates of chlorophyll degradation in San 6706 or difunon-treated leaves were 50–100% higher at 30 °C than at 0 °C. For all herbicide treatments bleaching of chlorophyll was virtually prevented when the 10 lx-grown leaf segments were exposed to 30000 lx (at 0 °C) in the absence of O_2 under a N_2-atmosphere (Fig. 6).

It has already been documented that in the presence of San 6706 and difunon also the NADP-glyceraldehyde-3-P dehydrogenase activity of 10 lx-grown leaves was inactivated within 6 h of exposure to 30000 lx both at 0 °C and at 30 °C [8]. Within the same time also the plastid rRNAs were largely destroyed at the high light intensity in the presence of San 6706 and difunon (Fig. 3). In contrast to the behavior of chlorophyll substantial photodestruction of the plastid rRNAs occurred also in aminotriazole-treated leaves at 30 °C and 30000 lx (Fig. 3) and was already quite significant within 6 h (data not shown). In haloxidine-treated leaves the plastid rRNAs were more stable, and rRNA degradation at 30000 lx was not established as significant (Fig. 3).

Isolation of plastids from herbicide-bleached leaves

In order to ascertain whether soluble chloroplast enzymes synthesized on cytoplasmic ribosomes, such as NADP-glyceraldehyde-3-P dehydrogenase or the

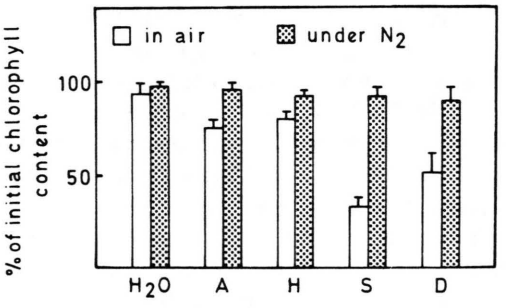

Fig. 6. Changes of the total chlorophyll contents in the middle sections of the first leaves of 6-day-old seedlings grown at 10 lx on H_2O or on different herbicide solutions during a 6 h illumination with white light of 30000 lx at 0 °C under air or under a nitrogen atmosphere. A, 0.3 mM aminotriazole; H, 0.15 mM haloxidine; S, 0.02 mM San 6706; D, 0.1 mM difunon.

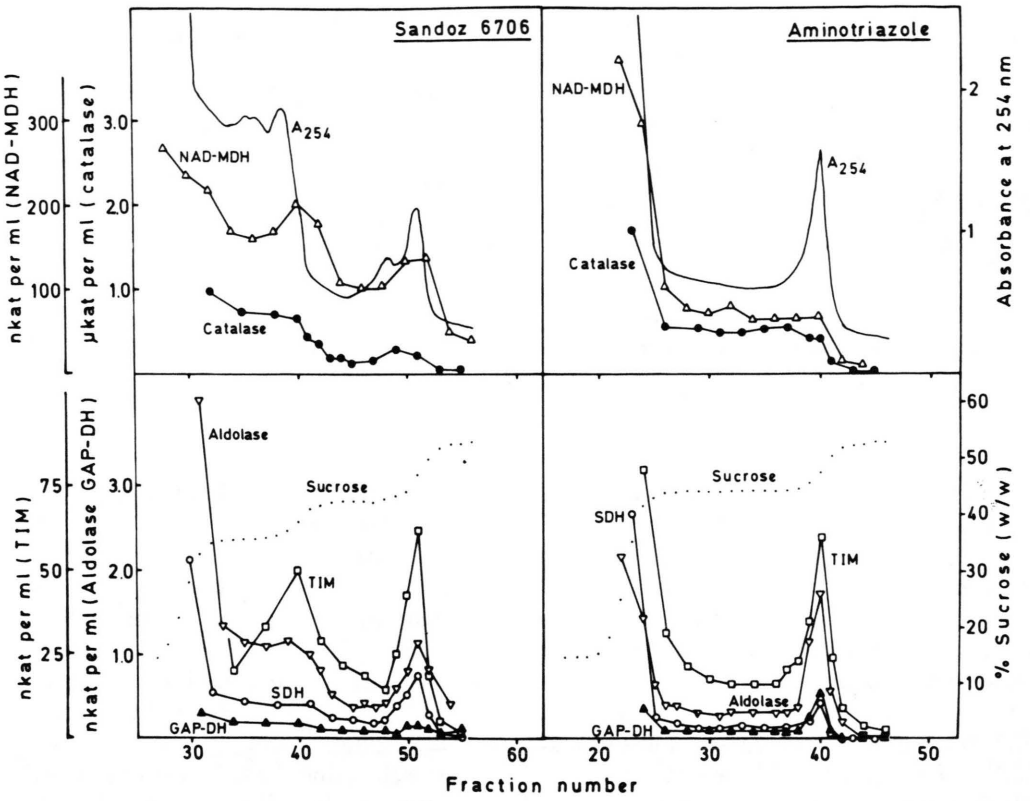

Fig. 7. Sucrose gradient isolation of intact plastids (indicated by the high density peaks of A_{254} and marker enzymes) from homogenates of first leaves of 6-day-old rye seedlings grown in continuous white light of 5000 lx on 0.01 mM San 6706 or 0.25 mM aminotriazole. The activities of catalase and NAD-specific malate dehydrogenase present in the intact plastid fraction are contaminations that could not be avoided in the herbicide-treated material and with the procedures applied. ● Catalase, △ NAD-specific malate dehydrogenase (NAD-MDH), ▽ fructose-1,6-bisP aldolase, ▲ NADP-specific glyceraldehyde-3-P dehydrogenase (GAP-DH), □ triose-P isomerase (TIM), ○ shikimate dehydrogenase (SDH), · sucrose concentration (w/w), —— absorbance at 254 nm (A_{254}).

plastidic forms of fructosebisphosphate aldolase or triosephosphate isomerase, were imported into the plastids of herbicide-bleached leaves, attempts were made to isolate such plastids from leaves grown either in the presence of aminotriazole or Sandoz 6706 at 5000 lx. The yields of such enzyme activities recovered in crude particulate fractions obtained by a 20 min centrifugation at $12000 \times g$ from homogenates of aminotriazole-bleached or Sandoz 6706-treated (in brackets) leaves were in a similar order of magnitude as for control or heat-bleached rye leaves [18]: 33% for NADP-glyceraldehyde-3-P dehydrogenase, 49 (73)% for fructosebisphosphate aldolase, 15% (9) for triosephosphate isomerase. After equilibrium density centrifugation on sucrose gradients chloroplast marker enzyme activities, if present at all, were found at a similar buoyant density range in preparations from herbicide-bleached as from control leaves (data not shown), though the peaks were broader and less prominent. In order to enrich intact plastids of higher purity, crude homogenates were centrifuged for only short times on stepped gradients. This procedure allowed intact plastids to accumulate at a step of high density while broken plastids stayed in the upper part of the gradient because of their lower density and mitochondria and peroxisomes because of their lower mobility (Fig. 7; see ref. [18]). An intact plastid fraction containing several soluble plastid-localized enzymes was isolated from both bleached aminotriazole- and Sandoz 6706-treated leaves (Fig. 7). The fraction identified by the marker enzyme activities as intact plastids in the preparation from Sandoz 6706-bleached leaves possessed hardly any

NADP-glyceraldehyde-3-P dehydrogenase activity since this enzyme was virtually missing in the whole leaves but they contained activities of some enzymes that are usually also found in proplastids, *e.g.* triosephosphate isomerase and shikimate dehydrogenase (Fig. 7).

Discussion

In previous work [2] we had demonstrated that among several chlorosis-inducing herbicides two groups were to be descriminated with regard to the extent of damage they caused in addition to the elimination of chlorophyll. Severe inactivations of chloroplastic and peroxisomal enzymes observed in the presence of those herbicides designated as group 2 (San 6706, difunon) were not seen in treatments with aminotriazole or haloxidine (group 1). The present results further substantiate the contention that the two groups of bleaching herbicides differ also in their mechanism of action. In dim light where herbicide-induced defects resulting secondarily from photodestructive damage were largely avoided (see also [1, 12]), a severe inhibition of carotenoid accumulation that is often regarded as a primary defect characteristic for all bleaching herbicides was only seen in treatmens with group 2 herbicides. Therefore, it appears doubtful whether in treatments with group 1 herbicides the bleaching was caused by a carotenoid-deficiency, although the composition and organization of the carotenoids might also have been disturbed. Besides impaired carotenogenesis, disorders of the photosynthetic electron transport, as indicated by the inability for O_2-evolution of dim-light-grown leaves from treatments with all four herbicides tested, have to be taken into account as initial effects of prolonged herbicide applications and as another conceivable source of harmful activated O_2 and photodestructive damage [14, 23, 24]. Otherwise dim-light-grown leaves were in most respects comparable to untreated controls and only the pyridazinone herbicides are known to show some direct interference with photosynthetic reactions [9].

The present comparative investigations of the photodestructive events after a transfer of dim-light-grown leaf segments to a high light intensity of 30000 lx further accentuate the differences between the two groups of herbicides with regard to the strengths of the photodegradative damages and the genesis of the chlorosis. Only in treatments with group 2 herbicides a rapid breakdown of existing chlorophyll was seen after exposure to the high light intensity. Since the photodegradation of chlorophyll required the presence of O_2, which had not been demonstrated for these herbicides before, and occurred also at 0 °C it has to be regarded as a true photooxidation arising predominantly from photochemical events, as expected in carotenoid-deficient leaf cells. The slight temperature dependence of the rate of chlorophyll photodestruction (maximal Q_{10} of about 1.4) conceivably resulted from secondary reactions augmenting the primary photodynamic effects [14, 23, 25]. For instance, lipid peroxidation occurred in the presence of group 2 herbicides and is possibly involved in the bleaching of chlorophyll [22]. In treatments with group 2 herbicides the chlorosis thus appeared to arise from a direct photodegradation of chlorophyll that was, in addition, accompanied by rapid and severe photodestructions of other chloroplast components such as 70S ribosomes and chloroplast enzymes, so that most of the biosynthetic functions of the chloroplast were, finally, destroyed, including that for new chlorophyll synthesis [8]. At least the inactivation of NADP-glyceraldehyde-3-P dehydrogenase, a chloroplast marker enzyme that is imported into the organelle from a cytoplasmic site of synthesis [18] appeared to be also mediated through photodynamic reactions since it took place at 0 °C [8].

Whereas in the presence of group 1 herbicides photodestructive reactions clearly appeared to be also involved in the genesis of the chlorosis because chlorophyll and 70S ribosomes were formed in dim light, a significant direct breakdown of chlorophyll was not seen within 12 h after exposure to bright light. Its further accumulation was, however, stopped. The significance of the slight photodegradation of chlorophyll occurring at 0 °C, where it could not be compensated by new synthesis, in treatments with very high and growth-reducing concentrations of group 1 herbicides is questionable because such high concentrations were not needed for the development of the chlorosis during continuous growth in a high light intensity (5000 lx [2]). During prolonged exposure of dim-light-grown leaves to bright light a slow decline of the chlorophyll content is to be expected, must, however, presumably be attributed to the natural turnover of chlorophyll [26] that will become apparent after termination of

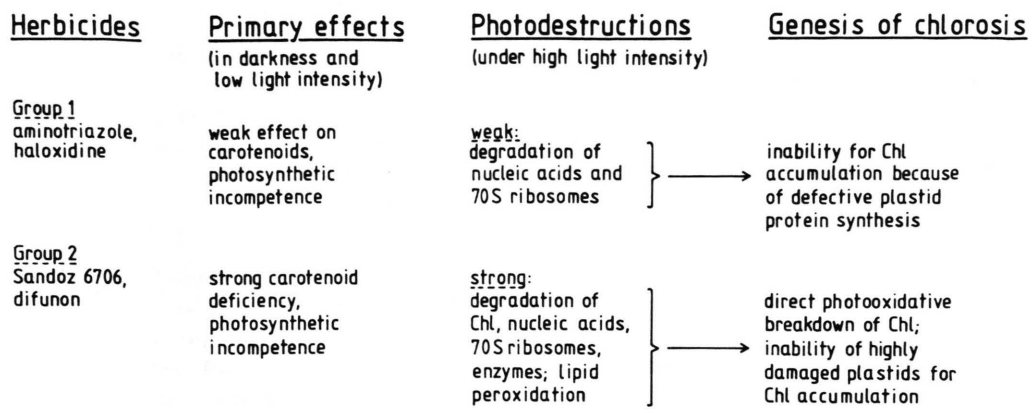

Fig. 8. Scheme to illustrate the difference in the sequence of events leading to chlorosis in the presence of the two groups of herbicides applied.

chlorophyll synthesis. This may be the reason why a decrease of chlorophyll has also been described in treatments with group 1 herbicides after exposure to bright light [1].

Photodestructive events appeared to be much weaker in the presence of herbicides of group 1 than of those of group 2. The former were not accompanied by significant lipid peroxidation [2] and not only insufficient for chlorophyll destruction but also for the inactivation of chloroplast enzymes, such as the NADP-glyceraldehyde-3-P dehydrogenase. The demonstration of the presence of the latter enzyme in the intact chloroplast fraction of aminotriazole-bleached leaves (Fig. 7) excludes the possibility that it escaped photoinactivation in the chloroplasts because its import into the organelle was prevented through the herbicide treatment and the enzyme had remained in the cytoplasm. Nucleic acids or components of the protein synthesis machinery appeared to be more sensitive to even weak photooxidative action than pigments and enzymes because the 70S ribosomes were also in aminotriazole-treated leaves destroyed after a transfer from low to high light intensity. In this respect it is of interest that the elimination of chloroplast DNA has also been described for aminotriazole-bleached leaves [11]. We have to conclude that in aminotriazole-treated leaves the chlorosis was predominantly not caused by a photooxidative breakdown of chlorophyll but through the induction of a 70S ribosome-deficiency that will prevent further chlorophyll accumulation [15, 16]. The differences in the sequence of events involved in the action of the two groups of chlorosis-inducing herbicides are summarized in Fig. 8. For haloxidine the reservation has to be made that its action still remains somewhat more uncertain because in haloxidine treatments an unequivocal destruction of plastid rRNA was not established within 24 h exposure to bright light whereas it is clear that during a prolonged exposure the 70S ribosomes are also eliminated [2]. It is to be expected and may be hypothesized that in haloxidine treatments only a few most sensitive but indispensable components of the chloroplast transcription-translation system are initially inactivated, thus blocking chloroplastic protein synthesis and thereby the accumulation of 70S ribosomes and, finally, of chlorophyll.

The observation that photodestructions appeared to be much stronger in red, than in blue, light, particularly with regard to the photoinactivation of enzymes (Fig. 1), is not yet clearly understood. It would appear reasonable that in the blue, chlorophyll is more protected from photodynamic reactions by the blue-absorbing carotenoids than in red light. Since red-light-excited chlorophyll can, however, also be quenched by carotenoids [27, 28] this protection is conceivably mainly due to a screening effect.

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