Comparison of the Action of Bleaching Herbicides

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Among chlorosis-inducing herbicides that interfere with carotenoid synthesis two groups of different potency can be discriminated (group 1: aminotriazole amd haloxidine; group 2 with more extensive photodestructions: pyridazinone herbicides and difunon). After application of herbicides of group 2 colored carotenoids were completely absent and preexisting chlorophyll was degraded by photochemical reactions requiring high light intensity and O_2, that occurred also at 0 °C. In treatments with group 1 herbicides direct photodegradation of chlorophyll was not sufficient to generate the chlorosis. Light-induced interference with constituents of the chloroplast protein synthesis apparatus being more sensitive to photooxidative damage than chlorophyll, appeared to indirectly mediate the chlorosis. In the absence of chloroplast protein synthesis further chlorophyll accumulation is prevented. Photodegradation of chlorophyll in the presence of group 2 herbicides involved the participation of $O_2^{\cdot-}$ radicals and was accompanied by lipid peroxidation. In all herbicide treatments the catalase activity of the leaves was very low. Only in the presence of group 2 herbicides chloroplast enzymes of cytoplasmic origin (*e.g.* NADP-glyceraldehyde-3-phosphate dehydrogenase) were also inactivated. Rapid inactivation of catalase as well as of NADP-glyceraldehyde-3-phosphate dehydrogenase was induced by exposure of dim-light-grown herbicide-treated leaves to bright light, also at 0 °C. In treatments with herbicides of group 2 also other peroxisomal enzymes (*e.g.* glycolate oxidate, hydroxypyruvate reductase) were affected. The elimination of these peroxisomal enzymes also appeared to depend on photooxidative processes of the chloroplast.

Introduction

Several bleaching herbicides have been produced that induce chlorosis in leaves developing in their presence but do not effect preexisting chlorophyll in mature leaves [1]. The chlorosis does not appear to result from a direct inhibition of chlorophyll biosynthesis since the formation and phototransformation of protochlorophyll(ide) were not blocked [2–4], and a report on the inhibition of an individual enzyme of chlorophyll biosynthesis [5] was not generally confirmed [6]. By contrast, carotenoid biosynthesis is regarded as the primary target of the herbicide actions, because virtually all chlorosis-inducing herbicides either totally prevent, or interfere with, the formation of colored carotenoids *in vivo* and usually lead to a comcomitant build-up of carotenoid intermediates, such as phytoene, phytofluene, ζ-carotene, or lycopene (for literature see [1, 7, 8]). This indicates that desaturation and cyclization reactions were inhibited. Pyridazinone herbicides and difunon inhibit the synthesis of β-carotene also in the fungus *Phycomyces* [9]. Recently, the *in vitro* inhibition by pyridazinone herbicides and difunon of defined enzymatic steps was demonstrated in cell-free carotenogenic systems from *Phycomyces* [10], and the blue-green alga *Aphanocapsa* [11, 12]. However, the sites of inhibition of carotenogenesis appear to differ in different organisms and for the different herbicides.

The deficiency or absence of protecting carotenoids is thought to give rise to photodestructive reactions initiating the chlorosis in herbicide-treated leaves because carotenoids are needed to quench excited triplet chlorophyll or activated oxygen produced by the reaction of the former with O_2, and thus to dissipate excess light energy [1, 7, 13, 14]. Our work has shown that the herbicide-induced chlorosis is not confined to deficiencies of carotenoids and chlorophyll. However, the extent of photodestructive damage accompanying the chlorosis as well as the detailed sequence of events giving rise to the chlorosis differ markedly among the bleaching herbicides and will be reviewed in the following.

Abbreviations: amitrole (aminotriazole), 3-amino-1,2,4-triazole; difunon, 5-(dimethyl-aminomethylene)-2-oxo-4-phenyl-2,5-dihydrofurane-carbonitrile-(3); haloxidine, 3,5-dichloro-2,5-difluoro-4-hydroxypyridine; metflurazon, 4-chloro-5-dimethylamino-2-(3-trifluoromethylphenyl)pyridazin-3(2H)one.

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Symptoms Accompanying the Herbicide-Induced Chlorosis

We have concentrated on a comparative investigation of mainly four examples of chlorosis-inducing herbicides among which two groups can be distinguished from the symptoms accompanying the chlorosis (Table I): aminotriazole and haloxidine were designated as group 1, pyridazinone herbicides, such as meflurazon (SAN 6706), and difunon (EMD IT 5919) were designated as group 2 [3, 15, 16]. Presumably also herbicides with intermediate properties exist [15]. Though deficiencies of carotenoids and chlorophyll accompany all herbicide treatments, after application of herbicides of group 1 the carotenoid-deficiency was less complete than in the presence of herbicides of group 2 [7, 8, 15, 16]. Notably, prenylquinones were not markedly diminished in herbicide-bleached radish leaves [8], but continued to accumulate in the plastoglobuli. Common to all herbicide treatments was that in the chlorotic leaves chloroplast 70S ribosomes and their translation products (*e.g.* ribulosebisphosphate carboxylase of which the large subunit is synthesized on plastid ribosomes) were missing and that the catalase activity was very low (Table I, [15]). Two sets of additional deficiencies were observed only in treatments with herbicides of group 2: in their presence also plastid enzymes that are synthesized outside the organelle on 80S ribosomes, such as NADP-glyceraldehyde-3-phosphate dehydrogenase [15] or enzymes of protochlorophyll(ide) synthesis [3], and further peroxisomal enzymes, such as glycolate oxidase or hydroxypyruvate reductase [15], were virtually eliminated (Table I).

Table I. Comparison of the symptoms accompanying the chlorosis in two groups of bleaching herbicides in rye leaves grown at 5000 lux. –, very low or absent; +, not or only little affected.

Parameter	Group 1 (aminotriazole, haloxidine)	Group 2 (metflurazon, difunon)
Chlorophyll	–	–
Carotenoids	low	–
70S Ribosomes	–	–
Products of 70 ribosomes (*e.g.* ribulosebisphosphate carboxylase)	–	–
Chloroplast enzymes synthesized on 80S ribosomes (*e.g.* NADP-glyceraldehyde-P dehydrogenase)	+	–
Peroxisomal enzymes		
Catalase	–	–
Glycolate oxidase	+	–
OH-Pyruvate reductase	+	–

Except for the interference with carotenoid synthesis, all defects summarized in Table I, appear to result from photodestructions because they were not observed in darkness nor in dim light of 10 lux ($0 \cdot 1\ W \cdot m^{-2}$). At 10 lux herbicide-treated leaves became pale green and contained 50–100% of the chlorophyll content of untreated control leaves, and in the presence of herbicides of group 1 even high amounts of carotenoids [16].

Mechanism of Bleaching

The genesis of the chlorosis is conveniently studied after transferring herbicide-treated leaves from low to high light intensity. In contrast to earlier observations [2], we observed a rapid and substantial bleaching of preexisting chlorophyll only in treatments with group 2 herbicides but not in treatments with group 1 herbicides after exposure of 10 lux-grown leaves to 30 000 lux. The photodestruction of chlorophyll in treatments with metflurazon and difunon required the presence of O_2 and occurred also at 0 °C. It had a low Q_{10} of 1.4. This documented that the breakdown of chlorophyll in treatments with herbicides of group 2 was of truly photooxidative nature and resulted, due to the absence of protecting carotenoids, from photochemical but not from enzyme-catalyzed reactions [16]. In these herbicide treatments also 70S ribosomes and chloroplast enzymes were degraded on exposure to high light intensity [16], and the photoinactivation of the NADP-glyceraldehyde-3-phosphate dehydrogenase occurred also at 0 °C [3]. While in leaves treated with metflurazon or difunon a direct photodegradation of chlorophyll appeared to be responsible for the chlorosis this cannot markedly contribute to the initiation of the chlorosis in the presence of group 1 herbicides. However, while preexisting chlorophyll was quite stable in treatments with group 1 herbicides, constituents of the protein synthesizing machinery appeared to be more sensitive to photodestruction. In aminotriazole-treated leaves the 70S ribosomes were within

one day degraded after transfer from 10 to 30 000 lux [16]. In haloxidine-treated leaves we did not observe such a rapid and significant breakdown of pre-existing 70S ribosomes [16]. However, inasmuch as the protein of the ribulosebisphosphate carboxylase, estimated by immuno-chemical methods (Fig. 1), did not further increase but even slightly decreased after exposure to 30 000 lux, we have to conclude that chloroplast protein synthesis was also in haloxidine-treated leaves immediately inactivated after exposure to bright light. A block of chloroplast protein synthesis appears to be the main reason for the chlorosis in the presence of group 1 herbicides because it prevents the accumulation of chlorophyll which obviously needs specific membrane polypeptides synthesized within the chloroplasts for complexing and stabilization [17]. With increasing photodestructive damage the capacity for protochlorophyll(ide) and chlorophyll synthesis is, in addition, also gradually declining because the enzymes of chlorophyll biosynthesis were in leaves treated with group 2 herbicides inactivated like other chloroplast enzymes [3, 4], and regulatory feedback inhibitions blocked in all treatments the synthesis of the precursor δ-aminolevulinic acid [3].

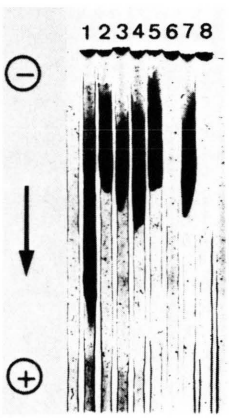

Fig. 1. Quantitative estimation of the amounts of ribulosebisphosphate carboxylase in soluble extracts (in 50 mM tricine-KOH, 5 mM $MgCl_2$, 4 mM dithioerythritol, pH 7.5) from the middle sections (top and basal quarter discarded) of the first leaves of 6-day-old rye seedlings by zone immunoelectrophoresis [29] in agarose gels containing 0.013% rabbit antiserum against rye ribulosebisphosphate carboxylase. Seedlings were grown on H_2O (1, 2, 3), 0.15 mM haloxidine (4, 5, 6), or 0.2 mM metflurazon (SAN 6706) (7, 8) at 10 lux (2–5, 7, 8) or 5000 lux (1, 6). In treatments 3, 5 and 8 the segments from 10 lux-grown leaves were for further 24 h exposed to 30 000 lux [16] at 25 °C. The length of the precipitation lines is proportional to the amount of the antigen.

It is notable that the marked difference in the potency of the two groups of herbicides to induce photodegradation of chlorophyll in bright light was only seen in intact leaves but not in isolated chloroplasts, *e.g.* from 10 lux-grown metflurazon- and aminotriazole-treated leaves, *in vitro* [18]. In treatments with group 1 herbicides some unknown additional protective mechanisms, except for the membrane-bound carotenoids, must be acting *in vivo* but not *in vitro*. One obvious difference between the two groups of herbicides possibly contributing to their different potency is that dim-light grown leaves treated with group 1 herbicides contained almost as high total carotenoid levels as untreated controls [16], so that it even appears questionable why photodestructive damage occurred at all. Certainly, the qualitative composition of the carotenoids was changed in favor of intermediates, such as lycopene [8], and the carotenoids were conceivably not properly integrated into pigment-protein-complexes [19]. This may explain that dim-light-grown leaves from all herbicide treatments had, though they were green, lost the capacity for photosynthetic oxygen evolution [16]. The disorders of the electron transport chain indicated by the photosynthetic incompetence may also act as a major source of photooxidative damage when the excitation energy of the photosystems is not properly drained through the flow of electron transport, as in leaves treated with DCMU-type herbicides [20]. Investigations of chlorophyll fluorescence indicated that in leaves treated with bleaching herbicides the energy transfer from the light-harvesting systems to the reaction centers was disturbed [19]. It is of interest that in some algae chlorotic cells develop in the presence of pyridazinone herbicides even in darkness and that bleaching was attributed to metabolic but not to photodynamic processes [21]. This difference is conceivably related to the observation that in algae but not in higher plants chlorophyll formation is controlled by the availability of carotenoids [22].

The Photooxidative Events

Several forms of activated O_2 have been discussed to arise from the reaction of triplet chlorophyll with O_2 under the influence of excess light energy and implicated in the oxidative decomposition of the pigment (for literature see [18]). Though $^1O_2$ is mostly believed to mediate chlorophyll

degradation [7, 13, 14], we did not find any evidence for its involvement in the bleaching of chlorophyll in the presence of group 2 herbicides. Known scavengers of $^1O_2$ did not prevent bleaching, and D_2O which greatly prolongs the lifetime of $^1O_2$ did not enhance the breakdown of chlorophyll [18].

Also hydroxyl radical scavengers were without effect. However, *p*-benzoquinone, a scavenger of free radicals and of triplet chlorophyll, and a penicillamine copper complex which exerts superoxide dismutase activity, totally prevented the photodestruction of chlorophyll in herbicide-treated leaves or even restored chlorophyll accumulation [18]. Such results are suggestive for involvements of triplet chlorophyll, as expected, and $O_2^{\cdot-}$ in herbicide-induced bleaching. From present knowledge it is not to be expected that the $O_2^{\cdot-}$ radical attacks chlorophyll directly. Either $O_2^{\cdot-}$ is converted to more reactive forms of activated O_2 or acts by mediating lipid peroxidation [23]. The formation of malondialdehyde indicated that lipid peroxidation accompanied photodestructions in the presence of group 2 herbicides but not in the presence of group 1 herbicides [18]. Intermediates of lipid peroxidation are regarded as particularly agressive and capable of destroying chlorophyll as well as proteins [24, 25]. This may explain that direct photodestruction of chlorophyll and of chloroplast enzymes was only in treatments with group 2 herbicides observed where lipid peroxidation occurred.

Inactivation and Peroxisomal Enzymes

Initially it was hard to imagine that photodynamic processes of the chloroplasts should be specifically transmitted to a single other organelle, and which relationships existed between the herbicide-induced chlorosis and the peroxisomal defects. Since photorespiration is also regarded as a means of protection against photooxidative damage, it was conceivable that the herbicide-induced inactivation of peroxisomal enzymes increased photodestructions and thus even contributed to the generation of the chlorosis because the photorespiratory pathway was blocked. However, evidences are now prevailing that the inactivations of peroxisomal enzymes are secondary consequences of photooxidative events in the chloroplasts [26], except for the inactivation of catalase by aminotriazole which is long known to bind specifically to the protein of this enzyme [27].

The inactivation of peroxisomal enzymes was always closely related to the induction of chlorosis and to the strength of photooxidative events. It accompanied, for instance, only treatments with a bleaching (SAN 6706) but not with a non-bleaching pyridazinone herbicide (SAN 9785), and occurred only in red, but not in blue, light were bleaching was less complete and photodestructions were obviously weaker than in red light [26]. When peroxisomal enzyme activities, particularly that of catalase, were kept low without herbicide applications in an atmosphere of low O_2 and high CO_2 concentration the stability of chlorophyll, tested by exposure to high light intensity in normal air, was not decreased. Further, peroxisomal enzyme activities were neither in darkness nor in dim light decreased, relative to corresponding untreated controls [15, 26]. After exposure of dim-light-grown leaves to bright light catalase was rapidly inactivated, even at 0 °C. At 0 °C catalase was also in untreated controls to the same extent inactivated as in the herbicide treatments [26]. This suggests that catalase either suffers from photoinactivation whenever the flow of photosynthesis is, by whatever means, blocked, or that its activity is even permanently inactivated in light but under physiological conditions continuously restored by reactivation or new synthesis. For the other peroxisomal enzymes, glycolate oxidase and hydroxypyruvate reductase, that were only in the presence of group 2 herbicides eliminated, only little inactivation of preexisting activities was observed after transfer of dim-light-grown herbicide-treated leaves to bright light. However, further increases of their activities were, in contrast to untreated controls and treatments with group 1 herbicides, immediately blocked in leaves treated with group 2 herbicides after exposure to a high light intensity. Our results support the assumption that photo-destructive damage is specifically transmitted from the chloroplast to the leaf peroxisomes, presumably by membrane contacts that are usually seen between these two organelles [28]. Products of lipid peroxidation occurring in the presence of group 2 herbicides conceivably spread through the membranes. This could explain that not so much pre-existing, but the newly formed, peroxisomal enzymes were affected, presumably when transported across the organellar membrane and exposed to lipid peroxidation products.

[1] C. Fedtke, Biochemistry and physiology of herbicide action, Springer, Berlin, Heidelberg, New York 1982.
[2] E. R. Burns, G. A. Buchanan, and M. C. Carter, Plant Physiol. **47,** 144–148 (1971).
[3] J. Feierabend, U. Schulz, P. Kemmerich, and T. Lowitz, Z. Naturforsch. **34c,** 1036–1039 (1979).
[4] B. Klockare, L. Axelsson, H. Ryberg, A. S. Sandelius, and K.-O. Widell, in: Photosynthesis (G. Akoyunoglou, ed.), **Vol. V,** pp. 277–284, Balaban Int. Sci. Service, Philadelphia 1981.
[5] R. Hampp, N. Sankhla, and W. Huber, Physiol. Plant. **33,** 53–57 (1975).
[6] K.-J. Kunert and P. Böger, Weed Sci. **26,** 292–296 (1978).
[7] S. M. Ridley, in: Carotenoid chemistry and biochemistry (G. Britton and T. W. Goodwin, eds.), pp. 353–369, Pergamon Press, Oxford 1982.
[8] K. H. Grumbach, Z. Naturforsch. **37c,** 642–650 (1982).
[9] G. Sandmann, K.-J. Kunert, and P. Böger, Z. Naturforsch. **34c,** 1044–1046 (1979).
[10] G. Sandmann, P. M. Bramley, and P. Böger, Pesticide Biochem. Physiol. **14,** 185–191 (1980).
[11] J. E. Clarke, G. Sandmann, P. M. Bramley, and P. Böger, FEBS Lett. **140,** 202–206 (1982).
[12] J. E. Clarke, P. M. Bramley, G. Sandmann, and P. Böger, in: Biochemistry and metabolism of plant lipids (J. F. G. M. Wintermans and P. J. C. Kuiper, eds.), pp. 549–554, Elsevier Biomedical Press, Amsterdam 1982.
[13] N. J. Krinsky, Phil. Trans. R. Soc. Lond. **B 284,** 581–590 (1978).
[14] E. Elstner, Ann. Rev. Plant Physiol. **33,** 73–96 (1982).
[15] J. Feierabend and B. Schubert, Plant Physiol. **61,** 1017–1022 (1978).
[16] J. Feierabend, Th. Winkelhüsener, P. Kemmerich, and U. Schulz, Z. Naturforsch. **37c,** 898–907 (1982).
[17] J. Feierabend, Planta **135,** 83–88 (1977).
[18] J. Feierabend and Th. Winkelhüsener, Plant Physiol. **70,** 1277–1282 (1982).
[19] C. Buschmann and K. H. Grumbach, Z. Naturforsch. **37c,** 632–641 (1982).
[20] S. M. Ridley, Plant Physiol. **59,** 724–732 (1977).
[21] M. M. Tantawy and L. H. Grimme, Pesticide Biochem. Physiol. **18,** 304–314 (1982).
[22] A. D. Pardo and J. A. Schiff, Can. J. Bot. **58,** 25–35 (1980).
[23] B. Halliwell, Cell Biol. Int. Rep. **2,** 113–128 (1978).
[24] H. W. Gardner, J. Agric. Food Chem. **27,** 220–229 (1979).
[25] G. D. Peiser and S. F. Yang, Phytochem. **17,** 79–84 (1978).
[26] J. Feierabend and P. Kemmerich, Physiol. Plant. **57,** 346–351 (1983).
[27] E. Margoliash and A. Novogradsky, Biochem. J. **74,** 339–348 (1960).
[28] B. Gerhardt, Microbodies/Peroxisomen pflanzlicher Zellen, Cell Biology Monographs. **Vol. 5,** pp. 16–21, Springer, Wien, New York 1978.
[29] O. Vesterberg, Hoppe-Seyler's Z. Physiol. Chem. **361,** 617–624 (1980).