Nature of Photooxidative Events in Leaves Treated with Chlorosis-Inducing Herbicides

JÜRGEN FEIERABEND AND THERESA WINKELHÜSENER
Botanisches Institut, J. W. Goethe-Universität, Postfach 11 19 32, D-6000 Frankfurt/am Main, Federal Republic of Germany

ABSTRACT
Leaves of rye seedlings (Secale cereale L.) grown in the presence of four chlorosis-inducing herbicides under a low light intensity of 10 lux formed chlorophyll. When segments of such dim-light-grown leaves were exposed to 30,000 lux at either 0°C or 30°C, treatments with aminotriazole or haloxadine (group 1) showed no or only minor changes of their chlorophyll contents. In treatments with San 6706 or difunon (group 2), however, rapid photodestruction of chlorophyll occurred both at 0°C and at 30°C and was accompanied by an increase of malondialdehyde that was not seen in the presence of group 1 herbicides. Unlike the in vivo behavior, virtually equal rates of chlorophyll breakdown were observed for aminotriazole and San 6706 treatments in suspensions of isolated chloroplasts from 10 lux-grown leaves after exposure to strong light. The free radical scavengers p-benzoquinone and hydroquinone and the ß-penicillamine copper complex exerting superoxide dismutating activity effectively prevented photooxidation of chlorophyll in 10 lux-grown herbicide-treated leaf segments or even restored an accumulation of chlorophyll at 30,000 lux. Ascorbate and several singlet oxygen or hydroxyl radical scavengers had no protective effects. Deuterium oxide and H2O2 did not enhance the degradation of chlorophyll. Superoxide dismutase activity was decreased in leaves bleached in the presence of group 2 herbicides.

In previous work, two groups of chlorosis-inducing herbicides of different potency were discriminated (10, 11). A representative of the pyridazine herbicides, San 6706, and difunon (group 2) induced a severe carotenoid deficiency and very intense photooxidative damage of treated leaves in light, including the degradation of Chl. 70S ribosomes, chloroplast enzymes, and even the inactivation of many or conceivably all peroxisomal enzymes. Photooxidative damage in the presence of other chlorosis-inducing herbicides (group 1: aminotriazole, haloxadine) was much weaker: photodegradation of Chl did not appear to be of major relevance in our system and, except for catalase, chloroplastic and peroxisomal enzymes were not inactivated.

Photooxidation of Chl, as occurring in the presence of group 2 herbicides, has been attributed to the fact that excited triplet Chl cannot be quenched by carotenoids in the carotenoid-deficient leaves and is, therefore, able to react with O2 generating reactive O2 radicals, which cause the degradation of Chl and possibly other chloroplast constituents (23, 29). The question of the nature of the activated O2 species involved in Chl bleaching is not yet settled. Singlet O2 (O2), superoxide (O2−) and hydroxyl radicals (OH) have been implicated by different authors (15, 18, 23, 27). To characterize the photooxidative events and the forms of activated O2 involved in the herbicide-induced bleaching we have compared the photodestruction of Chl occurring when intact leaf segments or isolated chloroplasts from plants grown under dim light were exposed to a high light intensity. Several scavengers for free radicals or for specific species of activated O2 were tested for their ability to prevent, and D2O that prolongs the lifetime of O2 (28) and H2O2 for their ability to promote, the degradation of Chl. Superoxide dismutase was assayed to study the influence of the herbicides on the activity of one of the protective mechanisms against O2 toxicity and malondialdehyde was estimated to elucidate whether photooxidation of Chl was related to lipid peroxidation in herbicide-treated leaves.

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 H2O, and placed in plastic boxes on filter paper (Schleicher and Schüll, No. 598) moistened with either distilled H2O or a herbicide solution, as indicated. The seedlings were grown for 6 d 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 5,000 ± 500 lux or 10 lux (Warmton de Luxe only). The following herbicides and concentration 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 (haloxadine), 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-dihydrofurane-carbonitrile (3, difumone, EMD-IT-5914).

For growing seedlings in the presence of dl-α-tocopherol acetate, 500 μmol each were dissolved in ethanol and applied to filter paper discs in glass boxes (20 cm diameter). After evaporation of the solvent the filter paper was moistened with the herbicide solution (50 ml).

Isolation of Chloroplasts. Leaves of 6-day-old rye seedlings grown at 10 lux in the presence of either 0.3 mM aminotriazole or 0.02 mM Sandoz 6706 were finely minced with razor blades in 2 to 3 volumes of ice-cold grinding medium and then briefly and gently ground in a mortar in the presence of a small quantity of sea sand. The grinding medium of Feierabend and Bevers (9) was used, except that Ficoll was omitted. The homogenate was pressed through four layers of muslin, and two layers of Miracloth. The sediment obtained after 20-s centrifugation at 270 g was discarded. The supernatant was centrifuged for 5 min at 3,000 g. The resulting sediment was resuspended in grinding medium without ascorbate and the suspension adjusted to a Chl concentration of 0.12 mg ml−1.

Bleaching Experiments at High Light Intensity. From 6-d-old
rye seedlings grown at 10 lux 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 (pH 6.0). Where indicated, additional substances to be tested for their influence on Chl destruction were dissolved in the buffer. 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 30,000 ± 1,000 lux provided by high pressure mercury lamps (Südlicht, HSL-S 250 W TT V3).

The 5,5-diphenyloxazoline copper complex was prepared according to Birker and Freeman (2). BHT and D,L-a-tocopherol acetate were dissolved in ethanol and DMSO and diluted with buffer to a final concentration of 0.5% ethanol and 1% DMSO. In the 94% deuterium oxide solution, the K-phosphate concentration was only 57 mm.

Suspensions of isolated chloroplasts were exposed to 30,000 lux in test tubes kept in a water bath at 25°C and resuspended at 30-min intervals.

Application of H₂O₂. The influence of H₂O₂ applications on Chl destruction was studied with middle sections of leaves of 6-d-old rye seedlings grown at 10 lux that were floated for 6 h at room temperature in darkness and under slow rotation on 12 ml 0.1 m K-phosphate (pH 6.0), containing 1 mm H₂O₂ in Petri dishes (10 cm diameter). In some experiments, as indicated, the leaf sections were for the first 1.5 h kept on 0.5 mm Fe₂SO₄ in 0.5 mm EDTA, and thereafter transferred for a further 4.5 h to 1 mm H₂O₂ in K-phosphate (pH 6.0).

Incubations with 10 mm glycolic acid (without neutralization or buffer) were performed in darkness in the same way as described for the H₂O₂ applications. Control leaf sections were floated on a 10 mm citric acid solution, adjusted with NaOH to the pH 2.8 of the glycolic acid solution.

Preparation of Cell-Free Extracts. The first leaves of 20 rye seedlings grown at 5,000 lux were completely homogenized under ice-cold conditions with mortar and pestle with 50 mm Tris-HCl buffer (pH 7.8) and the volume adjusted to 10 ml. After 5 min centrifugation at 120g the supernatant was used for the assay of superoxide dismutase.

Estimation of Lipid Peroxidation. Decomposition products of the oxidation of polyunsaturated fatty acids (malondialdehyde) were determined as thiobarbituric acid-reactive substances in leaf extracts or with samples of chloroplast suspensions according to Heath and Packer (21). Homogenates of the middle sections of leaves grown at 10 lux were prepared in 5% TCA with mortar and pestle (10 leaf sections per 5.0 ml). Measurements were corrected for unspecific turbidity by subtracting the A at 600 nm, and for A at 532 nm originating from the extract after incubation in the absence of thiobarbituric acid.

Analytical Methods. Pigments were extracted with 90% acetone. Chl was determined according to Arnon (1).

Superoxide dismutase activity was assayed by the method of Marshall and Worsfeld (25).

The experiments were performed 3 to 6 times. The data presented are averages of the measurements. Standard deviations of the mean are indicated.

RESULTS

Bleaching of Chl in Leaves. When grown at a low light intensity of 10 lux leaves of rye seedlings formed Chl (between 40 and 100% of its content in H₂O₂-grown controls) in the presence of the herbicides aminotriazole, haloxadine, San 6706 and difunon at concentrations that were high enough to induce a complete chlorosis at 5,000 lux. Sections from the middle of 10 lux-grown leaves were excised and exposed to a high light intensity of 30,000 lux on a buffer of pH 6.0. Tips and basal parts were discarded to have a tissue of fairly uniform differentiation and reactivity. Illuminations with 30,000 lux were performed both at 0°C and 30°C to discriminate photochemical reactions from those involving metabolic processes. In the controls the Chl contents increased during the exposure to bright light at 30°C and slightly decreased at 0°C where no Chl synthesis occurred (Fig. 1). In the herbicide treatments, two types of response were observed: in treatments with San 6706 and difunon strong photodestructions of Chl occurred within a few hours both at 0°C and at 30°C (Fig. 1). In treatments with aminotriazole and haloxadine, bleaching of Chl was relatively slow and only seen in the presence of rather high, growth-inhibiting, herbicide concentrations (as used in Fig. 1) and at 0°C where degradation could not be compensated by new synthesis of Chl. To get some information about the nature of the photooxidative events involved in the bleaching of Chl in herbicide-treated tissue, several quotients of excited Chl and scavengers of activated O₂ forms were added to the buffer during the illumination with bright light.

Involvement of Chl Radicals and Singlet Oxygen (O₂). Quinones are known as quenchers of triplet Chl (13, 27). Both p-benzoquinone and hydroquinone markedly slowed Chl breakdown in 10 lux-grown herbicide-treated leaf segments exposed to 30,000 lux at 0°C. At 30°C their effect was even greater (Fig. 1). In San 6706 treatments, the light-induced Chl breakdown was almost prevented at 30°C, in all other herbicide treatments the Chl content clearly increased in the presence of p-benzoquinone in spite of the high light intensity. Even in untreated controls the rate of Chl accumulation was increased. The efficiency of p-benzoquinone to prevent bleaching was presumably still greater but underestimated in our experiments because of incomplete penetration into the tissue, particularly at 0°C. The cut ends of the leaf sections were considerably greener than the inner parts

![Fig. 1. Changes of the total Chl contents in middle sections of the first leaves of 6-d-old rye seedlings grown on H₂O or herbicide solutions in continuous white light of 10 lux after exposure to 30,000 lux at either 0°C or 30°C on a phosphate buffer medium without addition (○), and in the presence of 10 mM p-benzoquinone (△), 10 mM hydroquinone (□), or 1 mM DABCO (△). Herbicide concentrations were: 0.3 mM aminotriazole; 0.15 mM haloxadine; 0.02 mM San 6706; 0.1 mM difunon.](image-url)
after exposure to bright light in the presence of p-benzoquinone.

A known scavenger for $O_3$ is α-tocopherol (16, 20). Therefore, an observation of Hilton et al. (22) that Chl accumulation was strongly increased when α-tocopherol acetate was applied to growing seedlings simultaneously with a treatment with pyridazinone herbicides deserves particular interest. We fully confirmed the protective effect of α-tocopherol acetate for San 6706-treated rye leaves (Fig. 2). However, in all other herbicide treatments applications of α-tocopherol acetate had no or only minor effects (Fig. 2). When applied to 10 lux-grown leaf sections during exposure to 30,000 lux α-tocopherol acetate did not protect against photodegradation of Chl even when its uptake was facilitated by the addition of DMSO (Fig. 3). Whereas DMSO can, in addition, act as a hydroxyl radical scavenger (5) this did not influence the bleaching of Chl. Other generally applied scavengers for $O_3$, such as DABCO (Fig. 1) (26), BHT (Fig. 2) or ascorbate (Fig. 5) (3) did not protect the Chl from photodegradation in herbicide-treated leaves. DABCO even appeared to somewhat promote the bleaching of Chl. Chl degradation in leaf segments grown at 10 lux with aminotriazole or San 6706 was not appreciably enhanced in the presence of 94% D$_2$O (Fig. 4) which greatly prolongs the lifetime of $O_2$ (28).

**Involvement of Superoxide.** Ascorbate is oxidized by $O_2^-$ and can thereby act as scavenger (16). The δ-penicillamine copper complex was shown to exhibit superoxide dismutating activity (24) and can, as a small molecule, be taken up by intact tissue (30). In the 10 lux-grown rye leaf tissue ascorbate was totally unable to prevent Chl bleaching after transfer to 30,000 lux but, instead, even appeared to promote it. By contrast, δ-penicillamine copper very markedly slowed high light-induced Chl degradation after all herbicide treatments at 0°C. At 30°C Chl breakdown was not only in all instances completely prevented but Chl even accumulated in the high light intensity (Fig. 5). The magnitude of Chl accumulation varied for the different herbicides. Also in untreated control leaves an enhanced Chl accumulation was observed in the presence of δ-penicillamine copper (Fig. 5).

**To investigate the influence of the herbicide treatments on the superoxide dismutating ability of the tissues, superoxide dismutase activity was estimated in extracts of leaves grown at 5,000 lux where all herbicides induced chlorosis. In the absence of herbi-
cides, superoxide dismutase activity was about twice as high in green leaves of light-grown, as in etiolated leaves of dark-grown seedlings. Chlorotic leaves from seedlings grown in light in the presence of aminotriazole or haloxidine exhibited as high activities as untreated green leaves, but leaves grown in the presence of San 6706 and particularly difunon had considerably lower superoxide dismutase activities (Fig. 6).

Involvement of Hydroxyl Radicals. Thiourea, formate, and Tris were applied as scavengers of hydroxyl radicals (5, 17). Chl bleaching of 10 lux-grown leaf segments at 30,000 lux (Fig. 7) was in no instance decreased by their presence. In San 6706 and difunon-treated leaf segments, even an enhanced Chl degradation was observed in the presence of some of the hydroxyl scavengers.

Influence of H2O2. H2O2 was applied to, or generated in, green 10 lux-grown untreated and aminotriazole-treated leaves in darkness in order to test its efficiency as a possible intermediate of photooxidative processes on the stability of Chl. Except for a slight Chl degradation in combination with a pretreatment of the leaf sections with an Fe2+ salt solution (FeSO4) external H2O2 applications had no effect, even when DMSO or propanol were present to facilitate the uptake (Fig. 8).

Externally applied glycolic acid is readily oxidized by leaf segments (8) and can, therefore, be expected to generate H2O2 inside the cells. In aminotriazole-treated tissue an even greater accumulation of the resulting internal H2O2 is to be expected because of the inactivation of catalase through aminotriazole (10). Nevertheless, Chl was not significantly degraded after application of glycolic acid, as compared to leaf sections floated as controls on a citric acid solution of the same pH as the glycolic acid solution applied.

Lipid Peroxidation. Photooxidative destruction of lipids can be assayed by the formation of ethane or thiobarbituric acid-reactive material ('malondialdehyde') (6). Only in leaf segments grown at 10 lux in the presence of San 6706 and difunon thiobarbituric acid-reactive material markedly increased during a 3 h illumination with 30,000 lux above the level measured before the strong light treatment. In the other herbicide treatments and in control leaves no change of the malondialdehyde content was seen (Fig. 9).

Bleaching of Chl in Isolated Chloroplasts. Chloroplasts were isolated from leaves grown at 10 lux in the presence of aminotriazole and San 6706 or, as controls, without herbicides on H2O. Chloroplast suspensions were illuminated with 30,000 lux at 25°C.
In vitro considerable photodestruction of Chl occurred in chloroplast suspensions from untreated control leaves, but in preparations from herbicide-treated leaves Chl degradation was much greater. However, in striking contrast to the in vivo behavior, chloroplasts from the aminotriazole treatments exhibited an equally high photodestruction of Chl as those from San 6706 treatments (Fig. 10) and in vitro malondialdehyde increased in both herbicide treatments and even in the untreated controls (Fig. 10).

**DISCUSSION**

After exposure of dim light-grown leaf segments to a high light intensity significant photodestruction of Chl was seen only in treatments with the herbicides designated here as group 2 (San 6706, difunon) but not in treatments with group 1 herbicides (aminotriazole, haloxydone). Therefore, we feel that the chlorosis induced by group 1 herbicides can at least not predominantly originate from a direct photodegradative degradation of Chl. The strong photodestruction of Chl in the presence of group 2 herbicides has been related to the severe carotenoid deficiency induced by them (for literature, see 10, 11, 29) which results in the occurrence of the reactive triplet state of Chl that is normally quenched by protective carotenoids. Triplet Chl is able to interact with O₂, yielding activated O₂ forms which backreact with Chl and cause its oxidative decomposition. That the strong photodestruction of Chl in the presence of group 2 herbicides occurred at 0°C demonstrates that it mainly resulted from photodynamic reactions. Initially only singlet O₂ was thought to be involved in the photodynamic decomposition of Chl (23). However, also the photochemical formation of superoxide and hydroxyl radicals has been assumed in other Chl-containing systems and discussed as being responsible for the photodestruction of Chl (15, 18, 27).

Inasmuch as photodestruction of Chl was in isolated chloroplasts as strong in treatments with a representative of group 1 herbicides, aminotriazole, as in treatments with group 2 herbicides, some protective mechanisms were obviously lost during isolation. Because the in vitro system did thus not truly reflect the in vivo situation we have used the intact leaf system for the application of free radical and activated O₂ scavengers, whereas this is much more problematic. In the in vivo system greater uncertainties exist whether the substances were sufficiently taken up by the tissue and whether they really reached the chloroplast membranes. A further disadvantage of the approach is that many of the scavenger substances are not truly specific for only one radical form. Ascorbate can, for instance, react with O₂, O₂⁻ and OH⁻ (3, 16).

Among the different substances tested only benzo- and hydroquinone and the d-penicillamine copper complex, a superoxide dismutase substitute (24), effectively protected Chl from photooxidation in the herbicide-treated tissue. The observation that in leaf segments from untreated controls or seedlings treated with the milder chlorosis-inducing herbicides of group 1, where no light-induced Chl bleaching was observable at 30°C, both the quinones and the penicillamine-copper complex significantly increased Chl accumulation at 30°C indicated that also under these conditions (i.e. even without herbicides) some slight photooxidation was usually occurring but fully compensated by new synthesis of Chl. Quinones had similarly been found to be most effective in preventing light-induced degradation of Chl in leaf discs of Cucumis, a chilling-sensitive plant, at low temperature (19) and to protect Chl from destruction in the presence of bisulfite and O₂ (27). The efficiency of the quinones is most probably attributable to their ability to scavenge free radicals and quench triplet Chl energy (13).

The strong protective action of the penicillamine copper points to an essential role of the superoxide radical in the herbicide-dependent (group 2) photodegradation of Chl, whereas inefficiency or even Chl breakdown enhancing action of ascorbate seems to question this result. It remains, however, uncertain whether externally applied ascorbate was in an unmetabolized form reaching the chloroplast membranes. In addition, when exposed to O₂⁻, autoxidizing ascorbate is known to produce highly reactive intermediates able to evoke the oxidative destruction of several cell constituents (16). Because the action of ascorbate can thus even enlarge cellular damage penicillamine copper appears to be the more specific O₂⁻ scavenger. Its protective effect suggests that the endogenous superoxide dismutase activity was not sufficient to break down the O₂⁻ generated under strong light in treatments with group 2 herbicides or that the enzyme was not accessible to its substrate. In San 6706- and difunon-bleached leaves, total superoxide dismutase activity was markedly lowered, relative to untreated controls, but by far not absent. It is to be expected that, similar to other chloroplast enzymes, such as NADP-glyceraldehyde-P dehydrogenase (11), that portion of superoxide dismutase which is localized in the chloroplasts (for literature, see 6) was inactivated. Presumably this inactivation was, however, not origin for, but itself consequence of, photooxidation in the chloroplast so that already the initial activity in the organelle must have been insufficient under high light conditions in the presence of the herbicides. A crucial involvement of superoxide was similarly deduced for paraquat-induced bleaching from its inhibition by the penicillamine copper complex in intact flax cotyledons (30), and for Chl destruction catalyzed by bisulfite (27).

Direct one-electron oxidation of Chl by O₂⁻ has been considered (27) but the O₂⁻ radical itself is mostly regarded as only poorly reactive (16) and mainly as a source for the generation of more aggressive forms of activated oxygen, such as singlet oxygen (12, 16, 30), Fenton type oxidants resulting from the interaction of Fe⁺⁺ and H₂O₂ (7, 17), hydroxyl radicals (17), or lipid peroxides (27, 30). The suppression of Chl bleaching by the presence of α-tocopherol acetate in the growth medium together with pyridazine-4-nitro-herbicides (Fig. 2) (22) appeared to point towards an involvement of α-tocopherol. It seems, however, rather to result from other than the O₂ scavenging properties of α-tocopherol because other chlorosis-inducing herbicides, including the very similar difunon, were not affected. In addition, direct application of α-tocopherol or other O₂ scavengers like DABCO and BHT to leaf segments were without effect on herbicide-dependent photooxidation of Chl. By contrast, in acetone solution, Chl was efficiently protected from photooxidation by α-tocopherol (20). Whereas the effectiveness of several of the O₂ scavengers might have been hampered by insufficient uptake into the intact leaf tissue, D₂O which strikingly
increases the lifetime of $^{18}O_2$ (28) should have been expected to
easily permeate to cells. However, inasmuch as $D_2O$ did not affect
the rate of Chl bleaching all our results speak against a partici-
pation of $O_2^-$ in the herbicide-dependent photooxidation of Chl in
intact leaves. In isolated chloroplast fragments $D_2O$ has been
shown to increase lipid peroxidation strongly (28).

The product of the dismutation of $O_2^-$, $H_2O_2$, is regarded as the
most toxic activated $O_2$ form (16) and our results with external
applications or intracellular generation of $H_2O_2$ even in combina-
tion with a Fe$^{3+}$-treatment thought to favor Fenton-type oxidant
and OH$^-$ radical formation did not suggest any significant role of
$H_2O_2$ in the oxidative degradation of Chl. Similarly, in solution
Chl remained stable in the presence of $H_2O_2$ in vitro (27). The
inefficiency of the OH$^-$ radical scavengers, though they were
applied at rather high concentrations, speaks against the conten-
tion of Harbour and Bolton (18) on the significance of the OH$^-$
radical for Chl photooxidation and it has now been questioned
whether OH$^-$-radicals are at all generated in biological systems
(7).

Superoxide appears to give rise to lipid peroxidation (12, 16, 27,
30) and the behavior of the malondialdehyde-like material indi-
cated that a concomitant lipid peroxidation occurred in those
herbicide treatments where Chl photodestruction was found. Evi-
dence has been presented that products of lipid peroxidation,
such as alkoxy radicals, destroy Chl (27). From this it is conceiv-
able that the photooxidative decomposition of Chl in treatments
with group 2 herbicides was mediated through lipid peroxidation
and that the differences in the extent of photodestructive damage
depend on unequal abilities of the various herbicides to induce
lipid peroxidation. This may not only apply to the bleaching of
Chl but also to the breakdown of other cell constituents. Whereas
the activated forms of $O_2$ are themselves known to attack nucleic
acids and proteins (4, 12, 16), intermediates of lipid peroxidation
are regarded as particularly aggressive against proteins leading to
crosslinking, scission, conjugation with lipids and amino acid
damage (14). This can be the reason that the chloroplasm enzyme
NADP-glyceraldehyde-3-P dehydrogenase was inactivated only
in treatments with group 2 herbicides where lipid peroxidation
took place (11). Conceivably, lipid peroxidation is even able to
spread through membrane contacts which have been regularly
observed between chloroplasts and leaf microbodies and can thus
specifically propagate photodestructive events from the chloro-
plasts to another organelle leading to the inactivation of peroxy-
somal enzymes that was also exclusively observed in treatments
with group 2 herbicides (10, 11). Our results attribute key roles in
the generation of photodestructive damage in the presence of the
chlorosis-inducing herbicides of group 2 to $O_2^-$ and lipid peroxi-
dation but their detailed action and interdependence need further
elucidation.

Acknowledgments—We are grateful to Celamerck, Ingelheim, Imperial Chemical
Industries Ltd., Brackett, Berkshire, and Sandoz AG, Basel, for supplying the
compounds tested. Technical assistance of Mrs. B. Fiolka and the help of Miss P.
Kemmerich during some of the experiments is greatly appreciated.

LITERATURE CITED

1. Arnion DI 1949 Copper enzymes in isolated chloroplasts. Polyphenoloxidase in

2. Birker PJMWL, HC Freeman 1977 Structure, properties, and function of a
copper(I)-copper(II) complex of d-phenylamine: phenanthathilium(1+)-chloro-do-
6890–6899

3. Bodannes RS, PC Chan 1979 Ascorbic acid as a scavenger of singlet oxygen.

components by hydroxyl radicals, singlet oxygen and superoxide anion radicals.
Photochem Photobiol 28: 661–667

5. Cederbaum AI, E Dicker, E Rubin, G Cohen 1977 The effect of dimethylsul-
foxide and other hydroxy radical scavengers on the oxidation of ethanol by rat
liver microsomes. Biochem Biophys Res Commun 78: 1254–1262

In JV Bannister, Hao Hill, eda, Biochemical Aspects of Superoxide and Superoxide

donor-hydrogen peroxide complex instead of free OH radicals? FEBS Lett 121:
219–221

8. Feerabend J 1975 Developmental studies on microbodies in wheat leaves. III.
On the photocontrol of microbody development. Planta 123: 63–77


10. Feerabend J, B Schmidt 1978 Comparative investigation of the action of
several chlorosis-inducing herbicides on the biogenesis of chloroplasts and leaf

11. Feerabend J, U Schulz, P Kemmerich, T Lowitz 1979 On the action of
chlorosis-inducing herbicides in leaves. Z Naturforsch 34c: 1036–1039


13. Fujimori E, R Livingstone 1957 Interactions of chlorophyll in its triple state
with oxygen, carotene, etc. Nature 190: 1036–1038

14. Gairder JW 1979 Lipid hydroperoxide reactivity with proteins and amino acids:

in systems containing chlorophyll. Photochem Photobiol 32: 793–798

16. Halliwell B 1978 Biochemical mechanisms accounting for the toxic action
of oxygen on living organisms: the key role of superoxide dismutase. Cell Biol Int
Rep 2:113–128

17. Halliwell B 1978 Superoxide-dependent formation of hydroxyl radicals in
the presence of iron chelates. FEBS Lett 92: 321–326

18. Harbort JR, B Bolton 1978 The involvement of the hydroxyl radical in the
destructive photooxidation of chlorophylls in vitro and in vitro. Photochem Photobiol
28: 231–234

19. Haselvet Van PR 1976 Protection of Cucumis leaf pigments against photo-

20. Haselvet Van PR, LJ de KOK, PJCE Kuiper 1977 Effect of a-tocopherol, $\beta$-
carotene, monogalactosyldiglyceride and phosphatidylycholine on light-induced
degradation of chlorophyll a in acetone. Physiol Plant 45: 475–479

and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125:
189–198

22. Hilton JL, JB St John, MN Christiansen, KH Norris 1971 Interactions of
lipidic materials and a pyrimidine inhibitor of chloroplast development.
Plant Physiol 48: 171–177

Soc Lond B 264: 581–599

24. Leflenfder E, BF Elstner 1978 Determination of the superoxide dissimulating

assay using the oxygen electrode. Anal Biochem 86: 561–573

26. Quannewh C, TH Wilson 1968 Quenching of singlet oxygen by tertiary alicyclic

27. Feher GD, SF Yang 1978 Chlorophyll destruction in the presence of bisulfite
and linoleic acid hydroperoxide. Phytochemistry 17: 79–84

28. Takahama U 1979 Stimulation of lipid peroxidation and carotenoid bleaching
by deuterium oxide in illuminated chloroplast fragments: participation of

29. Waith K, JR Corbett 1979 Biochemistry of herbicides affecting photosyn-
thesis. Z Naturforsch 34c: 966–972

30. Youngman RJ, AD Dodge 1979 Mechanism of paraxial action: inhibition of the
Lemneid effect by a copper chelate with superoxide dismutating activity.
Z Naturforsch 34c: 1032–1035