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Photosynthesis Involvement in the Mechanism of Action of Diphenyl Ether Herbicides[1]

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MICHAEL P. ENSMINGER[2] AND F. DAN HESS*
Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907

ABSTRACT

Photosynthesis is not required for the toxicity of diphenyl ether herbicides, nor are chloroplast thylakoids the primary site of diphenyl ether herbicide activity. Isolated spinach (*Spinacia oleracea* L.) chloroplast fragments produced malonyl dialdehyde, indicating lipid peroxidation, when paraquat (1,1′-dimethyl-4,4′-bipyridinium ion) or diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] were added to the medium, but no malonyl dialdehyde was produced when chloroplast fragments were treated with the methyl ester of acifluorfen (methyl 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid), oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene], or MC15608 (methyl 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-chlorobenzoate). In most cases the toxicity of acifluorfen-methyl, oxyfluorfen, or MC15608 to the unicellular green alga *Chlamydomonas eugametos* (Moewus) did not decrease after simultaneous treatment with diuron. However, diuron significantly reduced cell death after paraquat treatment at all but the highest paraquat concentration tested (0.1 millimolar). These data indicate electron transport of photosynthesis is not serving the same function for diphenyl ether herbicides as for paraquat. Additional evidence for differential action of paraquat was obtained from the superoxide scavenger copper penicillamine (copper complex of 2-amino-3-mercapto-3-methylbutanoic acid). Copper penicillamine eliminated paraquat toxicity in cucumber (*Cucumis sativus* L.) cotyledons but did not reduce diphenyl ether herbicide toxicity.

DPE[3] herbicides initiate lipid peroxidation and eventually disrupt cell membranes causing cell death (7, 13, 18). This cell death by DPE herbicides requires light (8, 15, 17); however, the mechanism of initiation of lipid peroxidation of the polyunsaturated fatty acids in the membrane is unknown. Orr and Hess (18) previously proposed that DPE herbicides directly become free radicals by interacting with carotenoids, and these activated DPE herbicides initiate lipid peroxidation in the membrane. However, this hypothesis can not be valid for all DPE herbicides. For example, Cl and H atoms that replace the NO_2 of AFM can not undergo free radical formation yet have the same mode of action as AFM (7). A different theory for DPE membrane damage has been proposed by Sandmann and Böger (22), where the DPE herbicide oxyfluorfen requires photosynthesis prior to initiating lipid peroxidation of the polyunsaturated fatty acids in the membrane of *Scenedesmus acutus*.

The purpose of this study was to determine if photosynthesis is involved in DPE herbicide membrane disruption and cell death as it is for cell death by paraquat and diuron (5). This research used isolated spinach chloroplast fragments, the unicellular green alga *Chlamydomonas*, and excised cucumber cotyledons to evaluate the requirement of photosynthesis for DPE caused cell death.

MATERIALS AND METHODS

Isolated Chloroplast Fragments. Spinach chloroplast fragments were prepared by the method of Takahama (24) with some modification. Large veins were removed from 25 to 30 g of commercially grown spinach leaves, and then the leaves were cut into 1 cm^2 pieces. Leaf pieces were blended 30 s in a Waring Blendor with 125 ml of 17 mM Tris·HCl buffer (pH 7.4). The debris was filtered through eight layers of cheesecloth and centrifuged at 2000*g* for 1 min in a Sorvall HB-4 rotor. The supernatant was centrifuged 10,000*g* for 10 min, and the pellet was resuspended in 17 mM Tris·HCl buffer (pH 7.4) which was again centrifuged at 10,000*g* for 10 min. The chloroplast fragments in the pellet were resuspended in 17 mM Tris·HCl buffer, pH 7.4. All isolations were conducted at 4°C, and Chl concentrations were determined using the absorption coefficients for Chl *a* and *b* as given by MacKinney (14). Photosynthetic electron transport activity in the chloroplasts was determined indirectly by measuring the reduction of DCPIP.

The basic reaction mixture for each experiment contained chloroplast fragments (200 μg of Chl) in 2 ml of 17 mM Tris·HCl buffer (pH 7.4) plus the appropriate herbicide. Nontreated samples contained the same amount of solvent as the treated samples (0.1% [v/v] ethanol or 0.1% [v/v] DMSO). To illuminate the chloroplast fragments, a light intensity of 150 $\mu E\ m^{-2}\ s^{-1}$ (all light measurements were PAR) was provided by a bank of twelve General Electric F48T12/CW/VHO fluorescent lamps and four 60-w incandescent bulbs.

After treatment MDA formation, indicating lipid peroxidation, was determined according to the method of Takahama and Nishimura (25). To detect MDA, 0.5 ml of 40% TCA, 0.25 ml of 5 N HCl, and 0.5 ml of 2% thiobarbituric acid were added to 2 ml of chloroplast fragments. The thiobarbituric acid was first dissolved in a small amount of 1 N NaOH (20) and brought up to volume with distilled H_2O. Final NaOH concentration was 0.35 N. After mixing, the chloroplast fragments were placed in a boiling water bath for 10 min, cooled on ice, and centrifuged at 2000*g* for 5 min. Absorbance of the supernatant, containing MDA, was recorded by a spectrophotometer at 532 nm.

Algal Bioassay. All experiments with algae were conducted using the unicellular green alga *Chlamydomonas eugametos* (Moewus). *Chlamydomonas* cells were maintained on agar slants, and liquid cultures were initiated and grown as described previ-

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[2] Present address: Stauffer Chemical Company, Mountain View, CA 94042.

[3] Abbreviations: DPE, diphenyl ether; AFM, acifluorfen-methyl; DCPIP, 2,6-dichlorophenol-indophenol; MDA, malonyl dialdehyde; ESR, electron spin resonance.

ously (7). The population of *Chlamydomonas* cells at the start of each experiment was adjusted to 1×10^6 cells ml^{-1}. To initiate a bioassay, 10 ml of the cells were added to 25-ml flasks. Cotton plugs were placed in the neck of each flask. These flasks were aerated and placed on a shaker (60 rpm) in a growth chamber. Light intensity was 175 $\mu E\ m^{-2}\ s^{-1}$. The *Chlamydomonas* cells were pretreated for 5 min with 1×10^{-6} M diuron prior to adding the other herbicides for 90 min.

Cell death was recorded using the fluorescein diacetate procedure, which has previously been shown to be a good indicator of cell death (7, 27). Relative fluorescence intensity was measured by an Aminco fluoro-colorimeter (model number JA-7439; American Instrument Company, Silver Springs, MD), using excitation filter 47B (360–500 nm) and cutoff filter 8 (transmittance greater than 470 nm).

Spinach and Cucumber Bioassays. To evaluate herbicide-induced Chl destruction in spinach, commercial spinach leaves were cut into 1 cm^2 pieces, avoiding major veins. Eight leaf segments were floated on 40 ml of distilled H_2O in a Petri dish (9 cm diameter) with the appropriate herbicide. Nontreated samples contained the same amount of solvent as the treated samples. Spinach leaf segments were illuminated with 600 $\mu E\ m^{-2}\ s^{-1}$. Solutions were changed after 24 h and Chl was extracted at 48 or 64 h by grinding the spinach leaf segments in 80% acetone with a hand homogenizer. The homogenate was centrifuged at 2000*g* for 5 min, and Chl was determined by measuring *A* at 645 and 663 nm. Chl concentrations in the supernatant were determined using the absorption coefficients of MacKinney (14).

For the cucumber bioassays, cucumber seeds were planted in vermiculite and grown in the growth chamber with a 12 h diurnal day (220 $\mu E\ m^{-2}\ s^{-1}$) and night. Vermiculite was kept moist with deionized H_2O. After 7 d, eight greened cucumber cotyledons were placed in a Petri dish (9 cm diameter) containing 40 ml of distilled H_2O. These cotyledons were pretreated 24 h (220 $\mu E\ m^{-2}\ s^{-1}$) with the superoxide scavenger copper penicillamine. Copper penicillamine was prepared according to the method of Birker and Freeman (4). After the 24 h copper penicillamine pretreatment, the appropriate herbicide plus copper penicillamine was added to the cucumber cotyledons for 24 h in the light (600 $\mu E\ m^{-2}\ s^{-1}$). At the termination of the cucumber cotyledon bioassays, herbicide toxicity and effectiveness of copper penicillamine to protect against Chl degradation was evaluated by determining Chl content in the tissue. Chl was extracted as described above for spinach leaf segments.

General Procedures and Statistics. All experiments were conducted at ambient temperatures (23–25°C). Experiments had at least two replications and were at least duplicated. Where appropriate the data were subjected to analysis of variance. The means were compared by Duncan's multiple range test, with significance at the 5% level.

Chemicals and Herbicides. MC15608 and AFM were gifts of Rhone-Poulenc, Inc. Diuron, paraquat, and oxyfluorfen were purchased from Chem Service (West Chester, PA). Penicillamine, FDA, and thiobarbituric acid were purchased from Sigma Chemical Company.

RESULTS

Photosynthesis Involvement in Isolated Chloroplast Fragments. It was necessary to observe if paraquat, diuron, and DPE herbicides were toxic to spinach prior to using spinach leaves as a source of chloroplasts. Both paraquat and diuron were toxic to spinach (Table I) and have been used previously in isolated chloroplast experiments (24–26). Concentrations of paraquat (1×10^{-4} M) and diuron (5×10^{-6} M) used in the isolated chloroplast fragment study were the same as previously published (24–26). However, in whole leaf tissue, a higher concentration of diuron (5×10^{-5} M) was needed to observe Chl degradation (Table I). In this study, Chl degradation caused by treating leaf segments with 1×10^{-5} M paraquat was visually evident by 24 h, and by 48 h more than 98% of the Chl had been degraded (Table I). In spinach leaf segments, Chl degradation by 1×10^{-4} M paraquat, the paraquat concentration used in isolated chloroplast fragments, was similar to the breakdown of Chl caused by 1×10^{-5} M paraquat, except the bleaching occurred earlier (data not shown). Bleaching symptoms induced by diuron occurred later than paraquat induced bleaching. After 48 h, little Chl degradation was evident (data not shown), but by 64 h the Chl content of spinach leaf segments treated with diuron had decreased by 43% (Table I). All of the DPE herbicides studied destroyed Chl in the spinach leaf segments within 48 h (Table I). The concentrations of AFM, MC15608, and oxyfluorfen used caused visual symptoms of Chl breakdown in 24 h and about 50% Chl breakdown in 48 h. One exception was 5×10^{-5} M AFM, which caused a 92% reduction of Chl by 48 h (Table I). In this case, a concentration of AFM that destroyed the same percentage of Chl as did 1×10^{-5} M paraquat was included. AFM (1×10^{-5} M), MC15608 (1×10^{-4} M), and oxyfluorfen (5×10^{-5} M) treatments decreased the Chl content of the leaf segments 68%, 66%, and 52%, respectively, after 48 h of exposure to light (Table I). These DPE herbicide concentrations were then used in the isolated chloroplast fragments.

Table I. *Chl Degradation by Paraquat, Diuron, and DPE Herbicides in Spinach Leaf Segments*

Spinach leaf segments were floated on distilled H_2O in the light (600 $\mu E\ m^{-2}\ s^{-1}$) for 48 h with paraquat, AFM, MC15608, or oxyfluorfen or for 64 h with diuron. Nonherbicide-treated samples contained the same amount of solvent as the herbicide-treated samples; different solvents (1.0% [v/v] DMSO or 1.0% [v/v] ethanol) were used depending on the solvent the herbicide was dissolved in. Herbicide toxicity induced by the herbicides was determined by the degradation of Chl.

Treatment	Chl Content
	$\mu g\ Chl\ ml^{-1}\ g^{-1}$ fresh wt
Control (water)	1370
Paraquat (1×10^{-5} M)	27
Control (ethanol)	1111
Diuron (5×10^{-5} M)	637
Control (ethanol)	1423
AFM (5×10^{-5} M)	119
AFM (1×10^{-5} M)	450
Control (DMSO)	1325
MC15608 (1×10^{-4} M)	456
Oxyfluorfen (5×10^{-5} M)	642

To observe the maximum time which chloroplast fragments could be used for biological studies, photosynthetic electron transport activity was measured by DCPIP reduction. Formation of measureable levels of MDA in isolated chloroplasts requires up to 100 min (25, 26). The isolated spinach chloroplast fragments had photosynthetic electron transport activity throughout the entire time course of MDA formation, although the rate of electron transport decreased by 22% after 35 min and continued to decrease to about 35% of the original level by 105 min. MDA formation occurred only in diuron (5×10^{-6} M) and paraquat (1×10^{-4} M) -treated chloroplast fragments (Fig. 1). The amount of MDA formed in the membranes increased after a 35 min lag phase with paraquat treatment and after a 20 min lag phase in the diuron treatment. By 105 min in the light (150 $\mu E\ m^{-2}\ s^{-1}$) paraquat and diuron produced 0.9 and 0.8 nmol of MDA, respectively, whereas control spinach chloroplasts without herbicide produced less than 0.05 nmol of MDA. No MDA formed in chloroplast fragments kept in the dark for 105 min after paraquat (1×10^{-4} M) or diuron (5×10^{-6} M) treatment (data

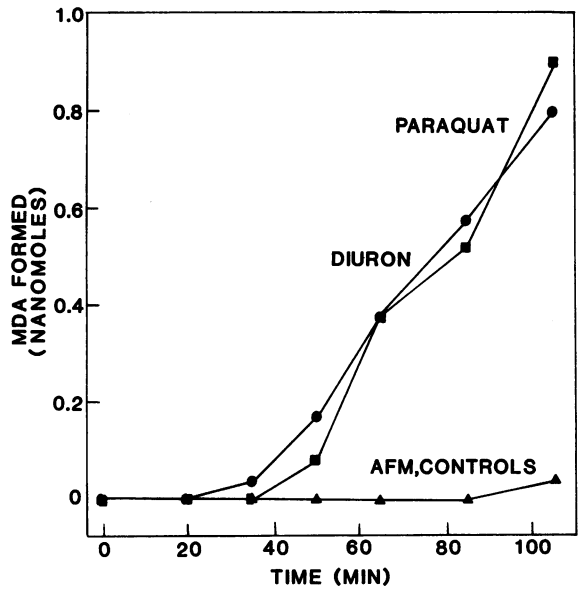

FIG. 1. MDA production by isolated chloroplast fragments treated with diuron (5×10^{-6} M), paraquat (1×10^{-4} M), or AFM (5×10^{-5} M) during 105 min in the light (150 μE m^{-2} s^{-1}). The basic reaction mixture of the isolated chloroplast fragments contained 200 μg of Chl in 2 ml of 17 mM Tris·HCl buffer (pH 7.4), plus the appropriate herbicide. Non-herbicide treated controls contained 0.1% (v/v) ethanol or distilled H_2O. At various times, MDA was measured by adding 40% TCA, 5 N HCl, and 2% thiobarbituric acid to the chloroplasts and incubating them 10 min in a boiling water bath.

not shown). AFM (5×10^{-5} M) did not elicit production of MDA in isolated chloroplast fragments during the entire 105 min (Fig. 1). In addition, no MDA formed in chloroplast fragments exposed to the DPE herbicides MC15608 (1×10^{-4} M) and oxyfluorfen (5×10^{-5} M) at 85 or 105 min (data not shown).

Photosynthesis Involvement of DPE Herbicides in *Chlamydomonas*. Herbicide toxicity by paraquat required photosynthetic electron transport in the unicellular alga *Chlamydomonas*. When diuron (1×10^{-6} M) completely inhibited (99%) photosynthetic electron transport, as measured by O_2 evolution, paraquat did not induce cell death until a concentration of 1×10^{-4} M (Fig. 2A). In the case where paraquat (1×10^{-5} M) killed about 65% of the cells in 90 min, the addition of 1×10^{-6} M diuron with the paraquat completely eliminated paraquat induced cell death (Fig. 2A). However, DPE herbicides remained active in the presence of diuron. When there was about 65% cell death with AFM (1×10^{-7} M), significant (58%) cell death still occurred when diuron was added to the assay medium (Fig. 2B). Partial protection of cell death was observed with diuron and 1×10^{-8}, 1×10^{-9}, and 5×10^{-10} M AFM, but the treatment of diuron plus AFM still killed significant numbers of cells (Fig. 2B). Similar observations were made with MC15608 (Fig. 2C). There was a significant decrease in the death of cells at 1×10^{-7} and 1×10^{-8} M MC15608, but as with AFM, significant cell death still resulted from treatment with these concentrations of MC15608 in the presence of 1×10^{-6} M diuron. With 5×10^{-8} M MC15608 and with all concentrations of oxyfluorfen tested, 1×10^{-6} M diuron failed to protect against cell death (Fig. 2, C and D).

Involvement of the Superoxide Radical in DPE Herbicide Toxicity. Copper penicillamine, a superoxide scavenger (9), provided additional evidence that paraquat toxicity differed from DPE herbicide toxicity. Copper penicillamine protected cucumber cotyledons from paraquat toxicity but not from AFM toxicity (Fig. 3). Without copper penicillamine added to cucumber cotyledons, paraquat (1×10^{-6} M) or AFM (1×10^{-6} M) caused Chl content to decrease by 60% and 49%, respectively, compared to Chl content in controls after a 24 h treatment in the light (600 μE m^{-2} s^{-1}) (Fig. 3). After treating the cotyledons with 1×10^{-6} M paraquat and 1 to 2×10^{-6} M copper penicillamine, Chl loss was only 20% and with 2.5 to 3×10^{-6} M copper penicillamine, Chl loss was only 10% greater than control levels of Chl. In contrast, the Chl content of cucumber cotyledons treated with AFM and copper penicillamine remained the same (Fig. 3). The highest copper penicillamine concentration tested (3×10^{-6} M) failed to protect the cotyledons against MC15608 (1×10^{-5} M) or oxyfluorfen (1×10^{-6} M) toxicity (Table II). MC15608 and oxyfluorfen, with or without copper penicillamine caused similar levels of Chl breakdown.

DISCUSSION

Two herbicides that cause membrane disruption (paraquat and diuron) require photosynthesis for activity (5). Paraquat diverts photosynthetic electron transport from reducing $NADP^+$ to reduction of the paraquat ion (5). In the presence of O_2, the univalently reduced paraquat radical is reoxidized to produce superoxide radical (9), which is normally present in chloroplasts and scavenged by superoxide dismutase enzymes (3). However, excess superoxide radial produced during the oxidation of paraquat radical overtaxes this protection system (9) and eventually causes membrane disruption as well as Chl and carotenoid breakdown (10, 28). Diuron inhibits electron transport, which prevents Chl from passing energy to P_{680}. Because light energy is still interacting with Chl, singlet Chl, which now cannot pass its energy to P_{680}, undergoes intersystem crossing to the longer lived triplet Chl state (5). Carotenoids normally quench triplet Chl (2), but this protective device is overloaded and the carotenoids are gradually destroyed (19). Triplet Chl may then induce membrane damage by electron or hydrogen abstraction from polyunsaturated fatty acids in the membrane or triplet Chl may interact with triplet O_2 to give singlet O_2 (5), which is capable of initiating lipid peroxidation (5, 23–25).

During photosynthesis, MDA formation increases in the presence of paraquat or diuron (Fig. 1). MDA is an indicator of lipid peroxidation, and this lipid peroxidation causes membrane dysfunction which eventually leads to cell death (5). The amount of MDA formed in these studies is similar to other published experiments (24, 25). In the presence of AFM (Fig. 1), MC15608, or oxyfluorfen and light, no MDA formation occurs in isolated chloroplast fragments. These data indicate that DPE herbicides differ from the action of paraquat and diuron. Yet, lipid peroxidation is one of the first symptoms of DPE herbicide toxicity (11, 13, 18). Therefore, within the confines of the experiments reported here, DPE herbicides do not solely require electron transport processes for induction of lipid peroxidation and the chloroplast thylakoids do not appear to be the primary site of DPE herbicide activity.

Photosynthesis is also not required for DPE herbicide toxicity in *Chlamydomonas* (Fig. 2). One $\times 10^{-6}$ M diuron inhibits electron transport within 5 min and this inhibition protects paraquat against cell death (Fig. 2A). Phenylurea herbicides inhibit the addition of electrons to paraquat from noncyclic photosynthetic electron transport in plants (16). Therefore, in *Chlamydomonas*, paraquat appears to function as in higher plants. If DPE herbicides require noncyclic photosynthetic electron transport for their primary toxicity, their activity with diuron in *Chlamydomonas* would be eliminated. But diuron does not substantially protect against DPE activity; AFM, MC15608, and oxyfluorfen remain active after diuron treatment (Fig. 2, B–D).

Kunert and Böger (11) and Lambert *et al.* (13) reported diuron decreases oxyfluorfen herbicide toxicity, and suggest this DPE herbicide accepts electrons from photosynthetic electron trans-

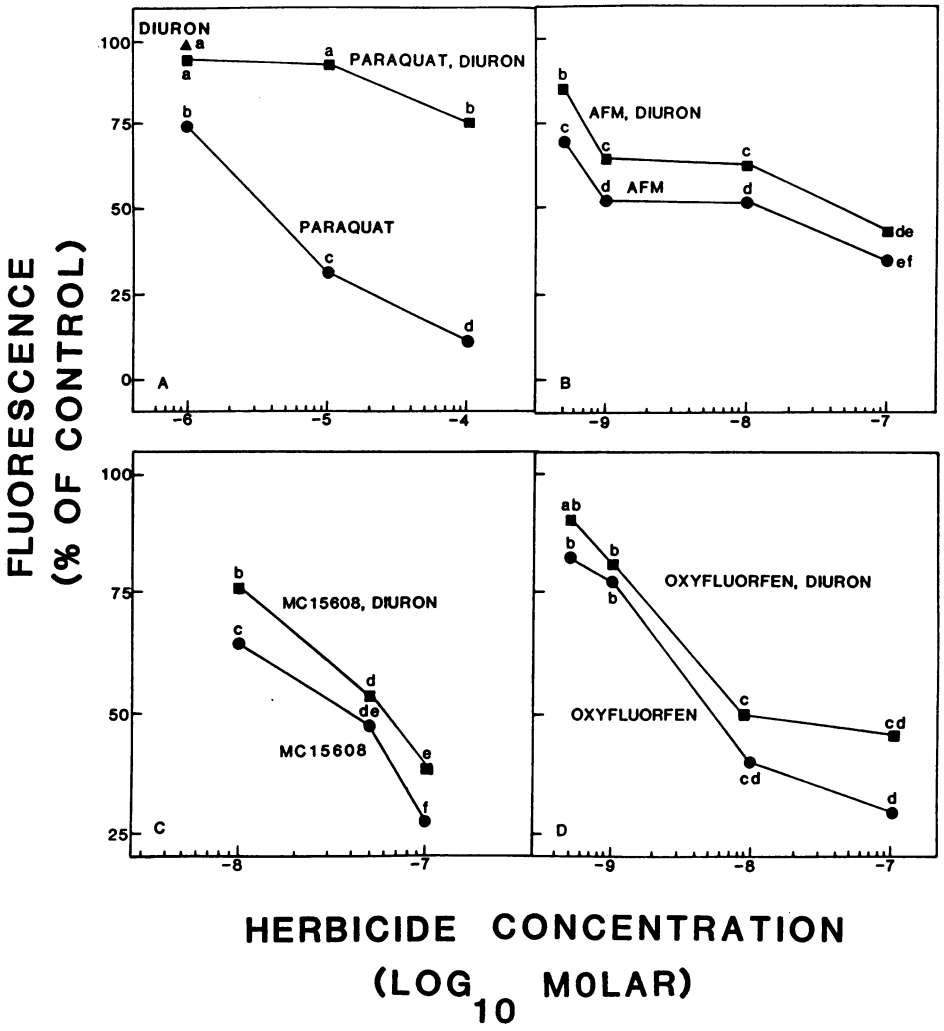

FIG. 2. Cell death of *Chlamydomonas* cells treated with paraquat (A) or the DPE herbicides AFM (B), MC15608 (C), or oxyfluorfen (D) alone or with simultaneous treatment with diuron. Cells were pretreated in the dark for 5 min with 1×10^{-6} M diuron, then placed in the light (175 μE m^{-2} s^{-1}) for 90 min with the appropriate herbicide plus diuron. Cell death was determined by the FDA method. Control treatments contained diuron (1×10^{-6} M) or 0.1% (v/v) ethanol and did not cause cell death. In each graph, data points containing the same letter are not significantly different according to Duncan's multiple range test (DMRT) at the 5% level. The no effect level was assigned the value 'a' by the DMRT test.

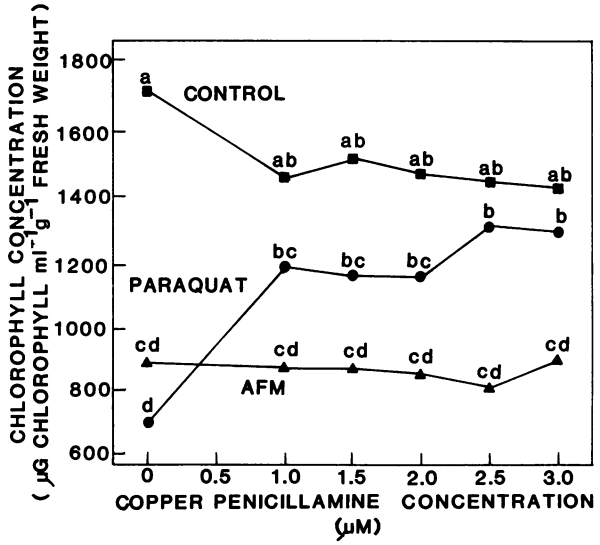

FIG. 3. Copper penicillamine protection of Chl degradation induced by paraquat or AFM. Cucumber cotyledons were pretreated for 24 h in the light (220 μE m^{-2} s^{-1}) with specified concentrations of copper penicillamine or with 0.1% (v/v) ethanol (control). AFM (1×10^{-6} M), paraquat (1×10^{-6} M), or ethanol (control) were added for an additional 24 h in the light (600 μE m^{-2} s^{-1}) in the presence or absence of copper penicillamine. Herbicide toxicity was determined by Chl degradation.

Table II. *Effect of DPE Herbicides Oxyfluorfen and MC15608 on Chl Degradation of Cucumber Cotyledons with and without 3×10^{-6} Molar Copper Penicillamine*

Cucumber cotyledons were floated on distilled H_2O and pretreated 24 h in the light (220 μE m^{-2} s^{-1}) with 3×10^{-6} M copper penicillamine or 0.1% (v/v) ethanol (control). Then 1×10^{-5} M MC15608 or 1×10^{-6} M oxyfluorfen was added with 3×10^{-6} M copper penicillamine to the cotyledons for an additional 24 h in the light (600 μE m^{-2} s^{-1}). Nontreated samples contained the same amount of solvent (0.1% [v/v] ethanol) as the treated samples. Herbicide toxicity induced by MC15608 and oxyfluorfen was determined by the degradation of Chl. Data points containing the same letter are not significantly different according to Duncan's multiple range test at the 5% level.

Treatment	Chl Content
	μg Chl ml^{-1} g^{-1} fresh wt
Control (0.1% [v/v] ethanol)	1496 a
1×10^{-5} M MC15608	941 cd
1×10^{-6} M oxyfluorfen	1076 c
Copper penicillamine control (copper penicillamine + 0.1% [v/v] ethanol)	1357 ab
Copper penicillamine + 1×10^{-5} M MC15608	877 d
Copper penicillamine + 1×10^{-6} M oxyfluorfen	951 cd

port as its mechanism of action. Diuron protection, measured by a decrease in short chain hydrocarbon gasses released, is observed after 15 h of simultaneous treatment of diuron with 1×10^{-5} M oxyfluorfen in the single cell alga *Bumilleriopsis filiformis* Vischer (phylum Chrysophyta) (13) or in 3 h of simultaneous treatment with diuron and 1×10^{-6} M oxyfluorfen in the green alga *Scenedesmus acutus* (11). Using ESR techniques with spin traps, Lambert *et al.* (12) found the presence of ESR signals from what might be alkyl or linolenic radicals in spinach chloroplasts treated with 1×10^{-4} M DPEs. Diuron (1×10^{-5} M) eliminates the ESR signals so photosynthetic electron transport is required. Because of differences in ESR signals between paraquat and DPEs, Lambert *et al.* (12) concluded the observed lipid peroxidation is caused by different mechanisms. Alscher and Strick (1) recently reported CO_2-dependent O_2 evolution is inhibited in intact chloroplasts from spinach by AFM and oxyfluorfen. The I_{50} concentration is approximately 8×10^{-6} M. At this concentration, coupled and uncoupled photosynthetic electron transport are not affected. These authors concluded a site of action may exist at the chloroplast envelope and perhaps an effect on the normal function of the envelope causes the inhibition of CO_2-dependent O_2 evolution. Matsunaka (15) reported monuron [3-(*p*-chlorophenyl)-1,1-dimethylurea] has no influence on the herbicide activity of the DPE herbicide nitrofen (2,4-dichlorophenyl *p*-nitrophenyl ether) in rice (*Oryza sativa* L.), Orr and Hess (18) indicated that diuron does not alter the herbicide toxicity of AFM in cucumber cotyledons, and Duke *et al.* (6) found PSII-inhibiting concentrations of diuron and atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] did not decrease ion leakage induced by the acid of AFM in cucumber cotyledon tissue. Prichard *et al.* (21), however, showed linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea] provides some protection against ion leakage when bean (*Phaseolus vulgaris* L.) leaf discs were treated with oxyfluorfen. The work by Duke *et al.* (6), Matsunaka (15), and Orr and Hess (18) are in agreement with data presented here (Fig. 2). Perhaps the algae used by Lambert *et al.* (12, 13) and Kunert and Böger (11) have a unique photosynthetic system. For example, *Scenedesmus acutus* does not respond to commonly used concentrations of paraquat (Hess, personal observation). Unfortunately, the ESR studies reported by Lambert *et al.* (12) required extremely high concentrations of DPE compounds. Perhaps, these concentrations induce a secondary toxic action directly involving photosynthesis. Pritchard *et al.* (21), for example, reported that 1×10^{-4} M oxyfluorfen has several effects on photosynthesis reactions. The work by Alscher and Strick (1) suggests the effect on photosynthesis is the result of an effect on the chloroplast envelope.

Cucumber cotyledons treated with the superoxide scavenger copper penicillamine suggest the superoxide radical does not have a major role in the induction of cell death by DPE herbicides as it does for paraquat. Copper penicillamine protects cotyledons from paraquat damage (28; Fig. 3) because it scavenges superoxide, the toxic species produced by paraquat (9). In our system copper penicillamine has no effect in preventing Chl degradation by AFM, oxyfluorfen, and MC15608 (Fig. 3; Table II). Duke *et al.* (6), however, reported that copper penicillamine (1×10^{-6} M) did yield a small decrease in ion leakage when discs from cucumber cotyledons were treated with the acid of AFM. Cytochemical analysis of DPE-treated tissue revealed increases in superoxide radical and H_2O_2 in the mitochondrion.

In conclusion, data presented here do not support the previous theory (22) that DPE herbicides primarily act in the chloroplast by accepting an electron from noncyclic photosynthetic electron transport or that photosynthesis is an obligate requirement for toxicity. Even though photosynthesis does not appear to be involved in DPE herbicide action, pigments are required for toxic activity; thus, the chloroplasts must be part of the mechanism of action of DPE herbicides. Perhaps the carotenoids in the outer chloroplast envelope are responsible for DPE herbicide toxicity, since these pigments are known to be necessary for plant death (8, 15), and the chloroplast outer envelope shows rapid structural disruption (18).

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