Mode of Action Studies on Nitrodiphenyl Ether Herbicides

I. USE OF BARLEY MUTANTS TO PROBE THE ROLE OF PHOTOSYNTHETIC ELECTRON TRANSPORT

JOHN R. BOWYER*, BEVERLY J. SMITH1, PATRICK CAMILLERI, AND SUSAN A. LEE
Department of Biochemistry, Royal Holloway and Bedford New College, Egham Hill, Egham, Surrey TW20 OEX (J.R.B., B.J.S.), and Shell Research Limited, Sittingbourne Research Centre, Sittingbourne, Kent ME9 8AG (P.C., S.A.L.), United Kingdom

ABSTRACT

5-[2-Chloro-4-(trifluoromethyl)phenoxy]-2-nitroacetophenone oxime-\(\text{o-}(\text{acid, methyl ester})\) (DPEI), is a potent nitrophenyl ether herbicide which causes rapid leaf wilting, membrane lipid peroxidation, and chlorophyll destruction in a process which is both light- and \(\text{O}_2\)-dependent. These effects resemble those of other nitrophenyl ether herbicides. Unlike paraquat, the herbicidal effects of DPEI are only slightly reduced by pretreatment with the photosynthetic electron transport inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethyleurea. DPEI is a weak inhibitor of photosynthetic electron transport (\(I_{50} \ 15 \text{ micromolar for water to paraquat}\) \textbf{in vitro}, with at least one site of action at the cytochrome \(b/f\) complex. Ultrastructural studies and measurements of ethane formation resulting from lipid peroxidation indicate that mutants of barley lacking photosystem I (PSI) \textit{(viridis-zb)} or photosystem II \textit{(viridis-zd)} are resistant to paraquat but susceptible to DPEI. The results indicate that electron transfer through both photosystems is not essential for the toxic effects of nitrophenyl ether herbicides. Furthermore, the results show that neither cyclic electron transport around PSI, nor the diversion of electrons from PSI to \(\text{O}_2\) when NADPH consumption is blocked are essential for the phytotoxicity of nitrophenyl ether herbicides.

The herbicidal effects of DPE 2 herbicides (rapid leaf wilting followed by necrosis) are caused by a light- and \(\text{O}_2\)-dependent peroxidation of unsaturated membrane lipids (13, 17, 20, 22, 28). However, the exact nature of the interactions which give rise to this activity are not yet understood. There are three principal hypotheses. The first hypothesis proposes that the DPE is reduced to a radical species by chloroplast PSI and the radical initiates lipid peroxidation. Evidence for this hypothesis is based largely on studies with the alga \textit{Scenedesmus obliquus} (14, 15). Pretreatment of the alga with the PSI electron transport inhibitor DCMU protects the algal cells from the herbicidal effects of the DPE oxyfluoren. The interpretation of this result is that by blocking photosynthetic electron transfer, reduction of the DPE by PSI is inhibited as in the case with the known PSI electron acceptor, paraquat. However, DCMU is only moderately effective at protecting higher plants and the alga \textit{Chlamydomonas eugametos} from DPEs (6, 17, 22), even though very high levels of protection against paraquat are observed. It has also been shown that chlorodiphényl ether analogs in which the \(\text{p}\)-nitro group is replaced by \(\text{p}\)-chloro have similar in \textit{vivo} herbicidal effects to the parent nitro compounds, although their in \textit{vivo} redox properties are very different and probably preclude radical formation by the herbicide molecule (6, 7, 25).

The DPE acifluorfen does not inhibit photosynthetic electron transport to artificial electron acceptors but it does inhibit \(\text{O}_2\)-dependent \(\text{O}_2\) evolution in intact chloroplasts (29). The site of this inhibition appears to be linked to an effect on the activation of stromal enzymes involved in \textit{CO}_2 fixation. The second hypothesis, proposed by Wetlauffer \textit{et al.} (29) is that an inhibition of \textit{CO}_2 fixation would result in an accumulation of NADPH and a shunting of electron transport to \(\text{O}_2\) generating superoxide, resulting ultimately in lipid peroxidation.

The third hypothesis proposes that carotenoids are involved in the activation of the nitrophenyl ethers, in a process independent of photosynthetic electron transfer, and which may involve exciplex formation (20, 21). Evidence for this hypothesis is based on the resistance to DPEs of plants depleted of carotenoid either as the result of mutation or of treatment with compounds which inhibit carotenoid biosynthesis (17, 28). These results are somewhat ambiguous because of the multiple effects of mutation and of the carotenoid biosynthesis inhibitors (e.g. depletion of unsaturated lipids) (3).

In this paper we introduce a novel DPE, 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitroacetophenone oxime-\(\text{o-}(\text{acid, methyl ester})\), referred to as DPEI, which we have shown using leaf discs to have herbicidal effects with the same characteristics as those of other DPEs. In an attempt to test the first two hypotheses described above and to learn more about the possible causes for the herbicidal activity of DPEs, we have studied the effect of DPEI on two mutants of barley, \textit{viridis-zb} and \textit{viridis-zd}. \textit{Viridis-zb} totally lacks PSI, but produces light-harvesting \textit{Chl} protein complexes and has normal levels of PSI1 activity (11, 19, 27); this mutant also has a lowered content of \(\beta\)-carotene compared to the 'normal' strain, and a very low xanthophyll content. \textit{Viridis-zd} lacks PSI activity (16) and has only 4% of the wild-type level of the particles attributed to PSI complexes in freeze fracture faces (26). It has more than 50% of the wild-type activity for PSI (16).

MATERIALS AND METHODS

Effect of Herbicides on Leaf Discs. \textit{Nicotiana tabacum cv} 'White Burley' (tobacco) plants were grown in a synthetic soil mix in a glasshouse with 18 h supplemental lighting per day from 400 W mercury lamps with one lamp per square meter. Eleven
mm diameter discs were cut from the leaves of 40 d old plants, avoiding the main vein. Sufficient discs from a number of plants were mixed together to obtain a random distribution and then used for various experiments. In initial experiments, leaf discs were floated on herbicide solutions in Petri dishes. This led to variable bleaching, depending on the extent to which the discs remained floating on the water surface. Submerged discs were less susceptible to bleaching, probably due to O₂ limitation. Therefore, to obtain more consistent effects, 15 or 20 discs were submerged in 30 ml water containing the herbicide, in a 125 ml Dreschel bottle bubbled with water-saturated O₂ at a rate of 100 ml min⁻¹. DPEI was used at 100 µm, and added as a stock solution in DMSO (0.1% v/v). Illumination, when used, was provided by three 20 W fluorescent strip lights at an intensity of 4.5 W m⁻². All light intensities quoted in W m⁻² were measured over the wavelength range 450 to 950 nm. After an appropriate period of illumination, the Chl content of the discs was estimated by grinding 10 discs in 10 ml of 4:1 v/v acetone:water using a Potter-Elvehjem homogenizer with a ground glass plunger, and measuring the absorption of the solution, after centrifugation (1700g for 4 min), at 652 nm (2). The results presented are the means from four replicates.

Preparation of Pea Thylakoid Membranes and Measurement of Electron Transport. Peas (Pisum sativum cv Feltham First) were grown in Fisons Levington compost in a glasshouse for 2 weeks. Aliquots of 15 g of leaves were homogenized for 15 s in an Atomix blender at full speed in 200 ml buffer (pH 7.3) at 1°C containing 0.4 M sucrose, 0.01 M MgCl₂, 0.03 M Hepes, and 0.5% w/v BSA. The homogenate was filtered through 4 layers of muslin and centrifuged at 300g for 3 min. The supernatant was centrifuged at 4000g for 3.5 min and the resulting chloroplast pellet resuspended in 0.4 M sucrose, 0.5% w/v BSA. The Chl content of the suspension was determined by extraction of an aliquot with 80% v/v acetone, and, after centrifugation (1700g for 4 min), measuring the absorption of the extract at 652 nm (2).

To measure the electron transport rate, chloroplast thylakoids were suspended to 50 µg/ml Chl in 50 mM Tricine buffer (pH 8.0) at 20°C containing 50 mM NaCl, 5 mM MgCl₂, 2 mM KH₂PO₄, 0.1 mM pararaut (electron acceptor), 2 mM NH₄Cl (uncoupler), and 1 mM NaN₃ (to inhibit endogenous catalase). The suspension was placed in a Claire type O₂ electrode (Rank Brothers, Cambridge) and illuminated with red light, provided by a 150 W floodlight filtered by a Schott RG610 filter and 10 cm of O₂, with an intensity greater than 200 W m⁻² (measured over the range 450 nm to 950 nm).

Electron transport from plastoquinol-1 to plastocyanin catalyzed by digitonin-treated pea chloroplast thylakoids was monitored spectrophotometrically at 595 nm minus 510 nm using a Perkin-Elmer 566 dual wavelength spectrophotometer, as described by Wood and Bendall (30).

Electron Microscopy of Herbicide-Treated Barley. Seeds were germinated and grown in vermiculite in a Conviron E7 Plant Growth Chamber under continuous illumination of 45 W m⁻² at 25°C, and watered daily. Seedlings with a mutant phenotype (double recessives, representing about 25% of the population) could easily be identified by their lighter color, but homozygous wild-type and heterozygous mutants cannot be distinguished.

After 7 d, phenotypically normal and mutant plants were treated with herbicide or control solutions where appropriate. Aqueous herbicide solutions were applied over the entire shoot using a small paint brush. Treated plants were returned to the growth chamber. The herbicides used were DPEI at 10 µm in water (added from a stock solution in DMSO to give 0.1% v/v final concentration) and pararaut at 0.5% v/v. All solutions contained 0.01% v/v Triton X-100. Control solutions contained 0.01% Triton X-100 and 0.1% v/v DMSO. For light-dependency experiments, the plants were treated with the herbicide under dim light, and then kept in complete darkness.

At appropriate times, pieces of leaf (1 x 3 mm) were excised from the shoot tip, and fixed for 2 h in 2% w/v glutaraldehyde in 60 mM sodium phosphate buffer (pH 7.4) containing 10 mM MgCl₂. After washing for 1 h in 60 mM phosphate buffer the samples were postfixed for 1 h in 1% w/v OsO₄ in 60 mM phosphate buffer. They were dehydrated through a graded series of ethanol dilutions (twice for 10 min in 30% v/v, 50% v/v, and 75% v/v ethanol:water, then three times for 10 min in absolute ethanol), infiltrated with propylene oxide (three times for 10 min) and left in propylene oxide:Taab resin (1:1, v/v) overnight. They were then placed in 100% Taab resin overnight, which was subsequently allowed to polymerize for 3 d at 60°C. Ultrathin sections were cut on an LKB III ultratome, stained for 20 min with saturated uranyl acetate solution and for 2 min in Reynolds’s lead citrate solution (1.33 g lead nitrate, 1.76 g sodium citrate, 50 ml H₂O, 8 ml 1 N NaOH), then viewed (at 60 kV) in a JEOL 100 CX electron microscope.

Hydrocarbon Formation in Herbicide-Treated Barley. Seeds were germinated and grown in vermiculite, under a continuous light of 35 W m⁻² at 25°C, and watered daily. After 6 d, “normal” and mutant plants were dipped in herbicide solutions or control solutions where appropriate. Great care was taken to support the stem after dipping to prevent it bending over. Dipping was carried out using the herbicide solution in a Petri dish. The solutions used were as in the electron microscope experiments, except that pararaut was used at 250 µm. Treated plants were then returned to the growth room. After appropriate periods of illumination in the growth room, two shoots per treatment were removed, placed in a 10 x 10 mm diameter test tube, sealed with a Subaseal No. 17 rubber seal, and placed under bright illumination (330 W m⁻²) for 4 h. Sample heating was prevented using a transparent perspex trough through which water was circulated, placed between the light source and the sample. The 4 h period is included in the time after herbicide application indicated in Figure 5. A 1 ml sample of the head space was removed using a gas tight syringe and analyzed by GC using a Pye Series 104 gas chromatograph with an alumina column at a temperature of 110°C. The results presented are the means of four replicates.

RESULTS

Herbicial Properties of DPEI. The results in Table 1 show that DPEI requires both light and O₂ in order to cause Chl bleaching in tobacco leaf discs. After 48 h, 100 µm DPEI caused a 60 to 70% fall in Chl content in the presence of light and O₂, but only minor effects were observed if either of these factors was absent. The concentration of DPEI used in these experiments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chl Content (mg g⁻¹ fresh wt) ± SE</th>
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<tbody>
<tr>
<td>Light, O₂</td>
<td>1.59 ± 0.15</td>
</tr>
<tr>
<td>Light, O₂ DPEI (100 µm)</td>
<td>0.55 ± 0.12</td>
</tr>
<tr>
<td>Dark, O₂</td>
<td>1.55 ± 0.17</td>
</tr>
<tr>
<td>Dark, O₂ DPEI (100 µm)</td>
<td>1.40 ± 0.09</td>
</tr>
<tr>
<td>Light, N₂</td>
<td>1.55 ± 0.03</td>
</tr>
<tr>
<td>Light, N₂ DPEI (100 µm)</td>
<td>1.74 ± 0.04</td>
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was much higher than that normally required to elicit herbicidal effects with DPEI because of the low oxygen tension used, and the submerison of the leaf discs into water. The results in Table II show that pretreatment of the leaf discs with 10 μM DCMU followed by exposure to light in the presence of DCMU caused only slight Chl bleaching and almost completely eliminated the effect of 100 μM paraquat (cf. Ref. 18). However, the presence of DCMU had little or no effect on the action of DPEI. These results indicate that the effects of DPEI on higher plant tissue have the same characteristics as those of other bleaching DPEs (8, 17, 20, 22, 28). Furthermore, as in the case with oxyfluorfen (14) the herbicidal effects of DPEI on Scenedesmus obliquus were greatly diminished by DCMU (JR Bowyer, J Howard, P Camilleri, unpublished data).

One possible mode of action of DPEI could be that it acts, like DCMU, by inhibiting photosynthetic electron transport. In experiments with isolated pea chloroplast thylakoids, we found that DPEI inhibited uncoupled electron transport from water to the PSI electron acceptor paraquat with an IC₅₀ of about 15 μM (Fig. 1). DPEI inhibited light-driven electron transport from duroquinol to paraquat (which involves the Cyt b₅f complex and PSI) but not electron transport from ascorbate/2,3,5,6-tetramethyl-p-phenylenediamine to paraquat (which involves only PSI) (result not shown). This implies that at least one site of inhibition is at the Cyt b₅f complex.

In digitonin-treated pea thylakoids, the Cyt b₅f complex catalyzes electron transport from added plastocyanin-1 to added plastoquinin (30). 4 μM DPEI inhibited the initial rate of this reaction (started by addition of plastoquinol-1) by 80%. This further indicates a site of inhibition at the Cyt b₅f complex but apparently digitonin treatment confers greater sensitivity. We have also shown that 50 μM DPEI completely inhibits CO₂-dependent O₂ evolution in barley protoplasts or intact chloroplasts within 4 min of its addition to the illuminated suspension (results not shown). This may in part result from an inhibition of photosynthetic electron transport, but may also involve direct effects on stromal enzymes involved in CO₂ fixation. Thus, Alschner and coworkers (29) have shown that 250 μM acifluorfen completely inhibits CO₂-dependent O₂ evolution in spinach chloroplasts but has no effect on electron transport.

**Effect of Herbicides on Barley Mutants.** When 7 d old, light-grown phenotypically wild-type barley seedlings were treated with either DPEI or paraquat under the conditions described for the EM studies, visible signs of necrosis and wilting were apparent after 24 h illumination, and considerable necrosis had occurred after 48 h. In phenotypically mutant vir-zb³ and vir-azd³ seedlings, the degree of necrosis observed with DPEI treatment was similar to that with the wild type but paraquat had no visible effects. Furthermore, no herbicidal effects were visible in any seedlings maintained in darkness after herbicide treatment.

The effects of DPEI and paraquat on the ultrastructure of the barley mutants were also investigated. Figure 2, A–D, shows the effects of DPEI and paraquat after 25 h in the light on phenotypically wild-type barley from the same stock as the vir-zb³ mutant. The untreated control has the typical morphology of wild-type barley (Fig. 2A) (23). The effects of DPEI on mesophyll cell morphology after 25 h are shown in Figure 2, B and C. Small vesicles appeared in the cytoplasm and chloroplast stroma, and the chloroplasts have become swollen and have moved away from the cell membrane (Fig. 2B). However, the thylakoid membrane system retained its normal organization, although occupying a smaller fraction of the swollen chloroplasts. In more severely affected cells, the tonoplast disrupted, loss of plastid envelope membrane occurred and the plasma membrane detached from the cell wall in places, but mitochondria and peroxisomes appear intact (Fig. 2C and one of the cells in Fig. 2B). Paraquat treated cells were severely necrotic (Fig. 2D). In the absence of light, neither DPEI nor paraquat had any effect on the ultrastructure (result not shown).

The ultrastructure of mesophyll cells in untreated vir-zb³ barley mutant is shown in Figure 3A (see also Simpson and von Wettstein [27]). The cells contain a large central vacuole, mitochondria, peroxisomes and chloroplasts. The chloroplasts contain well defined granal and stromal thylakoid lamellae, but there is a reduction in stromal lamellae which may result from the absence of PSI, whose surface charge properties are thought to restrict membrane appression. The granal stacks are randomly oriented in comparison to the arrangement seen in wild-type chloroplasts, possibly as a result of the deficiency of stromal lamellae, and this may be responsible for the more rounded appearance of the chloroplasts. Occasionally, dilation of the intrathylakoid spaces at the periphery of granal stacks and of stromal thylakoids was observed (Fig. 3B). The effects of DPEI (Fig. 3, C–E, showing increasing degrees of damage) were very similar to those seen in the wild type and, again, the effects were light-dependent. In contrast, paraquat had negligible effects on the ultrastructure of vir-zb³ in the light (Fig. 3F) or dark (results not shown).

**Table II. Chl Degradation by DPEI and Paraoquat in Tobacco Leaf Discs: Effect of DCMU**

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Treatment</th>
<th>Chl Content mg g⁻¹ fresh wt ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Control</td>
<td>1.41 ± 0.16</td>
</tr>
<tr>
<td>Control</td>
<td>DPEI (100 μM)</td>
<td>0.60 ± 0.20</td>
</tr>
<tr>
<td>DCMU</td>
<td>DCMU</td>
<td>0.25 ± 0.19</td>
</tr>
<tr>
<td>DCMU</td>
<td>DPEI + DCMU</td>
<td>0.64 ± 0.19</td>
</tr>
<tr>
<td>DCMU</td>
<td>Paraoquat + DCMU</td>
<td>1.23 ± 0.20</td>
</tr>
</tbody>
</table>

**Fig. 1. Effect of DPEI on photosynthetic electron transport from water to paraquat (0.1 mM) in isolated pea thylakoids. For each concentration, DPEI was added from an appropriate stock solution in DMSO to give 0.1% v/v of the solvent. The control rate of electron transport (in the presence of 0.1% v/v DMSO) was 80 μmol O₂·mg⁻¹Chl·h⁻¹.**
Fig. 2. Electron micrographs of mesophyll cells from wild-type barley after various treatments and illuminated for 25 h. A, Leaves treated with 0.1% v/v DMSO, showing two adjacent mesophyll cells with cell organelles at the periphery close to the cell wall and the large central vacuole (v). B and C, Leaves treated with 10 μM DPEI. The chloroplasts are misshapen and small vesicles (vc) have appeared in the cytoplasm. All cell organelles have moved toward the center of the cell. The tonoplast (t) and the chloroplast envelopes (e) have been disrupted in places (arrowed), but the thylakoid membranes, peroxisomes (pe) and mitochondria (m) remain intact. The plasma membrane (p) has detached from the cell wall in places (arrowed). D, Leaves treated with 0.5 mM paraquat and illuminated for 25 h, showing necrotic cells with clumping of cell organelles.
FIG. 3. Electron micrographs of mesophyll cells from barley mutant vir-zb^63 after various treatments and illuminated for 25 h. A and B, Leaves treated with 0.1% v/v DMSO. A, Adjacent mesophyll cells with chloroplasts and other organelles at the cell periphery. B, Chloroplasts showing dilation of intrathylakoid spaces at the periphery of the grana stacks and of the stromal thylakoids. C–E, Leaves treated with 10 μM DPEI. C, Part of a single mesophyll cell, showing vesicle formation in the cytoplasm (vc), and, at this stage, the intact tonoplast and swollen chloroplast. D, the tonoplast is disrupted and only a few fragments remain (arrow). The thylakoid system of the chloroplast is still intact. E, Part of a more severely damaged mesophyll cell. The cytoplasm has pulled away from the cell wall (arrow) and there is also disruption of and vesicle formation (vc) in the cytoplasm. F, Leaves treated with 0.5 mM paraquat, showing chloroplasts in adjacent mesophyll cells.
FIG. 4. Electron micrographs of mesophyll cells from barley mutant vir-zd9 after various treatments and illuminated for 25 h. A, Leaves treated with 0.1% v/v DMSO, showing two adjacent mesophyll cells with chloroplasts and other organelles located at the periphery of the cell, the large central vacuole (v), and the tonoplast membrane (arrow). B, Leaves treated with 10 μM DPEI, showing severely necrotic mesophyll cells. Only effete membrane is present; no cell organelles can be distinguished. C, Leaves treated with 0.5 mM paraquat. The apparent grana stacks are misshapen (arrow), as are the chloroplasts (c), which have moved away from the cell periphery.
The ultrastructure of mesophyll cells in controls of vir-zd^69 is shown in Figure 4A (see also Simpson and von Wettstein [27]). The cells differ from the wild type in that the apparent grana stacks have a much greater diameter. Mesophyll cells in tissue treated with DPEI and exposed to light for 24 h were severely damaged with complete disruption of all organelles (Fig. 4B). No ultrastructural effects were seen in vir-zd^69 seedlings treated with DPEI in darkness (results not shown). In the light, chloroplasts in paraquat-treated vir-zd^69 were slightly misshapen as were the grana stacks, and they had moved away from the cell periphery, but the chloroplast envelopes and tonoplast were intact (Fig. 4C). No effects of paraquat on vir-zd^69 ultrastructure were seen in dark-treated seedlings (results not shown).

The herbicidal actions of paraquat and nitrophenyl ethers, although probably resulting from different primary processes, both lead to the peroxidation of unsaturated lipids. These peroxides decompose to produce hydrocarbon gases which can be measured to provide a more quantitative assay of herbicidal toxicity (4, 13). The results of such experiments are shown in Figure 5, A–D. Although there are considerable variations in the levels of ethane produced, it is clear that hydrocarbon production reflects the visible effects of the herbicides, i.e. paraquat caused marked ethane formation in the wild-type strains but had little or no effect on mutants with an incomplete photosynthetic electron transport chain (Fig. 5, B and D) whereas DPEI enhanced ethane formation from both mutants (Fig. 5, A and C). The variations in extents of herbicide-induced ethane formation reflect the great variation in the rate of necrosis from one leaf to another which to some extent must reflect uneven distribution of herbicide on the leaves. Rapid necrosis after 1 d probably accounts for the fall in ethane formation after 2 d from the viridis-zd^69 parent wild type treated with paraquat (Fig. 5D).

**DISCUSSION**

The results demonstrate that DPEI behaves as a typical nitrodiphenyl ether herbicide, *i.e.* it causes rapid necrosis, Chl degradation and lipid peroxidation in a light and O₂-dependent process. Furthermore, the effects are not reduced by treatment with DCMU, in contrast to paraquat. The fact that DPEI is a very much poorer inhibitor of non-cyclic photosynthetic electron transport than DCMU (I₅₀ 0.24 μM (12)) whereas it elicits necrosis and Chl bleaching in vivo more rapidly and at a concentration which is about 10⁵ to 10⁷ times lower than that of DCMU and other PSII herbicides (unpublished observations, Biological Evaluation Division, Sittingbourne Research Centre) argues against inhibition of electron transport as being responsible for the herbicidal effects of DPEI. A similar conclusion was reached by Pritchard et al. (24).

The ultrastructural effects of DPEI are very similar to those reported elsewhere for other DPE herbicides (20, 22). They can be attributed to weakening and disruption of various membranes within the cell as a result of lipid peroxidation. Damage to the tonoplast and plasma membrane upsets the osmotic balance of the cell and is probably the cause of the swelling and rupture of chloroplasts and mitochondria, and ultimately to the loss of turgor. Similar effects are observed with paraquat (9). The effects of DPEs on cellular ultrastructure, although implicating membrane disruption, have so far not yielded any clues as to the initial molecular target of the herbicide within the cell. For example, the early effects resemble those in a catalase-deficient mutant of barley in which H₂O₂ accumulates (23). However, H₂O₂ accumulation does not appear to be the primary agent in DPE toxicity (14, 29). Ultrastructural effects may simply reflect those membranes which are most susceptible to lipid peroxidation or to changes in cell turgor (20, 22).

Our results do appear to establish clearly that electron transfer through PSI is not essential for DPE toxicity in barley. The viridis-zb^63 mutant appears to totally lack PSI activity and redox centers (11, 19, 27), so that we can rule out the hypothesis that DPEI must be reduced by PSI in order to elicit its herbicidal effects. Since paraquat toxicity primarily requires reduction by PSI it is not unexpected that vir-zb^63 is almost unaffected by paraquat.

**Viridis-zb^63** has normal levels of PSII activity, and a role for PSII in DPE toxicity could not be ruled out by the experiments with vir-zb^63. The experiments with mutant vir-zb^63 are not quite as unequivocal as those with vir-zb^63, since it may retain some PSII reaction centers (about 4% of the wild type). This was probably responsible for the minor ultrastructural effects observed with paraquat, since significant reduction of paraquat requires the participation of PSII to provide reducing equivalents to PSI. However, since vir-zb^63 appeared to be at least as sensitive to DPEI as wild-type tissue, it appears likely that PSI activity is also not essential for DPE toxicity. Our experiments do not, of course, preclude the possibility that either PSI or PSI is required; experiments to test this are in progress. Our results are therefore in agreement with those obtained using chlorophenyl ethers which cannot be reduced by PSI (6, 7). They are also consistent with the results of Ensminger and Hess (6), who have shown that the addition of DPE to isolated illuminated thylakoid fragments is insufficient to initiate lipid peroxidation, even though the pigments which drive photosynthesis (Chl and carotenoid) also appear to sensitize DPE toxicity (5).

Our results also argue against the hypothesis of Wettlaufer et al. (29) that the inhibition of CO₂ fixation by nitrodiphenyl ethers would cause NADPH to accumulate, so...
that electrons from PSI would be diverted to O₂, generating H₂O₂. Since photosynthetic O₂ reduction is almost entirely through PSI (10), it seems unlikely that the diversion of electrons to O₂ when NADP⁺ is unavailable is the cause of DPE toxicity. In any case, it would be expected that any H₂O₂ produced as a result of this process in ‘wild-type’ plants would be removed by the NADPH-dependent glutathione reductase/ascorbate peroxidase system (1). Furthermore, the results with vir-zf63 appear to rule out a key role for cyclic electron transport around PSI in DPE toxicity.

The mode of action of DPE herbicides remains a mystery. The action spectrum of the effects (5) and the varying degree of inhibition by DCMU (6, 14, 15, 17, 22, 24) indicate that the photosynthetic apparatus is involved in potentiating the primary effect of the herbicide, probably in a number of ways. Our own results suggest that this potentiating effect does not require a complete photosynthetic electron transport chain. In addition, the results of Ensminger and Hess (6) indicate that thylakoid fragments are not sufficient to elicit DPE toxicity. It seems that further studies on DPE toxicity will require studies on intact plastids and their interactions with other organelles.

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