Plant Physiol. (1988) 86, 672–676
0032-0889/88/86/0676/05/$01.00/0

The Generation of Singlet Oxygen ($^1O_2$) by the Nitrodiphenyl Ether Herbicide Oxyfluorfen Is Independent of Photosynthesis

Received for publication July 17, 1987 and in revised form October 5, 1987

PHIL HAWORTH* AND F. DAN HESS
Zoecon Research Institute, Sandoz Crop Protection Corporation, 975 California Avenue, Palo Alto, California 94304

ABSTRACT

The mechanism of action of the *p*-nitrodiphenyl ether herbicides has remained ambiguous because of conflicting reports in the literature. The diphenyl ether herbicide oxyfluorfen causes a light induced consumption of oxygen which resembles the electron acceptor reaction of paraquat. However, this reaction is not linked to the transport of electrons through photosystem I. This conclusion is based on the observation that the rate of oxygen consumption, in the presence of oxyfluorfen, does not demonstrate a first order rate dependence on light intensity. Using the bleaching of *N,N*-dimethyl *p*-nitrosoaniline as a specific detector of singlet oxygen, we demonstrate that oxyfluorfen is a potent generator of this toxic radical. The production of singlet oxygen occurs in the presence of inhibitors of photosynthetic electron transport (oxyfluorfen at 10^{-4} molar and paraquat) and also under temperature conditions (3°C) which prevent electron transport. This light induced reaction results in oxygen consumption and is the primary cause of lethality for oxyfluorfen. The production of singlet oxygen occurs rapidly and at low herbicide concentrations (10^{-9} molar). The reaction occurs without photosynthetic electron transport but does require an intact thylakoid membrane.

The widely used DPE[1] herbicides cause rapid chlorosis and necrosis of sensitive plant species, which is triggered by a light dependent oxidative destruction of membrane components (16–18). The mechanism of the light activation and the role of the photosynthetic membrane remain unclear since evidence exists for and against the involvement of photosynthetic electron transport in the initiation of the oxidative process (5, 6).

Because the symptoms of DPE injury resemble those of the dipyridinium herbicides (*e.g.*, paraquat), the initial search for a mechanism of action focused on photosynthetic electron transport (see Refs. 1 and 6 for review). Although some involvement with the photosynthetic process has been suggested (6, 11, 29), the mechanism of action of DPE herbicides and paraquat are clearly different. Lambert *et al.* (17) demonstrated, by electron spin resonance spectroscopy, that the peroxidative activity of paraquat and diphenyl ethers are caused by different mechanisms. Dipyridinium herbicides act through the generation of superoxide (O_2^-) (4), however, scavengers of O_2^-, SOD (16) and copper penicillamine (8), do not protect against the toxic effects of DPE. Other mechanisms proposed for DPEs include a direct chemical role of the herbicide in lipid peroxidation through abstraction of hydrogen from unsaturated lipids, but such a hypothesis is inconsistent with available physical-chemical data (9). Despite the confusion, there is broad agreement that the lethal effect of DPE herbicides is the result of lipid peroxidation which leads to extensive membrane destruction (4–6). The peroxidation of membrane lipid ultimately depends on the generation of a toxic oxygen species (4, 12, 23, 26); O_2^-, singlet oxygen ($^1O_2$), peroxide (O_2^{2-}) or hydroxyl radical (·OH). Since the involvement of O_2^- in the activity of DPEs has already been eliminated an alternative species must be identified.

Two significant questions on the mechanism of action of these herbicides remain unanswered; what is the nature of the toxic oxygen species generated and through what route is the light requirement mediated? In this report we demonstrate that the DPE herbicide oxyfluorfen is a potent generator of $^1O_2$. Furthermore the generation of $^1O_2$ does not require photosynthetic electron transport.

MATERIALS AND METHODS

Intact thylakoids were isolated from 10 to 14 d old pea seedlings (*Pisum sativum* L.) according to previously published procedures (13), before final resuspension in buffer consisting of 25 mM tricine-NaOH (pH 7.8), 10 mM NaCl, 5 mM $MgCl_2$, and 0.1 M sorbitol. Gramicidin (1 μM) was included as an uncoupler. Photosynthetic electron transport in these membranes was measured by an oxygen electrode (YSI Model 53, Yellow Springs, Ohio). Triton-solubilization of the thylakoid membrane into three major pigment protein complexes (light-harvesting complex-II, PSII, and PSI), was achieved according to documented procedures (20–22). Chl determination was performed according to the method of Mackinney (19).

Singlet oxygen was detected chemically by monitoring the bleaching (decrease in A at 440 nm) of RNO. The procedure was adapted from the published protocol of Joshi and Pathak (14). Intact thylakoids were diluted in resuspension buffer containing 0.01 M L-histidine and approximately 300 μM RNO, then illuminated with white light (1.3×10^3 $\mu E \cdot m^{-2} \cdot s^{-1}$ PAR). Aliquots (1 ml) were removed at various times from these samples and centrifuged at 3000*g* to pellet the membranes. The supernatant was decanted and the absorption at 440 nm measured. Once removed from direct illumination the A_{440} nm remained constant for several hours. Cercosporin a known generator of $^1O_2$ (3), was included as a positive control. All the herbicides used in this study were obtained from Chem Service (West Chester, PA).

RESULTS

Oxygen Consumption in the Presence of Oxyfluorfen. PSI electron transport in isolated thylakoids can be estimated by oxygen consumption via the Mehler reaction, with ascorbate/DCPIP as electron donor and methyl viologen (paraquat) as acceptor (Fig.

[1] Abbreviations: DPE, *p*-nitrodiphenyl ether; RNO, *N,N*-dimethyl *p*-nitrosoaniline; SOD, superoxide dismutase; DCPIP, 2,4-dichlorophenolindophenol.

1A, trace 1). Assuming that reducing equivalents are in excess, then the rate of PSI electron transport is a first order reaction with respect to light intensity (10). When paraquat is replaced by (10^{-7} M) oxyfluorfen, a proposed alternate acceptor (16), a qualitatively similar consumption of oxygen occurs (Fig. 1A, trace 2). This observation suggests oxyfluorfen acts as a PSI electron acceptor in a manner similar to paraquat. The data in Figure 1B illustrates that the PSI electron transport rate, as defined by ascorbate/DCPIP to paraquat, saturates at low levels of ascorbate. The rate of oxygen consumption is the same at 0.3 mM ascorbate (trace 1) and 10 mM ascorbate (trace 3). A similar experiment using oxyfluorfen (Fig. 1C) shows that oxygen consumption increases with ascorbate concentration (*cf.* trace 1 and trace 3). Since PSI electron transport saturates at low levels of ascorbate, this observation suggests oxyfluorfen dependent oxygen consumption is not linked to the flow of electrons through PSI. This conclusion does not agree with previously suggested hypotheses which link oxyfluorfen to PSI electron transport as either a primary or secondary electron acceptor (1, 11). Further support for the hypothesis that PSI electron transport and oxyfluorfen activity are separate is given in Figure 2. Since the rate of PSI electron transport is 1st order with respect to actinic light (10), a light saturation curve of the type seen in Figure 2 (closed circles) is expected when paraquat is the electron acceptor. The oxyfluorfen light saturation curve (closed diamonds) does not show the same first-order dependency on light but continues to increase beyond the light saturation point of PSI electron transport. Because the oxyfluorfen experiment was carried out at 10 mM ascorbate, a comparison curve of PSI electron transport with paraquat (open symbols) was included with ascorbate at 10 mM. This curve saturates at a lower light intensity than seen with 1 mM ascorbate because of an increased dark rate at the higher concentration, but the data still demonstrate a first order dependency. In all of these experiments the thylakoid membranes were isolated under identical condition so that the relative absorption cross sectons (*i.e.*, Chl/P700) are identical. Although these observations argue against direct involvement of PSI electron transport, they do demonstrate the light dependent nature of oxyfluorfen activity.

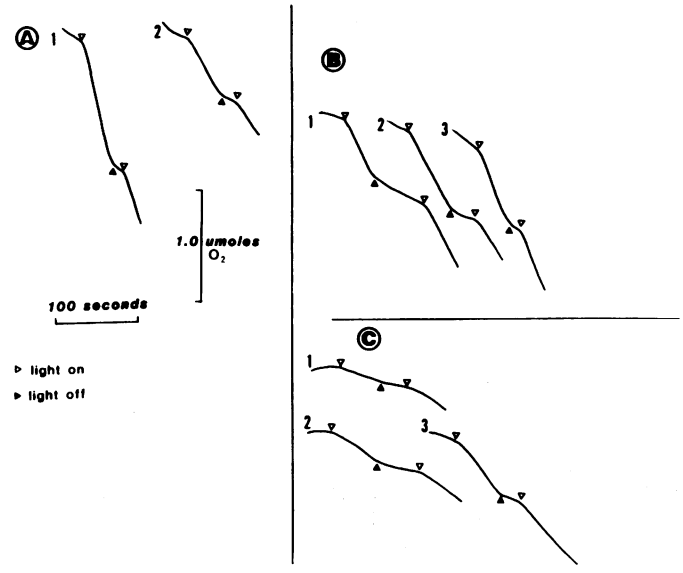

FIG. 1. PSI electron transport, measured with pea thylakoids. Chl concentration 30 μg/ml, volume 3 ml, temperature 30°C, actinic light intensity 1.3×10^3 $\mu E \cdot m^{-2} \cdot s^{-1}$ PAR. Resuspension buffer, 25 mM tricine-NaOH (pH 7.8), 10 mM NaCl, 5 mM $MgCl_2$, 0.1 M sorbitol, 1 μM gramicidin S, and 10^{-5} M DCMU. Electron donor: 1 mM ascorbate + 0.3 mM DCPIP. A, Electron acceptor: 1, 0.25 mM paraquat; 2, 10^{-7} M oxyfluorfen (rates = 1.12 and 0.58 mmol O_2/mg Chl·h, respectively). B, Electron acceptor: 0.25 mM paraquat; 1, 0.3 mM ascorbate, 2, 1 mM ascorbate; 3, 10 mM ascorbate. C, Electron acceptor: 10^{-7} M oxyfluorfen 1, 2 and 3 as in B.

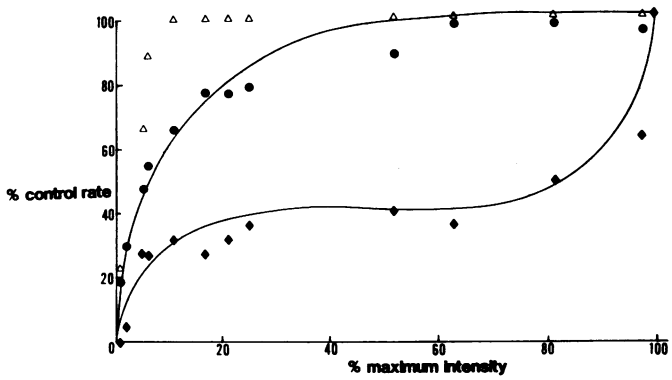

FIG. 2. Intensity dependence of oxygen consumption in the presence of, (●——●) 0.25 mM paraquat + 1 mM ascorbate (rate = 0.80 m/mol O_2mg Chl·h), 10^{-7}M oxyfluorfen (◆——◆) + 10 mM ascorbate (rate = 0.40) and (△——△) 0.25 mM paraquat + 10 mM ascorbate (rate = 0.82). Maximum illumination $1.3 \times 10^3 \mu E \cdot m \cdot^{-2} s^{-1}$ PAR, other experimental conditions are as described in Figure 1.

Singlet Oxygen Production by Oxyfluorfen. Scavengers of O_2^- do not protect against the toxic effects of DPE herbicides (8, 16). If O_2^- is not the toxic oxygen species involved, the most likely alternative is $^1O_2$. Accordingly, we have investigated the ability of oxyfluorfen to generate $^1O_2$. To carry out this investigation a positive control (*i.e.* a known generator of $^1O_2$) is required. For this study we have used the fungal toxin cercosporin (2, 3). In the presence of lights cercosporin will generate $^1O_2$ and rapidly bleach RNO, this reaction occurs directly via the cercosporin chromaphore (2). Host plants when infected with *Cercospora* fungal cells show membrane disruption induced by toxic oxygen species (3), similar to that caused by paraquat and the DPEs. Cercosporin therefore, provides an alternative chemical model on which to base mechanism of action studies for oxyfluorfen.

Figure 3 compares the ability of cercosporin, oxyfluorfen and paraquat to generate $^1O_2$ in the presence of isolated thylakoid membranes. As expected cercosporin generates $^1O_2$ independently of the amount of photosynthetic pigment, compare (closed circles) Figure 3A (300 μg Chl/ml) and Figure 3B (30 μg Chl/ml), since the light activation is direct. Oxyfluorfen (10^{-7} M) is also a potent generator of $^1O_2$ (closed triangles), but does show some dependence on the amount of photosynthetic membrane present. Singlet oxygen production by oxyfluorfen is not observed when thylakoid membranes are omitted from the reaction medium (data not shown). Paraquat shows little or no generation of $^1O_2$ under either Chl concentration (closed squares), which strongly supports the earlier conclusion of Lambert *et al.* (17). A significant amount of RNO bleaching *i.e.* $^1O_2$ production, occurs in the thylakoid control experiments (open circles), particularly at the higher Chl concentration. Because the assay system used in this experiment does not contain electron acceptors, the production of $^1O_2$ by the pigment bed under strong illumination is predictable (4, 24). Under conditions where an electron acceptor is present (*i.e.* paraquat), little or no $^1O_2$ generation from the pigment bed is observed. Since the inclusion of an electron acceptor most closely resembles the intact system, experiments where paraquat is present represent the most appropriate control.

In many mechanism of action studies the concentration of herbicide used does not accurately reflect the low levels known to cause *in vivo* injury. Ensminger *et al.* (9) have determined I_{50}

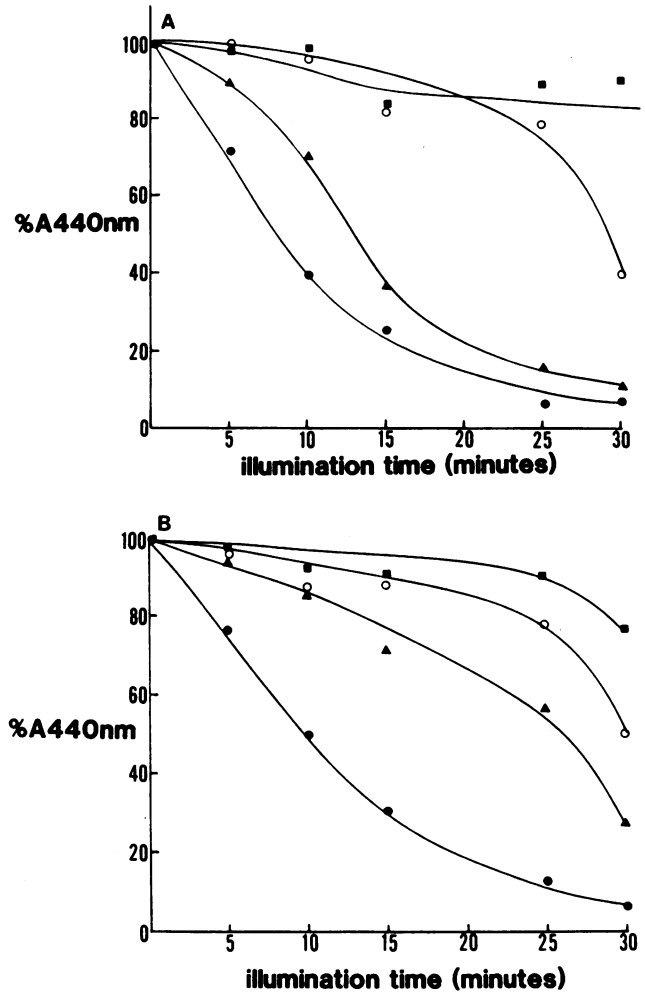

FIG. 3. Bleaching of RNO by singlet oxygen ($^1O_2$) A = 300 μg Chl/ml, B = 30 μg Chl/ml (■——■) + 0.25 mM paraquat, (○——○) = thylakoid membranes alone, (▲——▲) = 10^{-7} M oxyfluorfen. (●——●) = 10^{-5} M cercosporin. Samples resuspended in 25mM tricine-NaOH (ph 7.6) 10 mM NaCl 5 mM $MgCl_2$ and 0.1 M sorbitol at room temperature. The assay mixture contained 0.1 M L-histidine and approximately 300 μM RNO.

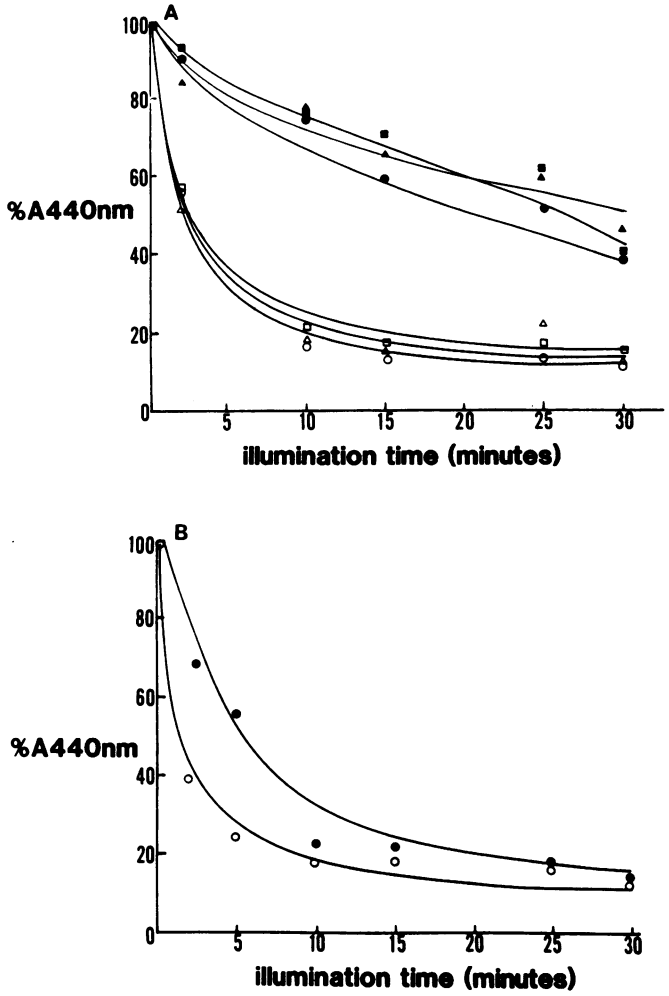

FIG. 4. Effect of paraquat on singlet oxygen production by (A) oxyfluorfen and (B) 10^{-5} M cercosporin. Open symbols oxyfluorfen (or cercosposin) alone, closed symbols oxyfluorfen (or cercosposin) plus 0.25 mM paraquat. A (○——○) 10^{-4} M oxyfluorfen, (□——□) 10^{-7} M oxyfluorfen, (△——△) 10^{-9} M oxyfluorfen. Experimental conditions described in Figure 3.

values (the molar concentration causing 50% inhibition of *Chlamydomonas* cell growth) as low as 10^{-9} M for some DPE herbicides. If the generation of $^1O_2$ is the primary cause of oxyfluorfen lethality, we would expect to see activity at or below 10^{-9} M in this *in vitro* system. The data in Figure 4A shows that oxyfluorfen can produce $^1O_2$ over a concentration range from 10^{-4} M to 10^{-9} M. No bleaching of RNO was observed at 10^{-10} M oxyfluorfen (data not shown). The same figure demonstrates the effect of paraquat addition on the ability of oxyfluorfen to generate $^1O_2$ (closed symbols, Fig. 4A). The addition of paraquat reduces, but does not abolish generation of $^1O_2$ by oxyfluorfen. The effect of paraquat (0.25 mM) does not depend on the concentration of oxyfluorfen present. This clearly demonstrates that oxyfluorfen and paraquat do not act through a competing mechanism, *i.e.* paraquat and oxyfluorfen cannot both be acting as electron acceptors at either the same or different sites (11).

The difference in the rate of RNO bleaching between oxyfluorfen (open symbols) and oxyfluorfen plus paraquat (closed symbols) in Figure 4A can in part be explained by the reduction of background $^1O_2$ production within the pigment bed. The level of this background production can be estimated. Figure 4B demonstrates the effect of paraquat addition on the generation of $^1O_2$ by cercosporin when thylakoid membranes are present. The small difference between the open and closed symbols in this experiment reflects the production of $^1O_2$ within the pigment bed, a reaction which occurs independently of the cercosporin produced $^1O_2$. The production of $^1O_2$ in the pigment bed, estimated from Figure 4B, does not explain the entire difference seen between the open symbols and the closed symbols observed in the oxyfluorfen experiment Figure 4A. One possible explanation for the additional effect upon the oxyfluorfen dependent production of $^1O_2$ could be linked to the overall redox state of the photosynthetic membrane in the presence or absence of an electron acceptor. In the absence of an electron acceptor the thylakoid membrane will develop a higher redox (more negative) potential than when the acceptor is present, due to a build up of electrons in the plastoquinone pool (10). Thus experiments using oxyfluorfen alone utilize a membrane system with more reducing power than a system in which paraquat is included. As seen in Figure 1C, the activity of oxyfluorfen does appear to be sensitive to the redox state of this system. Therefore, the inclusion of paraquat could slow the rate of $^1O_2$ production indirectly, by changing the redox potential of the activating complex as-

sociated with this membrane. Because the RNO assay system is sensitive to chemical reducing agents (*e.g.* ascorbate) we cannot test this hypothesis directly.

Elstner and Zeller (7) were the first investigators to report the use of RNO to investigate generation of excited oxygen species by chloroplast membranes. In their study a number of inhibitors of photosynthetic electron transport prevented the bleaching of RNO. As a result, these workers concluded that excited oxygen species were not involved in the light dependent bleaching of RNO observed in the presence of chloroplast membranes (7). As an alternative hypothesis they proposed that the bleaching of RNO occurred directly at the reducing side of PSI. In our study we have conducted a number of experiments to investigate this possibility. In agreement with these authors (7), inhibitors of PSII electron transport (diuron and atrazine) also completely inhibited RNO bleaching by oxyfluorfen. However, atrazine and diuron also inhibited bleaching induced by cercosporin, even in the absence of thylakoid membranes (data not shown). Since cercosporin bleaching is independent of photosynthetic electron transport, the effect of atrazine and diuron does not relate to their herbicidal properties. We attribute this surprising observation to either a direct effect of these herbicides on the assay system or the ability of atrazine and diuron to quench $^1O_2$. No inhibition of RNO bleaching was observed when paraquat (0.25 mM) was included with cercosporin in the absence of thylakoid membranes. More importantly the bleaching of RNO observed in the presence of either cercosporin or oxyfluorfen is inhibited by known singlet oxygen quenchers, quercetin and azide (25, 27) see Figure 5. The degree to which quercetin and azide inhibit this bleaching is consistent with their previously reported $^1O_2$ quenching ability (25, 27).

Kenyon *et al.* (15) have recently reported that the activity of the DPE herbicide acifluorfen is independent of temperature over a range of 3°C to 30°C. They conclude from this observation that metabolism is not required for the activity of acifluorfen. The temperature dependence of $^1O_2$ production by oxyfluorfen is depicted in Figure 6, which compares the degree of RNO bleaching by cercosporin, oxyfluorfen and paraquat at 30°C (closed symbols) and 3°C (open symbols). At 30°C both cercosporin (closed circles) and oxyfluorfen (closed triangles) display a rapid rate of bleaching, which is slowed, but not abolished at 3°C. We have measured whole chain photosynthetic electron transport (water to methylviologen) at 30°C, electron transport was not detectable at the lower temperature.

The dependence of oxyfluorfen activity on the absorption of light by photosynthetic pigments is a hypothesis which can be tested simply. An absolute dependence on the intact membrane system can be demonstrated by separating the thylakoid into its major pigment-protein complexes (20–22); PSI, PSII and light-harvesting complex. When thylakoid membranes are replaced with any of the isolated complexes, no light dependent $^1O_2$ production by oxyfluorfen is observed (data not shown). This final observation suggests that the activation of oxyfluorfen is dependent on the integrity of a membrane associated complex rather than a simple pigment, which is not preserved under the detergent isolation conditions.

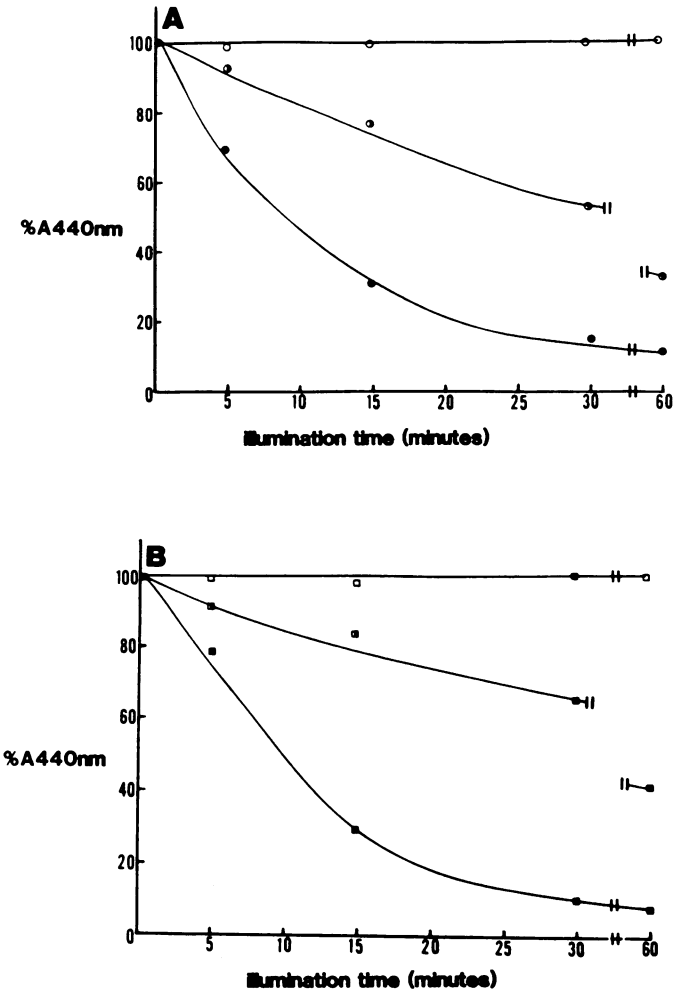

FIG. 5. Inhibition of RNO bleaching by singlet oxygen quenchers. A, 10^{-5} M cercosporin (●——●), + 100 μM quercetin (◑——◑), + 5 mM sodium azide (○——○). B, 10^{-7} M oxyfluorfen (■——■), + 100 μM quercetin (◨——◨) + 5 mM sodium azide (□——□). Experimental condition described in Figure 3.

DISCUSSION

Although we report in this study that oxyfluorfen does cause a light dependent oxygen consumption, the activity of DPE herbicides is distinct from a similar phenomenon caused by paraquat. The lethal effects of paraquat are associated with the production of O_2^-, resulting from direct chemical reduction at PSI. The production of O_2^- results in massive lipid peroxidation and cell death (4). This injury can be protected by SOD (16) and copper penicillamine (9). The lethal effects of oxyfluorfen, however, are not inhibited by these agents (6, 9). Since O_2^- is not involved in the toxicity of oxyfluorfen an alternative toxic oxygen species must be considered. Using the fungal toxin cercosporin as a model, we have demonstrated that oxyfluorfen will generate $^1O_2$ in the presence of thylakoid membranes and light. The oxyfluorfen induced generation of $^1O_2$ occurs at 10^{-9} M oxyfluorfen (Fig. 4), a concentration which is consistent with the I_{50} values determined by Ensminger *et al.* (9) for the inhibition of *Chlamydomonas* growth. Production of $^1O_2$ by oxyfluorfen occurs without photosynthesis as demonstrated by the data of Figures 4 and 5, but the reaction does require an intact membrane system. The observed consumption of oxygen (Fig. 1) can be explained by the production of $^1O_2$, as demonstrated by Reszka *et al.* (25). We are able to detect oxygen consumption under the RNO assay conditions (data not shown) which supports our hypothesis. The oxygen consumed under these conditions, however, can arise directly from $^1O_2$ or indirectly through oxidative events initiated by $^1O_2$ (4, 12, 24). Although $^1O_2$ quenchers will prevent the bleaching of RNO at 440 nm (Fig. 5) these molecules do not prevent oxygen consumption since the quenching mechanism itself consumes O_2 (25). The RNO assay system used in this study eliminates the possibility of ·OH as the toxic species. Although the RNO is sensitive to ·OH we only observe bleaching in the presence of an imidazole derivative (histidine). Bleaching

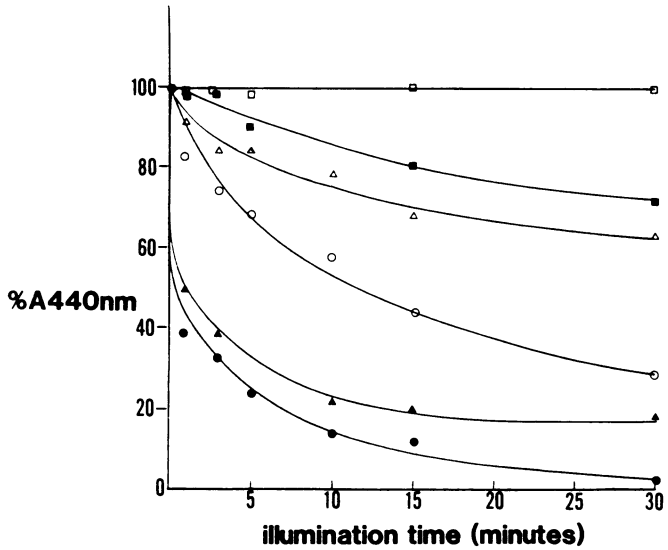

FIG. 6. Temperature dependency of RNO bleaching by singlet oxygen. Closed symbols, 30°C; open symbols, 3°C; circles, 10^{-5} M cercosporin; triangles, 10^{-7} M oxyfluorfen; squares, 0.25 mM paraquat.

of RNO by OH occurs in the absence of histidine (7). The conclusion is in contrast to the results of Upham and Matzios (28) who observed production of OH in the presence of oxyfluorfen. The reported production of OH only occurred under conditions where photosynthesis was active. It is possible that the production of OH occurred as a secondary effect of $^1O_2$ production and lipid peroxidation (12, 24).

As a consequence of these studies, we feel confident in proposing a mechanism to explain the lethal effects of oxyfluorfen which may be applicable to other DPE herbicides. The generation of $^1O_2$ by oxyfluorfen occurs through the interaction of the herbicide with an *in situ* pigment complex in the thylakoid membrane. This complex is not preserved during triton solubilization of thylakoid membranes. At this stage of our investigation it is not possible to describe the nature of this *in situ* pigment complex and it may exist in systems other than the thylakoid. All the data presented in this study are consistent with the light dependent nature of this reaction. In addition, the results presented in Figures 1 and 4 suggest the ability of the *in situ* oxyfluorfen-pigment complex to generate $^1O_2$ might depend on the redox potential of the membrane, at least in the immediate environment of the complex. Addition of ascorbate (Fig. 1C) and omission of paraquat (Fig. 4A) both serve to increase the redox potential of the membrane and also stimulate $^1O_2$ production. This association with redox state does not imply a requirement for active metabolism (Fig. 6) but does suggest active metabolism (photosynthesis) might enhance production of $^1O_2$ by the *in situ* oxyfluorfen-pigment complex (1). Active photosynthesis will reduce the plastoquinone pool and elevate the redox potential of the membrane. It is this relationship between the production of $^1O_2$ and the redox potential of the cell which has confused mechanism of action studies with DPE herbicides in general and oxyfluorfen in particular (6).

Singlet oxygen is known to poise a toxic threat to immediate biological systems because of its role in lipid peroxidation (12, 23, 26). It is proposed to be a direct initiator of lipid peroxidation by a concerted addition-abstraction reaction with the diene bonds of unsaturated lipids, giving rise to lipid hydroperoxides (12, 23, 26). The inevitable consequences of this peroxidation are membrane destruction and cell death, a pattern of injury which is entirely consistent with the effects of the DPE herbicides (6).

Acknowledgments—Cercosporin used in this study was a gift of Northrup King, Stanton MN. The authors wish to acknowledge the technical assistance of W. Miller.

LITERATURE CITED

1. BÖGER P 1984 Multiple modes of action of diphenyl ethers. Z Naturforsch 39c: 468–475
2. DUAB ME 1982 Cercosporin, a photosensitizing toxin from *Cercospora* species. Phytopathology 72: 370–374
3. DAUB ME, RP HANGARTER 1983 Light induced production of singlet oxygen and superoxide by the fungal toxin cercosporin. Plant Physiol 73: 855–857
4. DODGE AD 1983 Toxic oxygen species and herbicide action. *In* S Matsunaka, D Hutson, SE Murphy, eds. Pesticide Chemistry, Human Welfare and the Environment, Vol 3, Mode of Action, Metabolism and Toxicology. Pergamon Press, New York, pp 59–66
5. DUKE SO, WH KENYON 1986 Photosynthesis is not involved in the mechanism of action of acifluorfen in cucumber (*Cucumis sativus* L.). Plant Physiol 81: 882–888
6. DUKE SO, WH KENYON 1987 A non-metabolic model of acifluorfen activity. Z Naturforsch 42c: 813–818
7. ELSTNER EF, H ZELLER 1978 Bleaching of *p*-nitrosodimethylanaline by photosystem I of chloroplast lamellae. Plant Sci Lett 13: 15–20
8. ENSMIGER MP, FD HESS 1985 Photosynthesis involvement in the mechanism of action of diphenylether herbicides. Plant Physiol 78: 46–50
9. ENSMINGER MP, FD HESS, LT BAHR 1985 Nitrofree radical formation of diphenyl ether herbicides is not necessary for their toxic action. Pest Biochem Physiol 23: 163–170
10. GHIRARDI M, A MELIS 1984 Photosystem electron transport capacity and light harvesting antenna size in maize chloroplasts. Plant Physiol 74: 993–998
11. GILLMAN DJ, MW EVANS, AD DODGE 1985 Oxyfluorfen activation by photosynthetic eljectron transport. Proc Br Crop Protect Conf Weeds 7: 1195–1201
12. HALLIWELL B, JMC GUTTERIDGE 1984 Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J 219: 1–14
13. HAWORTH P 1983 Protein phosphorylation induced state I-state II transitions are dependent on thylakoid membrane microviscosity. Arch Biochem Biophy 226: 145–154
14. JOSHI PC, MA PATHAK 1984 The role of active oxygen ($^1O_2$ and O_2) induced by crude coal tar and its ingredients used in photochemotherapy of skin diseases. J Invest Dermatol 82: 67–73
15. KENYON WH, SO DUKE, RN PAUL 1987 Effects of temperature on the activity of the *p*-nitrosubstituted diphenyl ether herbicide acifluorfen in cucmber (*Cucumis sativus* L.) Pest Biochem Physiol (in press)
16. KUNERT KJ, P BÖGER 1987 The bleaching effect of the diphenyl ether herbicide oxyfluorfen. Weed Sci 29: 169–173
17. LAMBERT R, PMH KRONECK, P BÖGER 1984 Radical formation and peroxidative activity of phytotoxic diphenyl ethers. Z Naturforsch 39c: 486–491
18. LAMBERT R, G SANDMANN, P BOGER 1983 Mode of action of nitrodiphenylethers affecting pigments and membrane Integrity. *In* S Matsunaka, D Hutson, SD Murphy, eds, Pesticide Chemistry, Human Welfare and the Environment, Vol 3, Mode of Action, Metabolism and Toxicology. Pergamon Press, New York, pp 97–102
19. MACKINNEY G 1941 Absorption of light by chlorophyll solutions. J Biol Chem 140: 544–522
20. MULLET JE, JJ BURKE, CJ ARNTZEN 1980 Chlorophyll proteins of photosystem I. Plant Physiol 65: 814–822
21. MULLET JE, CJ ARNTZEN 1980 Stimulation of grana stacking in a model membrane system. Mediation by a purified light-harvesting pigment protein complex from chloroplasts. Biochim Biophys Acta 589: 100–117
22. MULLET JE, CJ ARNTZEN 1981 Identification of a 32-kilodalton polypeptides as herbicide receptor protein in photosystem II. Biochim Biophys Acta 635: 236–238
23. PERCIVAL MP, AD DODGE 1983 Photodynamic damage to chloroplast membranes, photosensitized oxidation of chloroplast acyl lipid. Plant Sci. Lett 29: 255–264
24. RABINOWITCH HD, I FRIDOVICH 1983 Superoxide radicals, superoxide dismutases and oxygen toxicity in plants. Photochem Photobiol 37: 679–690
25. RESZKA K, PG TSOUNGAS, JW LOWN 1986 Photosensitization by antitumor agents. 1. Production of singlet oxygen during irradation of anthrapyrazoles with visible light. Photochem Photobiol 43: 499–504
26. SVINGEN BA, FO O'NEAL, SD AUST 1978 The role of superoxide and singlet oxygen lipid peroxidation. Photochem Photobiol 28: 803–809
27. TAKAHAMA U 1983 Supression of lipid photoperoxidation by quercetin and its glycosides in spinach chloroplasts. Photochem Photobiol 38: 363–367
28. UPHAM BL, HC HATZIOS 1987 Potential involvement of alkoxyl and hydroxyl radicals in the peroxidative reaction of oxyfluorfen. Pest Biochem Physiol 28: 248–256
29. WETTLAUFER SH, R ALSCHER, C STRICK 1985 Chloroplast-diphenyl ether interactions II. Plant Physiol 78: 215–220