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Inhibition of Photosynthetic Electron Transport by Diphenyl Ether Herbicides[1, 2]

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ABSTRACT

The effects of the diphenyl ether herbicides HOE 29152 (methyl-2[4-(4-trifluoromethoxy) phenoxy] propanoate) and nitrofluorfen (2-chloro-1-[4-nitrophenoxy]-4-[trifluoromethyl]benzene) on photosynthetic electron transport have been examined with pea seedling and spinach chloroplasts. Linear electron transport (water to ferricyanide or methylviologen) is inhibited in treated chloroplasts, but neither photosystem II activity (water to dimethylquinone plus dibromothymoquinone) nor photosystem I activity (diaminodurene to methylviologen) is affected. Cyclic electron flow, catalyzed by either phenazine methosulfate or diaminodurene, is resistant to inhibition by nitrofluorfen. In diphenyl ether-treated chloroplasts the half-time for the dark reduction of cytochrome *f* is increased 5- to 15-fold. These data indicate that the site of inhibition for the diphenyl ethers is between the two photosystems in the plastoquinone-cytochrome *f* region.

Diphenyl ether herbicides are potent inhibitors of growth in higher plants (10, 12). Inhibition of respiration and photosynthesis appears to be associated with the phytotoxic effects of these compounds (11, 15). Based on the observation that in diphenyl ether-treated chloroplasts over-all electron transport is inhibited without affecting PSI activity, Moreland *et al.* (15) proposed that diphenyl ethers block chloroplast electron flow close to PSII. In this study we describe experiments designed to characterize further the site of inhibition for diphenyl ethers in the photosynthetic electron transport chain. Our results indicate that the diphenyl ethers HOE 29152 (methyl-2[4-(4-trifluoromethoxy)phenoxy]propanoate) and nitrofluorfen (2-chloro-1-[4-nitrophenoxyl]-4-[trifluoromethyl]benzene) inhibit electron flow *between* the two photosystems in the plastoquinone-Cyt *f* region of the chain.

MATERIALS AND METHODS

Chloroplasts were isolated either from pea seedlings or spinach, grown under controlled environmental conditions, as previously described (4, 16). Chl concentration was determined as described by Arnon (2).

O_2 evolution in the presence of a Hill acceptor was monitored using a Beckman No. 39065 electrode mounted in a thermostatically controlled Lucite chamber. The actinic light, which was saturating (8×10^5 ergs cm^{-2} s^{-1}), was provided by a 650-w tungsten-iodide lamp filtered by a copper sulfate solution and an orange cinemoid filter. The same apparatus was used to monitor O_2 consumption associated with MV[6] reduction. Photophosphorylation and proton uptake, measured with a glass electrode, were assayed as described elsewhere (4). Light-induced changes in Cyt absorbancy were measured using a single beam spectrophotometer in conjunction with a digital signal averager (24).

The diphenyl ether herbicides were generously supplied by Dr. P. Robinson of the American Hoechst Corp., Somerset, N.J., and Dr. R. Yih of the Rohm and Haas Company. Stock solutions of the herbicides were prepared in ethanol; the final concentration of ethanol in reaction mixtures did not exceed 1%. All other chemicals were of reagent grade.

RESULTS

Both HOE 29152 (HOE) and nitrofluorfen are inhibitors of whole chain electron transport (H_2O to MV) in isolated pea seedling chloroplasts (Fig. 1). The concentration required for 50% inhibition of uncoupled electron flow is 5 to 10 μM for nitrofluorfen and 50 to 70 μM for HOE. Both compounds also inhibit over-all electron transport in spinach, maize mesophyll, sorghum mesophyll, and duckweed chloroplasts (data not shown). The concentration dependency for inhibition of chloroplast electron flow by HOE is similar to that observed with trifluralin (18); even at saturating concentrations complete inhibition is not observed. The residual electron flow observed in the presence of 500 μM HOE is completely sensitive to either 4 μM DCMU or 2 μM DBMIB. Both coupled and uncoupled electron flow are inhibited to the same extent by HOE and nitrofluorfen suggesting that these compounds do not act secondarily as energy transfer inhibitors (14).

Table I summarizes the results of a number of experiments in which we compared the effects of HOE, nitrofluorfen, and DBMIB on electron transport in pea seedling chloroplasts with a number of acceptors. Note that when over-all electron flow (H_2O to ferricyanide or MV) is blocked, the reduction of ferricyanide in the presence of class III acceptors (DAD or DMQ) is only partially affected. Also note in Table I that neither PSII activity (H_2O to DMQ_{ox} + DBMIB) nor PSI activity ($DADH_2$ to MV) is affected by HOE or nitrofluorfen.

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[6] Abbreviations: MV: methylviologen; DBMIB: 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DAD: diaminodurene, 2,3,5,6-tetramethyl-*p*-phenylenediamine; DMQ: 2,5-dimethyl-*p*-benzoquinone; DQ: duroquinone, 2,3,5,6-tetramethyl-*p*-benzoquinone; TMPD: *N,N,N',N'*-tetramethyl-*p*-phenylenediamine; PYO: pyocyanine; PMS: phenazine methosulfate; Q: primary electron acceptor of photosystem II; EDAC: 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide.

Further evidence for the site of inhibition in diphenyl ether-treated chloroplasts was provided by analyzing the effect of the two compounds on the PSI-dependent oxidation of durohydroquinone (DQH_2). Based on the observation that the oxidation of DQH_2 is extremely DBMIB-sensitive, it has been suggested that DQH_2 donates electrons to the plastoquinone region of the photosynthetic electron transfer chain (9, 22). The PSI-catalyzed oxidation of DQH_2, in contrast to the oxidation of DAD, is markedly sensitive to inhibition by nitrofluorfen, and partially sensitive to inhibition by HOE (Table II).

Consistent with a site of inhibition for diphenyl ethers between the two photosystems is the ability of 0.1 mM TMPD (in the absence of ascorbate) to reverse the inhibition (21) of over-all electron flow (H_2O to MV) in nitrofluorfen-treated chloroplasts (data not shown).

Under conditions where noncyclic photophosphorylation catalyzed by ferricyanide is severely inhibited by nitrofluorfen, phosphorylation catalyzed by DAD plus ferricyanide or DMQ plus ferricyanide is only partially inhibited (Table III). Cyclic photophosphorylation, assayed in the presence of DCMU, is resistant to inhibition by nitrofluorfen (Table IV). When PYO-catalyzed phosphorylation is assayed in the absence of DCMU an inhibition of ATP synthesis by nitrofluorfen is observed. This inhibition may reflect an effect of nitrofluorfen on PSII redox poising of PYO (1, 6). In other experiments we observed that HOE had basically similar effects on phosphorylation.

The extent of proton uptake (PMS-catalyzed) is not altered in nitrofluorfen-treated chloroplasts at pH 7.5, but the apparent first order rate constant for proton efflux is increased from $0.037\ s^{-1}$ to $0.050\ s^{-1}$ (data not shown).

Cyt f in chloroplasts is normally reduced in the dark and is rapidly oxidized upon illumination. When the actinic light is turned off the Cyt returns to the reduced state utilizing electrons from the plastoquinone pool (24). By monitoring the A change in the α-band region of Cyt f one can observe the time course for dark reduction and determine if an inhibitor is having an effect on the rate of reduction. Figure 2A shows the kinetics of the A increase in spinach chloroplasts subsequent to a 0.3-s red actinic flash. The effects upon the A increase of adding nitrofluorfen and HOE are shown in Figure 2, B and C, respectively. These A increases are shown as a function of wavelength in Figure 3. The spectrum, with a peak at 553 nm, is similar to the reduced-oxidized spectrum of the α-band of isolated Cyt f (24) indicating that the A increase observed at 554 to 542 nm is due primarily to Cyt f. In

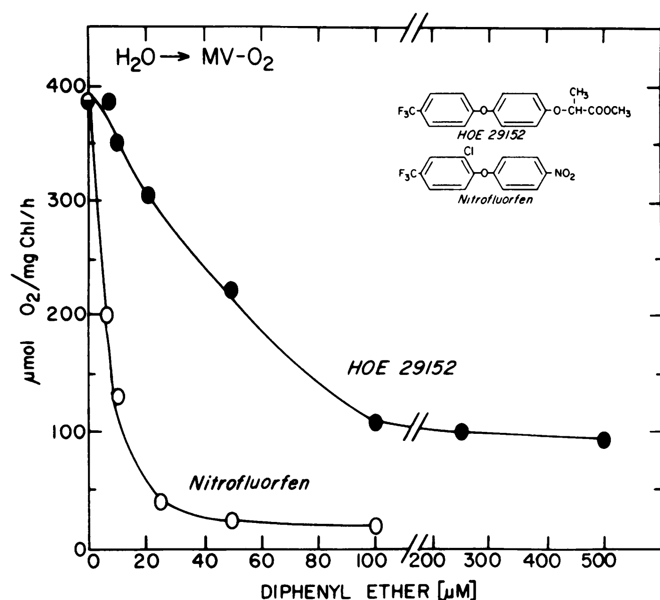

FIG. 1. Effect of HOE 29152 and nitrofluorfen on uncoupled electron transport in pea seedling chloroplasts. The reaction mixture (in 5 ml) contained: 100 mM sorbitol, 50 mM Tricine-NaOH (pH 7.6), 20 mM KCl, 5 mM $MgCl_2$, 0.5 mM EDTA, Na_2, 0.1 mM MV, 0.5 mM NaN_3, 4 µg/ml gramicidin D, 2 mM NH_4Cl, and chloroplasts equivalent to 105 µg Chl. Temp, 23 C.

Table II. *Effect of HOE 29152 and Nitrofluorfen on Durohydroquinone Oxidation*

The reaction mixture was similar to the one in the legend to Table I employed to assay DAD oxidation, except 0.5 mM durohydroquinone replaced DAD, and ascorbate was omitted.

Inhibitor	O_2 Consumed	Inhibition
µM	*µmol/mg Chl·h*	*%*
None	629	
HOE 29152		
50	453	
100	433	
200	391	38
Nitrofluorfen		
10	320	
50	179	
100	126	80
DBMIB		
0.1	35	
1.0	26	96

Table I. *Effect of HOE 29152 and Nitrofluorfen on Electron Transport with Different Electron Acceptors*

Conditions as described in the legend to Figure 1. Electron transport was assayed under uncoupled conditions with the exception of DADox reduction which was assayed under coupled conditions (1 mM ADP and 5 mM K_2HPO_4 replaced gramicidin and NH_4Cl in the reaction mixture). The acceptor systems were: H_2O to FeCy (1.5 mM $K_3Fe[CN]_6$), H_2O to MV-O_2 (0.1 mM MV plus 0.5 mM NaN_3), H_2O to DADox (0.5 mM DAD plus 1.5 mM $K_3Fe[CN]_6$), H_2O to DMQox (0.5 mM DMQ, 1.5 mM $K_3Fe[CN]_6$, +/− 20 µM DBMIB), $DADH_2$ to MV-O_2 (1 mM DAD, 2.5 mM neutralized ascorbate, 0.1 mM MV, 0.5 mM NaN_3, 40 µM DCMU). The control rates in µmol O_2 evolved or consumed/mg Chl/h: H_2O to FeCy (290–370), H_2O to MV-O_2 (270–400), H_2O to DADox (160–200), H_2O to DMQox (370–450), H_2O to DMQox + DBMIB (60–150), $DADH_2$ to MV-O_2 (900–1600).

Inhibitor	Electron Transport Pathway					
	$H_2O \rightarrow$ FeCy	$H_2O \rightarrow$ MV-O_2	$H_2O \rightarrow$ DADox	$H_2O \rightarrow$ DMQox	$H_2O \rightarrow$ DMQox (+ DBMIB)	$DADH_2 \rightarrow$ MV-O_2
	% inhibition					
50 µM HOE	34	32		29		
500 µM HOE	79	75	43	36	0	3
10 µM Nitrofluorfen	70	70	23	60		
50 µM Nitrofluorfen	90	91	42	63	2	8
2 µM DBMIB	91	98	49	74		11

Table III. *Effect of Nitrofluorfen on Noncyclic Photophosphorylation*

The reaction mixture (in 1 ml) contained: 50 mM KCl, 50 mM Tricine-NaOH (pH 8.1), 5 mM $MgCl_2$, 1 mM ADP, 5 mM K_2HPO_4 (containing 1 × 10^6 cpm of ^{32}P), and pea chloroplasts equivalent to 23 μg/ml. Temp, 22 C. The acceptor systems were H_2O to FeCy (1.5 mM $K_3Fe[CN]_6$), H_2O to DADox (0.5 mM DAD plus 1.5 mM $K_3Fe[CN]_6$), H_2O to DMQox (0.5 mM DMQ plus 1.5 mM $K_3Fe[CN]_6$). Samples were illuminated for 2 min with heat-filtered white light (incident light intensity = 2 × 10^6 ergs cm^{-2} s^{-1}).

Electron Transport Pathway	ATP Synthesis		
	Control	200 μM Nitrofluorfen	2 μM DBMIB
	μmol/mg Chl·h		
H_2O → FeCy	319	15	9
H_2O → DADox	477	127	35
H_2O → DMQox	409	180	37

the control chloroplasts (Fig. 2A) in the presence of 2 μM gramicidin the half-time for the dark reduction of Cyt *f* is 29 ± 3 ms. This value typically ranges from 25 to 32 ms. Upon the addition of 100 μM nitrofluorfen the dark reduction half-time is increased to 430 ± 40 ms. Under the same conditions the rate of electron transport from H_2O to MV is inhibited approximately 90% (data not shown). The addition of 400 μM HOE 29152 increased the dark reduction half-time to 150 ± 15 ms. Under the same conditions the rate of electron transport is inhibited by 70 to 80% (data not shown). The extent of the dark *A* increases presented here corresponds to a stoichiometry of Cyt *f* to Chl of 1:660–730.

DISCUSSION

The majority of photosynthetic electron transport inhibitors act near PSII between Q and plastoquinone (7, 17, 21). A few inhibitors have been discovered that are capable of inhibiting electron transfer between the secondary acceptors of PSII and the secondary donors of PSI, *i.e.* near the rate-limiting step; these include DBMIB (8), EDAC (13), bathophenanthroline (3), and trifluralin (18). The locus of impairment in chloroplasts treated with HOE and nitrofluorfen also appears to be in the region of the electron transport chain between plastoquinone and Cyt *f*. This conclusion is based on the following evidence: (*a*) both electron transport and phosphorylation mediated by class III acceptors (H_2O to DMQox or DADox) are partially sensitive to inhibition by the diphenyl ethers, (*b*) the diphenyl ethers have little or no effect on PSII electron transport activity; (*c*) HOE and nitrofluorfen inhibit the PSI-dependent oxidation or durohydroquinone but do not affect other PSI-dependent reactions, such as the transport of electrons from DAD to MV or cyclic photophosphorylation with a variety of mediators; (*d*) the level of inhibition is similar at saturating and nonsaturating light intensities (W. S. Cohen, unpublished), sug-

Table IV. *Effect of Nitrofluorfen on Cyclic Photophosphorylation with Various Mediators*

The reaction mixture was similar to the one described in the legend to Table III. The mediators were: H_2O to MV-O_2 (0.1 mM MV plus 0.5 mM NaN_3), PYO (0.025 mM PYO, 2.5 mM neutralized ascorbate, +/− 2 μM DCMU), PMS (50 μM PMS plus 2 μM DCMU), DAD (0.5 mM DAD plus 2 μM DCMU).

Electron Transport Pathway	ATP Synthesis	
	Control	200 μM Nitrofluorfen
	μmol/mg Chl/h	
H_2O → MV-O_2	354	69
PYO	878	460
PYO/DCMU	88	97
PMS/DCMU	860	879
DAD/DCMU	839	831

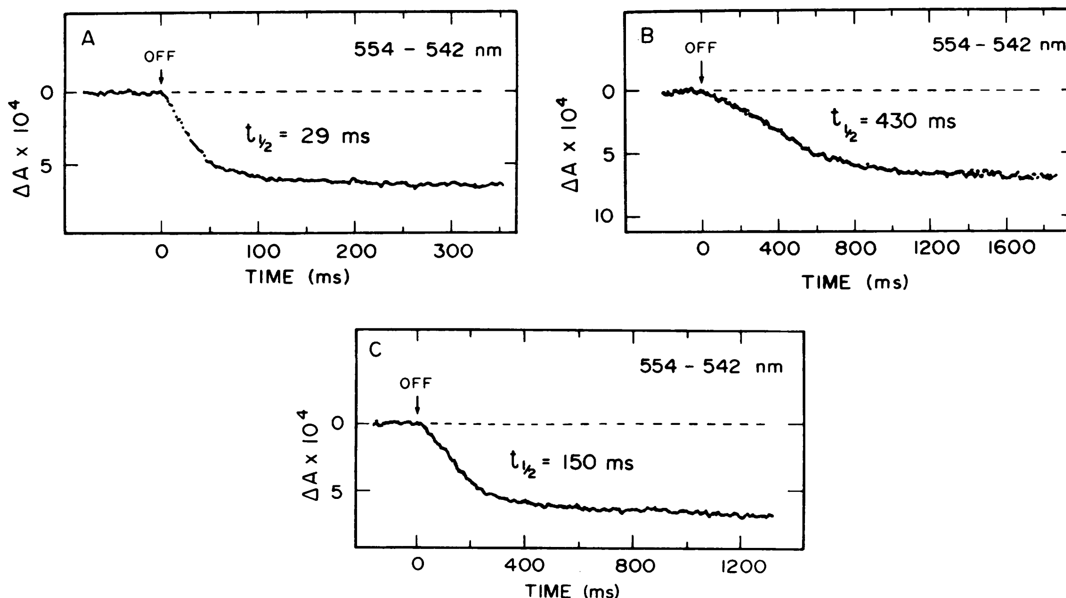

FIG. 2. A: kinetics of *A* increase, at 554 to 542 nm, subsequent to illumination of spinach chloroplasts by a 0.3-s flash. The trace shown is the average of 300 sampling runs and has been curve-smoothed one time. The half-bandwidth of the measuring beam was 2 nm. The time constant of the instrument was 2 ms. The repetition rate of the flashes was 0.33 Hz. The reaction mixture was kept at 18 C and contained 0.2 M sucrose, 30 mM Tricine-NaOH (pH 7.8), 10 mM KCl, 2 mM $MgCl_2$, 0.1 mM MV, 2 μM gramicidin, and 23 μg Chl/ml. Actinic light, filtered by a heat filter and a Corning 2-58 blocking filter, was 4 × 10^5 erg cm^{-2} s^{-1}. B: kinetics of *A* increase, at 554 to 542 nm, subsequent to illumination of chloroplasts by a 0.3-s flash in the presence of 100 μM nitrofluorfen. The trace shown is the average of 320 sampling runs and the time constant was 5 ms. All other experimental conditions were as described in Figure 2A. C: kinetics of *A* increase, at 554 to 542 nm, subsequent to illumination of chloroplasts by a 0.3-s flash in the presence of 400 μM HOE. The trace shown is the average of 384 sampling runs. All other experimental conditions were as described in Figure 2A.

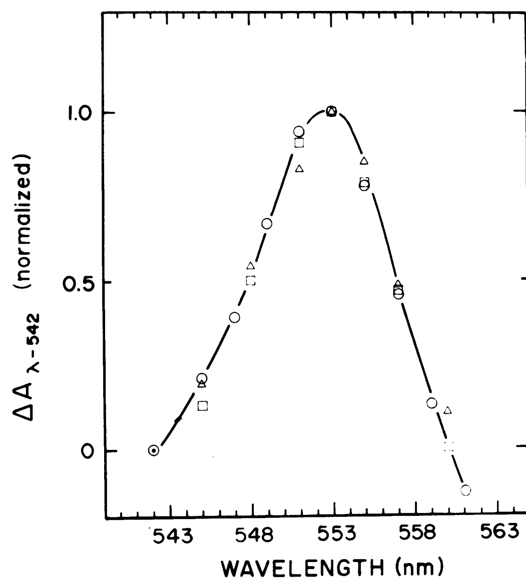

FIG. 3. Wavelength dependence of A increase observed subsequent to a 0.3-s actinic flash in spinach chloroplasts: (○), control; (□), nitrofluorfen; (△), HOE 29152. Experimental conditions were as described in Figure 2. The reference wavelength was 542 nm.

gesting that the block is at a point in the electron transfer chain beyond the place where electrons are shared in the common pool of plastoquinone (5, 19, 23); and (*e*) the half-time for the reduction of Cyt *f* by the plastoquinone pool is increased 5- to 15-fold in diphenyl ether-treated chloroplasts.

In addition to HOE and nitrofluorfen we observed that nitrofen(2-chloro-1-[4-nitrophenoxy]-4-chlorobenzene)—one of the diphenyl ethers used in Moreland's original study (15)—also acts in the plastoquinone-Cyt *f* region (W. Bugg and W. S. Cohen, unpublished) rather than at the DCMU site as has been suggested (15). Data have been presented recently suggesting that the dinitrophenylethers of bromo- and iodonitrothymol also act at a site close to plastoquinone (20, 21). Experiments are currently in progress to determine more precisely the relationship between the chemical structure of diphenyl ethers and their site of inhibition in the chloroplast electron transport chain.

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LITERATURE CITED

1. ANDERSON M, RE MCCARTY 1969 The effects of plastocyanin on photophosphorylation. Biochim Biophys Acta 189: 193–206
2. ARNON DI 1949 Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol 24: 1–15
3. BERING L, RA DILLEY, FL CRANE 1977 Inhibition of energy transducing functions of chloroplast membranes by lipophilic iron chelators. Biochim Biophys Acta 430: 327–335
4. COHEN WS 1978 The coupling of electron flow to ATP synthesis in pea chloroplasts stored in the presence of glycerol at −70°C. Plant Sci Lett 11: 191–197
5. GOULD JM, S IZAWA 1973 Photosystem II electron transport and phosphorylation with dibromothymoquinone as the electron acceptor. Eur J Biochem 37: 185–192
6. HAUSKA G, RE MCCARTY, E RACKER 1970 The site of phosphorylation associated with photosystem I. Biochim Biophys Acta 197: 206–218
7. IZAWA S 1977 Inhibitors of electron transport. Encycl Plant Physiol 5: (new series) 266–282
8. IZAWA S, JM GOULD, DR ORT, P. FELKER, NE GOOD 1973 Electron transport and phosphorylation in chloroplasts as a function of the electron acceptor. III. A DBMIB-insensitive phosphorylation associated with photosystem II. Biochim Biophys Acta 305: 119–128
9. IZAWA S, RL PAN 1978 Photosystem I electron transport and phosphorylation supported by electron donation to the plastoquinone region. Biochem Biophys Res Commun 83: 1171–1177
10. LANGELUDEKKE P, F SCHWERDTLE, J TROSKEN, H BIERINGER 1977 HOE 29152—a new selective herbicide for the control of annual and perennial grasses in dicotyledonous crops. Z Pflanzenkrankeiten Pflanzenshutz 8: 429–435
11. KOCHER H, K LOTSCH 1975 A note on the mode of action of the phenoxy propionic acid derivatives. *In* Symposium on Status, Biology and Control of Grass Weeds in Europe, Paris, 1975, Vol I, pp 430–437
12. MATSUNAKA S 1969 Acceptor of light energy in photoactivation of diphenyl ether herbicides. J Agric Food Chem 17: 171–175
13. MCCARTY RE 1974 Inhibition of electron transport in chloroplasts between the two photosystems by a water soluble carbodiimide. Arch Biochem Biophys 161: 93–99
14. MCCARTY RE, E RACKER 1967 Partial resolution of the enzymes catalyzing photophosphorylation. II. The inhibition and stimulation of photophosphorylation by *N,N'*-dicyclohexyl carbodiimide. J Biol Chem 242: 3435–3439
15. MORELAND D, W BLACKMON, H TODD, F FARMER 1970 Effects of diphenyl ether herbicides on reactions of chloroplasts and mitochondria. Weed Sci 18: 636–641
16. ORT D, S IZAWA 1973 Studies on the energy-coupling sites of photophosphorylation. II. Treatment of chloroplasts with NH_2OH plus ethylenediaminetetraacetate to inhibit water oxidation while maintaining energy-coupling efficiencies. Plant Physiol 52: 595–600
17. RADOSEVICH SR, KE STEINBACK, CJ ARNTZEN 1979 Effect of photosystem II inhibitors on thylakoid membranes of two common groundsel (*Senecio vulgaris*) biotypes. Weed Sci 27: 216–218
18. ROBINSON SJ, CF YOCUM, H IKUMA, F HAYASHI 1977 Inhibition of chloroplast electron transport by trifluralin and diallate. Plant Physiol 60: 840–844
19. SIGGEL U, G RENGER, HH STIEHL, B RUMBERG 1972 Evidence for electronic and ionic interaction between electron transport chains in chloroplasts. Biochim Biophys Acta 256: 328–335
20. TREBST A, W DRABER 1979 Structure activity correlations of recent herbicides in photosynthetic reactions. *In* H Geissbuhler, ed, Advances in Pesticide Science, Part 2. Pergamon Press, Oxford, pp 223–234
21. TREBST A, H WIETOSKA, W DRABER, HJ KNOPS 1978 The inhibition of photosynthetic electron flow in chloroplasts by the dinitrophenyl ether of bromo- or iodo-nitrothymol. Z Naturforsch 33: 919–927
22. WHITE CC, RK CHAIN, R MALKIN 1978 Duroquinol as an electron donor for chloroplast electron transfer reactions. Biochim Biophys Acta 502: 127–137
23. WHITMARSH J, W CRAMER 1978 A pathway for the reduction of cytochrome b-559 by photosystem II in chloroplasts. Biochim Biophys Acta 501: 83–93
24. WHITMARSH J, WA CRAMER 1979 Cytochrome *f* function in photosynthetic electron transport. Biophys J 26: 223–234