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EFFECTS OF DDT ON PHOTOSYNTHETIC ELECTRON FLOW IN *SECALE* SPECIES*

SHER AKBAR† and LYNDON J. ROGERS

Department of Biochemistry and Agricultural Biochemistry, The University College of Wales, Aberystwyth, Dyfed, SY23 3DD, Wales, U.K.

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Key Word Index—*Secale* species; Gramineae; rye; DDT; photosynthesis; photosynthetic electron flow.

Abstract—Following a survey of a range of varieties of rye, mainly *Secale cereale*, for reaction to DDT, the mode of action of the pesticide in a susceptible variety was studied. Two sites of interaction of DDT with the photosynthetic electron transport chain were demonstrated. The first site of inhibition was on the oxidizing side of photosystem 2, between the sites of electron donation from diphenylcarbazide at pH 6.0 and pH 8.0 in Tris-washed chloroplasts. The second site of DDT inhibition was in the intermediate electron transport chain, and was demonstrated by using dichlorophenol-indophenol and phenyldiamines as electron donors in chloroplasts where electron flow from photosystem 2 was inhibited by 3-(3,4-dichlorophenyl)-1,1-dimethylurea. The sites are distinct from those characteristic of herbicides which affect photosynthetic electron flow.

INTRODUCTION

Following its discovery more than a century ago, DDT found extensive application in insect irradication programmes. It has been regarded as a model compound for studying the mode of action of organochlorine insecticides. However, because of concern about the effects of residues in the environment it has gradually been withdrawn from agricultural use in a number of countries, including the U.K. Following its wide use in agriculture it became evident that varietal differences in susceptibility to DDT existed in several plant species including *Hordeum vulgare* (barley) [1], *Secale* species (rye) [2] and *Triticum* species (wheat) [3]. In these cereals the reaction to the pesticide is controlled by a single gene, with susceptibility the dominant character. For barley the biochemical mode of action of the pesticide has been established previously [4–6] and the biochemical explanation for DDT toxicity in rye can now be reported.

RESULTS AND DISCUSSION

Incidence of susceptibility

We surveyed the reaction to DDT of some twenty varieties of rye grown from seed provided by local suppliers or from the Crops Research Division, US Department of Agriculture small grain collection. Preliminary studies had indicated that the phytotoxic response included an inhibition of photosynthesis. A variety was therefore classed as susceptible if the Hill activity of chloroplasts isolated from seedlings two days after DDT treatment was inhibited about 40% compared to chloroplasts from untreated seedlings. The conclusion was confirmed from the chlorosis which susceptible seedlings developed about 8–10 days after spraying. In this survey (Table 1) three varieties were susceptible and two varieties gave an equivocal reaction. The observation differs somewhat from an earlier survey [2] in which few varieties proved to be resistant and the reason for this is unclear. The only varieties common to the two investigations were Lovazpatonai and Rheidol, both susceptible in the earlier survey. In our tests Lovazpatonai was clearly susceptible but Rheidol proved to be resistant to spraying with DDT as judged by inhibition of photosynthesis and from physiological response in greenhouse trials. Rheidol is reported to be a mixture of DDT resistant and susceptible plants in which the latter greatly predominate [2]. The significant difference in treatment in the two surveys was a single spraying with DDT in the present survey compared to two treatments, a week apart, in the earlier work [2]. Supplies of most of the varieties were too limited to permit our studies to be extended to other spraying programmes. To define the biochemical basis of susceptibility subsequent investigations were confined to a single susceptible variety, Lovazpatonai, and a resistant variety, Rhayader.

Effect on photosynthetic electron flow

From the data in Table 1 it was evident that in chloroplasts from DDT-treated susceptible rye both DCIP and ferricyanide photoreductions were inhibited, showing a site of inhibition of DDT associated with photosystem 2 electron flow. This was located by exper-

*Part 1 in the series "Susceptibility of Cereals to Organochlorine Pesticides and Biochemical Mode of Action".

†Present address: Department of Agricultural Chemistry, NWFP Agricultural University, Peshawar, Pakistan.

Abbreviations used: DAD, 2,3,5,6-tetramethyl-*p*-phenylenediamine(diaminodurene); DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DPC, diphenylcarbazide; DCIP, 2,6-dichlorophenolindophenol; DDT, (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; MV, methyl viologen; PMS, phenazine methosulphate; TMPD, *N,N,N',N'*-tetramethyl *p*-phenylenediamine.

Table 1. Susceptibility of rye to DDT as assessed by inhibition of DCIP and ferricyanide photoreduction in chloroplasts isolated from DDT-treated and untreated seedlings

Variety	DCIP photoreduction			$Fe(CN)_6^{3-}$ photoreduction			Reaction to DDT
	UT	T	% inhibition	UT	T	% inhibition	
Adams	73	70	4	77	79	−3	R
Balbo	47	46	2	65	58	11	R
Centeno DeLa Estan Zuela	46	43	6	51	52	−2	R
Grande Crouelle	46	52	−13	63	66	−5	R
Greenfold	78	67	14	92	80	13	R
Hungarian Giant	96	42	56	92	39	58	S
Irlanda I	64	66	−3	78	79	−1	R
K 5836	51	56	−10	72	76	−5	R
Kirszkajh	63	66	−5	81	76	6	R
Korean	77	69	10	81	79	2	R
Lovaspatonai	93	53	43	106	61	42	S
Nemelorsrag	55	57	−4	79	87	−10	R
Perevaya	70	56	20	76	63	17	(S)
Rhayader	95	88	7	106	105	1	R
Rheidol	74	78	−5	95	102	−7	R
TK 15576-67	49	31	37	64	41	36	S
Tetraploide Vilmorin	67	68	−1	74	74	0	R
Vita-graze	52	55	−6	59	65	−10	R
Von Lochow	61	44	28	71	55	23	(S)
1-3-127	58	59	−2	70	68	3	R
5-SC-12	82	76	7	89	86	3	R

Varieties are *Secale cereale*, except 5-SC-12 (*S. segetale*). Rates for DDT treated (T) and untreated (UT) samples are given as μmol acceptor reduced hr^{-1} mg^{-1} chlorophyll. In the final column S and R indicate susceptible and resistant, respectively.

iments in which chloroplasts were Tris-washed to abolish electron donation from water and where DPC was used as an artificial electron donor to photosystem 2 [7, 8]. Data from three separate experiments are summarized in Fig. 1. When assays were carried out at pH 6.0 there was a 50% inhibition of electron flow from water by DDT in chloroplasts isolated from susceptible rye whereas this inhibition was not evident in Tris-washed chloroplasts when DPC was used as the electron donor. At progressively higher pH values the restoration of electron flow by DPC became less effective and at pH 8.0 and above the inhibition of electron flow to DCIP was close to that for inhibition of electron donation from the physiological donor before Tris-washing. For chloroplasts from DDT-treated and untreated resistant rye there was no difference in rates of electron flow from DPC to DCIP at these pH values (data not shown). These investigations demonstrated that the site of inhibition by DDT responsible for the decreased ability to photoreduce DCIP is on the oxidising site of photosystem 2 before (i.e. on the water side of) the site of electron donation from DPC at pH 6.0, but after its site of donation at pH 8.0. These data also show that changes in pH alter the site of electron donation by DPC. The oxidation of this donor does involve a proton transfer suggesting that at the lower pH electrons could be donated to an electron carrier with a higher redox potential. The implication that there is more than one site for electron donation between water and P680 would be in accord with suggestions from other investigations [e.g. 9].

Consistent with these observations, it was found that non-cyclic photophosphorylation concomitant with ferricyanide photoreduction was also inhibited in chloroplasts from DDT-treated rye (Table 2). This inhibition was somewhat greater than for the accompanying photoreduction of the electron acceptor suggesting that the pesticide may have some uncoupling effect over and above its action as an inhibitor of electron transport. The term inhibitory uncoupler has been introduced [10] for a number of herbicides which show similar behaviour. The P/2e ratio for chloroplasts from untreated rye was about 0.8 which is appreciably lower than the best ratios obtained for chloroplasts from spinach [11]. An inhibition of phenazine methosulphate catalysed cyclic photophosphorylation in chloroplasts from DDT-treated susceptible seedlings was also evident. Neither type of photophosphorylation was inhibited in chloroplasts from resistant rye treated with the pesticide.

The observation that photosystem 1-associated cyclic photophosphorylation was inhibited could be due to uncoupling or to inhibition by DDT at an additional site to that on the oxidising side of photosystem 2. That a site of inhibition was present was shown by studies of electron donation to photosystem 1 by DCIP, TMPD or DAD, all in the presence of ascorbate, using MV or $NADP^+$ as electron acceptor. In these experiments electron donation from water via photosystem 2 was inhibited by DCMU. The results presented in Table 3 show that electron donation from $DCIPH_2$ to either acceptor was within experimental error the same in chloroplasts from DDT-treated and untreated seedlings. However, when TMPD or DAD were used as electron donors electron transport

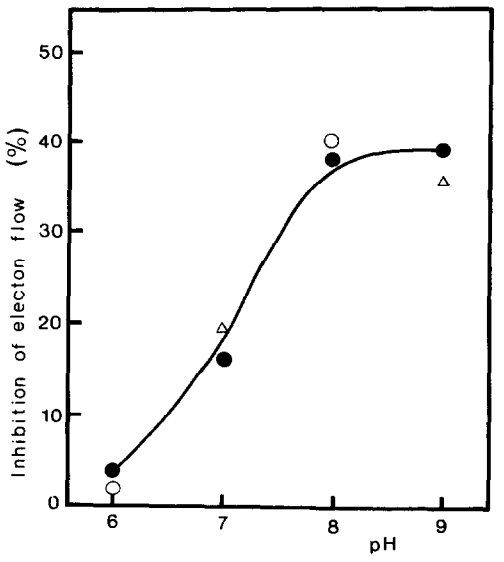

Fig. 1. Effect of pH on the site of electron donation by DPC in relation to a site of inhibition by DDT. Rates are given as per cent inhibition by DDT compared with data for DCIP photoreduction using DPC as donor at the corresponding pH in chloroplasts from untreated plants. In accompanying controls not subjected to Tris-washing electron transport from H_2O was inhibited some 50% in chloroplasts from DDT-treated seedlings. The symbols indicate results from experiments on three separate occasions. For chloroplasts from untreated seedlings the rates of electron transport (μmol DCIP reduced hr^{-1} mg^{-1} chlorophyll) for H_2O as donor were about 80 at pH 6.0 and 40 at pH 8.0, and for DPC as donor were about 120 at pH 6.0 and 55 at pH 8.0.

in chloroplasts from DDT-treated seedlings was inhibited. In conjunction with the experiments with DCIP, this suggests that these two donors were supplying electrons to a carrier on the oxidizing side of a site of inhibition by DDT in the intermediate electron transport chain. We noted that for DAD, and to a lesser extent TMPD, the inhibition of methyl viologen reduction was somewhat greater at pH 7.0 than pH 8.0. The simplest interpretation of these data is that the inhibition of PMS-catalysed cyclic photophosphorylation which was observed results from this site of inhibition. On this basis, cyclic electron flow from the reducing side of photosystem 1 via PMS would involve a component of the intermediate electron transport chain on the oxidizing side of the DDT inhibition site. From the high rates of photophosphorylation obtained with PMS it has been deduced that electron donation would be directly to the reaction centre P700 or to plastocyanin [12]. One further conclusion from these experiments, noting the data for $DCIPH_2$ with $NADP^+$ as acceptor, is that these cannot be a site of inhibition by DDT on the reducing side of photosystem 1.

A further experiment confirmed that the second site of inhibition involved directly a component of the intermediate electron transport chain. In this case, DPC at pH 6.0 was used as electron donor, by-passing the site of inhibition by DDT before photosystem 2, and $NADP^+$ as electron acceptor. In chloroplasts from DDT-treated plants there was an appreciable inhibition in electron flow compared to the untreated control (Table 4). The conclusions from these several experiments exploiting natural and artificial electron donors and acceptors are shown in Fig. 2.

In general, these data and conclusions are similar to those reported previously for the effect of DDT on

Table 2. Photophosphorylation by chloroplasts isolated from DDT-treated susceptible and resistant varieties of rye

	Non-cyclic photophosphorylation						Cyclic photophosphorylation		
	$Fe(CN)_6^{3-}$ photoreduction			Pi esterification			Pi esterification		
Variety	UT	T	% inhibition	UT	T	% inhibition	UT	T	% inhibition
Lovaspatonai	540	370	31	210	90	55	730	430	41
Rhayader	625	605	4	215	210	2	745	735	1

Chloroplasts were isolated 2 days after treating seedlings with DDT (T) or a control spray (UT). Rates are given as μmol Pi esterified or $Fe(CN)_6^{3-}$ reduced hr^{-1} mg^{-1} chlorophyll.

Table 3. Effect of DDT on photosystem 1 activity in susceptible rye with $DCIPH_2$, TMPD and DAD as electron donors and MV or $NADP^+$ as electron acceptor

Electron donor	DCIP			TMPD			DAD		
Electron acceptor	MV	MV	$NADP^+$	MV	MV	$NADP^+$	MV	MV	$NADP^+$
pH	7	8	8	7	8	8	7	8	8
DDT-treated	149	177	30	184	250	15	144	368	17
Untreated	137	195	29	307	378	26	301	561	25
% inhibition	−9	9	−2	40	33	42	52	34	32

Rates are expressed as μmol acceptor reduced hr^{-1} mg^{-1} chlorophyll.

Table 4. Electron transport from DPC to $NADP^+$ or DCIP in Tris-washed chloroplasts from susceptible rye at pH 6.0

Treatment	None	None	Tris-washed		Tris-washed	
Electron donor	H_2O	H_2O	H_2O	DPC	H_2O	DPC
Electron acceptor	DCIP	$NADP^+$	DCIP	DCIP	$NADP^+$	$NADP^+$
Untreated rye	83	15	5	78	1	19
DDT-treated rye	46	6	2	70	1	10
% inhibition	44	60	—	10	—	48

Rates are expressed as μmol DCIP or $NADP^+$ reduced hr^{-1} mg^{-1} chlorophyll.

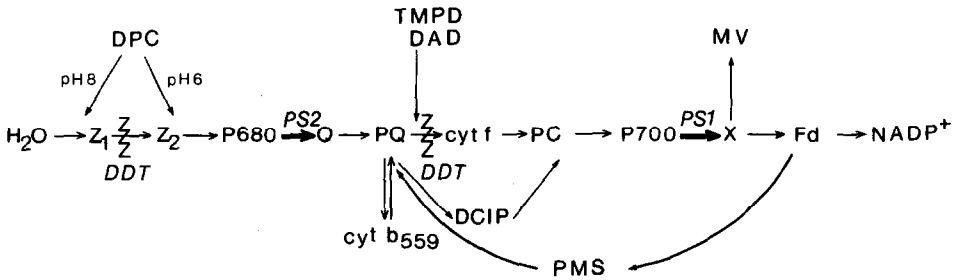

Fig. 2. Sites of inhibition of photosynthetic electron flow by DDT in relation to interaction of natural and artificial electron donors and acceptor systems. P_{680} and P_{700} are the reaction centres for photosystem 2 (PS2) and photosystem 1 (PS1), respectively, with primary electron acceptors Q and X. Z_1 and Z_2 are hypothetical sites; PQ, plastoquinone; PC, plastocyanin; Fd, ferredoxin.

photosynthetic electron flow in a susceptible barley variety [1, 2] where two sites of interaction of DDT with the electron transport chain also occur. In that case DDT was shown [13] to have a marked effect on cytochrome *f* responses due to an inhibition on the oxidizing side of the cytochrome, through there were also decreases in the concentrations of the other photosynthetic cytochromes. This would be consistent with DDT acting at or close to the DBMIB site.

We believe these investigations have two points of general interest. Apart from the bipyridyls, herbicides acting on photosynthetic electron flow interact between photosystems and 1 and 2 either in the Q_B-protein region (the 32kD herbicide-binding protein) or at the Fe:S-cyt *f*/cyt b_{553} complex (the DBMIB site) [14]. A site of inhibition on the oxidizing side of photosystem 2, such as is shown by DDT, has however been suggested for some other compounds. Two sites of cation interaction with the oxygen evolving site have been reported [15], and sulphydryl reagents affect a site on the DCMU-insensitive silicomolybdate pathway inhibiting electron flow from DPC to DCIP [16]. These reagents also inhibit on the oxidising side of the DBMIB site. However, closest relationship in mode of action is shown by the calcium chelator, ethyleneglycol bis(d-aminoethyl ether)-*N,N'*-tetraacetic acid [17]. Here there is one site of inhibition after DPC donates electrons and a further site of interaction in the intermediate electron transport chain. It is of interest in the present context that electron flow from DCIP to MV is not affected while that from TMPD to this acceptor is inhibited by some 50%. There is an evident similarity to the data for DDT inhibition (Table 3) suggesting both DDT and the chelator act at the same or closely related sites. Other compounds which also inhibit electron flow between photosystem 2 and photosystem 1 other than at the Q_B site are bentazon [18], picrate [19] and β-pinene [20]. Herbicides which may inhibit at the DBMIB site are the halogenated naphthoquinones [21] and substituted diphenyl ethers [22], while trifluralin inhibits both at this site and the Q_B site [23]. The diphenylamines may inhibit at a different site in this region [24].

Another feature of particular interest concerning the inhibition of photosynthetic electron flow by DDT reported in these studies with rye, and in the earlier work with barley [4, 5], is the recognition of its genetic basis. When in evolution species resistant to the pesticide developed is unknown, and it is difficult to see how mutations conferring the DDT trait would give a selective advantage to the species. However, in this context it is of interest to note development in recent years of resistance to the s-triazines and other herbicides in a range of weed species [25], where the trait is inherited in a non-Mendelian pattern. Here the basis of sensitivity to the herbicide can be traced to a single amino acid change in the 32kD herbicide-binding protein in the Q_B region [26]. The molecular basis for the DDT response in cereals may also be due to a change in structure of a membrane constituent. However, two sites of involvement of such a component with the photosynthetic electron transport

chain would be implicated. It is also conceivable that DDT may also perturb other cellular membranes but this has not been investigated.

EXPERIMENTAL

Treatment of plants with DDT. Seeds of *Secale* sp. were sown in John Innes No. 2 compost and seedlings grown in an environmental growth chamber under a 16 hr day regime (day temp. 23°, night temp. 17°) with fluorescent and tungsten lighting giving *ca* 200 $\mu Em^{-2} sec^{-1}$ at the leaf surface. Approx. 10 days after germination, when seedlings were at the two or three leaf stage of growth, they were sprayed with a 0.25% (w/v) emulsion of technical DDT in 0.1% (v/v) Tween 60–Me_2CO (9:1 by vol.). Seedlings used as controls were treated with 0.1% Tween 60–Me_2CO (9:1 by vol.).

Isolation of chloroplasts. Small pieces of leaf tissue (4–6 g wet wt) were homogenized in 40 ml ice-cold buffer in an MSE top-drive homogenizer operated at maximum speed for three × 10 sec. The medium was either 0.5 M sucrose and 50 mM $KHCO_3$, adjusted to pH 7.5 or, for studies of photophosphorylation, the media given in method B of ref. [27]. The homogenate was squeezed through four layers of muslin and centrifuged at 200 *g* for 90 sec, the pellet being discarded. The supernatant was centrifuged at 800 *g* for 7 min and the sedimented chloroplasts were washed and resuspended in the appropriate medium. Suspensions were maintained at 2–4° and used within 10 min of preparation. Chlorophyll was determined by measurements of $A_{645\,nm}$ and $A_{663\,nm}$ on 80% (v/v) Me_2CO extracts [28].

Assay of photosynthetic activities. Assays of DCIP or ferricyanide photoreduction, and of cyclic photophosphorylation or non-cyclic photophosphorylation, at saturating light intensity were as in ref. [4]. Preparation of Tris-washed chloroplats was as in ref. [5], using 0.5 M Tris in the washing medium. Photoreductions of $NADP^+$ involving photosystem 1 alone or both photosystems were as in ref. [5] except Porphyra umbilicalis ferredoxin was used. Assays of photooxidations of DCIP, DAD and TMPD by the oxygen electrode technique were as in ref. [5].

When DPC was used as electron donor over the pH range 6.0–9.0 and DCIP as electron acceptor the reaction cuvette contained KH_2PO_4, 70 μmol; sucrose, 500 μmol; DCIP, 0.16 μmol; DPC, 1.5 μmol and chloroplasts equivalent to about 40 μg chlorophyll, in a total volume of 3.0 ml.

Cuvettes were illuminated for a series of 30 sec intervals, the $A_{620\,nm}$ being measured initially, and after each illumination, against a control lacking DCIP. The rate of DCIP photoreduction was determined from the time interval over which the reduction proceeded linearly. The $A_{620\,nm}$ of DCIP at pH 6 was taken as 13 400 l. mol^{-1} cm^{-1} [5]. Cuvettes supplemented with 17 nmol DCMU were used to check that electron flow through photosystem 2 accounted for the photoreduction of DCIP.

REFERENCES

1. Wiebe, G. A. and Hayes, J. D. (1960) *Agron. J.* **52**, 685.
2. Jones, J. M. and Hayes, J. D. (1967) *Plant Pathol.* **16**, 139.
3. Briggle, L. W. (1964) *Crop Sci.* **4**, 457.
4. Owen, W. J., Rogers, L. J. and Hayes, J. D. (1975) *J. Exp. Botany* **26**, 692.
5. Owen, W. J., Delaney, M. E. and Rogers, L. J. (1977) *J. Exp. Botany* **28**, 986.
6. Delaney, M. E., Owen, W. J. and Rogers, L. J. (1977) *J. Exp. Botany* **28**, 1153.
7. Vernon, L. P. and Shaw, E. R. (1969) *Plant Physiol.* **44**, 1645.
8. Blankenship, R. E. and Sauer, K. (1974) *Biochim. Biophys. Acta* **357**, 252.
9. Velthuys, B. R. (1980) *Annu. Rev. Plant Physiol.* **31**, 545.
10. Moreland, D. E. (1980) *Annu. Rev. Plant Physiol.* **31**, 597.
11. Heathcote, P. and Hall, D. O. (1975) *Biochem. Biophys. Res. Commun.* **56**, 767.
12. Avron, M. (1977) *Annu. Rev. Biochem.* **46**, 143.
13. Delaney, M. E., Jones, M. and Rogers, L. J. (1978) *J. Exp. Botany* **29**, 25.
14. Moreland, D. E. and Novitzky, W. (1984) *Z. Naturforsch.* **39c**, 329.
15. England, R. R. and Evans, E. H. (1983) *Biochem. J.* **210**, 473.
16. Barr, R. and Crane, F. L. (1982) *Biochim. Biophys. Acta* **681**, 139.
17. Barr, R., Troxel, K. S. and Crane, F. L. (1980) *Biochem. Biophys. Res. Commun.* **92**, 206.
18. Suwanketnikorn, R., Hatzios, K. H., Penner, D. and Bell, D. (1982) *Can. J. Botany* **60**, 402.
19. Oettmeier, W. and Masson, K. (1982) *Eur. J. Biochem.* **122**, 163.
20. Pauly, G., Douce, R. and Carde, J.-P. (1981) *Z. Pflanzenphysiol.* **104**, S199.
21. Pfister, K., Lichtenthaler, H. K., Burger, G., Musso, H. and Zahn, M. (1981) *Z. Naturforsch.* **36c**, 645.
22. Draber, W., Knops, H. J. and Trebst, A. (1981) *Z. Naturforsch.* **36c**, 848.
23. Droppa, M., Dementer, S. and Horvath, G., (1981) *Z. Naturforsch.* **36c**, 853.
24. Oettmeier, W. (1981) *Z. Naturforsch.* **36c**, 1024.
25. Hirschberg, J., Bleecker, A. and Kyle, D. J. (1984) *Z. Naturforsch.* **39c**, 412.
26. Hirschberg, J. and McIntosh, L. (1983) *Science* **222**, 1346.
27. Plesnicar, M. and Bendall, D. S. (1973) *Biochem. J.* **136**, 803.
28. Arnon, D. I. (1949) *Plant Physiol.* **24**, 1.