Phytochemistry, Vol. 25, No. 5, pp. 1003–1008, 1986.
Printed in Great Britain.

0031-9422/86 $3.00 + 0.00

INHIBITION OF PHOTOSYNTHESIS BY THE ORGANOCHLORINE PESTICIDE TOXAPHENE*

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(*Received* 8 *July* 1985)

Key Word Index—*Avena* species; Gramineae; oat; camphechlor; Hill reaction; photosynthesis; toxaphene.

Abstract—Susceptibility to toxaphene, an extensively used organochlorine pesticide, was evident in five cereal types and was widespread in oat (*Avena* spp.) and barley (*Hordeum* spp.). In these cereals most of the varieties tested were susceptible, this being assessed biochemically from inhibition of the Hill reaction in chloroplasts from treated plants. Studies with the susceptible *Avena sativa* variety Blyth showed two sites of inhibition of photosynthetic electron flow. The first site was on the oxidizing side of photosystem 2, and the second in the intermediate electron transport chain between the two photosystems. As a consequence of the latter, cyclic photophosphorylation was inhibited and toxaphene may additionally have some uncoupler activity.

INTRODUCTION

The organochlorine pesticide toxaphene is widely used in a number of countries, and particularly the USA where more than 5×10^5 tons have been used since 1947 [1]. Applications to plants include major use in cotton insect control programmes and other applications in the treatment of small grains, soy beans and vegetables. It has also been used in control of external insects on livestock, and for fish eradication [2]. In the UK it has been used as a worm killer on turf and lawns, but currently has limited agricultural or horticultural application.

Toxaphene is a complex mixture of more than 200 constituents [1] with an average elemental composition of $C_{10}H_{10}Cl_8$ [2]. It is produced by chlorination of camphene to an overall chlorine content of about 68%. The major constituents are polychlorobornane isomers (76%) and polychlorobornenes (18%) with small amounts of polychlorobornadienes, chlorinated hydrocarbons and some non-chlorinated compounds [1]. Some of the major toxic constituents to insects, fish and mammals [3–5] have been isolated and identified (Fig 1). Toxicity to a photosynthetic organism, the cyanobacterium *Anacystis nidulans*, has also been shown [6].

Despite its importance in terms of usage, and in view of its apparent persistence in the environment [7] and possible health hazard to animals [4, 8, 9], little is known about the mode of action of toxaphene or of its effects against non-target organisms. However, greenhouse and field trials have shown previously that some oat (*Avena* spp.) varieties are susceptible to toxaphene [10]. Reaction was controlled by a single major gene with susceptibility conditioned by the dominant allele. Study of the F2 and F3 generations of crosses between susceptible and resistant parents suggested that there was no cytoplasmic effect on the reaction to toxaphene and that a single locus conditioned the response.

There have been a few reports of the susceptibility of plants to other insecticides. These include the reactions of sorgo to chlorinated hydrocarbons and phosphate insecticides [11] and of cereal species to 1,1,1-trichlorobis(*p*-chlorophenyl)ethane (DDT) [12, 13]. In all these, reaction to the pesticide is genetically based. Also of interest in this context is the cytoplasmic genome effect linking susceptibility of methomyl to male sterility in a

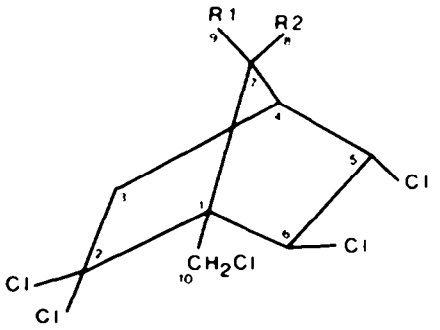

Fig. 1. Representative structure of toxaphene. Toxic components that have been identified are 2,3,5-endo,6-exo,8,9,10-heptachlorobornane where both R_1 and R_2 are $-CH_2Cl$ and the corresponding 8,8,9,10- and 8,9,9,10-octachlorobornanes.

*Part 3 in the series "Susceptibility of Cereals to Organochlorine Pesticides and Biochemical Mode of Action". For Part 2 see ref. [13].

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Abbreviations: DAD, 2,3,5,6-tetramethyl-*p*-phenylenediamine; 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; MV, methyl viologen; PMS, phenazine methosulphate; TMPD, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine.

maize (*Zea mays*) variety [14]. Only in the case of susceptibility to DDT in barley (*Hordeum* spp.) [12] and rye (*Secale* spp.) [13] has the mode of action of the pesticide been elucidated. The biochemical basis of the susceptibility of oat to toxaphene has now been defined.

RESULTS AND DISCUSSION

Using inhibition of photosynthetic electron flow as an index of susceptibility the responses to toxaphene of a wide range of varieties of five cereals were examined. Two days after treatment, and before visible leaf damage was evident, leaves of seedlings were harvested, chloroplasts isolated, and rates of DCIP and ferricyanide photoreductions determined. Varieties where chloroplasts from treated plants showed an inhibition of photosynthetic electron flow of about 40% or more were classed as susceptible. On this basis almost all the barley and oat, and about half the rye varieties tested, were susceptible. In contrast, maize and wheat (*Triticum* spp.) varieties were with few exceptions resistant to toxaphene. The data from this extensive survey is to be summarized elsewhere, and subsequent studies were concentrated on the response of oat to the pesticide (Table 1). This survey was based on a selection from the US Department of Agriculture Small Grains Collection, supplemented by several UK commercial varieties. All but two of the varieties were susceptible, with parallel inhibitions of DCIP and ferricyanide photoreductions. The two exceptions were the varieties Moregrain and New Nortex, which gave an intermediate response. For Moregrain, DCIP photoreduction was inhibited 27% and ferricyanide photoreduction was inhibited 8%, while for New Nortex, the corresponding inhibitions were 33% and 16%, respectively. Supplies of seeds were insufficient to permit repeated tests. These two varieties were classed as resistant in the earlier field trials defining the genetic basis of the response to toxaphene [10]. Possibly in the present studies, which used seedlings at the two- to three-leaf stage, toxaphene may have caused some generalized membrane damage unrelated to the gene effect. The earlier studies [10] were with plants nearing maturity and the distinction between susceptibility and resistance was based on observations of visible damage to the sprayed leaves. In susceptible plants leaves became salmon-coloured some 3 days after spraying and leaf tissue contacted by the spray subsequently became brown and died.

Studies on the biochemical mode of action of toxaphene were confined to a single susceptible variety, Blyth, one of the commercially available UK varieties. To define the most appropriate time after spraying to harvest leaves, the time-course of inhibition of the Hill reaction over an 8 day period was followed (Fig. 2). This showed that inhibition of photosynthesis was a maximum at 2–4 days after spraying and thereafter there was a slow recovery. For subsequent studies seedlings were harvested usually 2 days after spraying with a 2% v/v emulsion of toxaphene or the control spray. The recovery in chloroplast function might suggest that some detoxication of toxaphene occurs. Metabolism of toxaphene by dechlorination and dehydrochlorination has been shown to occur in mammals [15, 16] and a bacterium [17]. It is also relevant that these studies were made on plants maintained in growth chambers at light intensities which were low compared to natural daylight. Under these conditions the excitation energy absorbed by the photosynthetic pigments which, because of inhibition by toxaphene, could not be used for photosynthetic electron flow, is presumably dissipated effectively by the carotenoid protective system. At high intensities this might not be the case and cellular deteriorative processes [18] could result in death of the seedling despite any partial detoxication of the pesticide.

Studies using DPC as an artificial electron donor to the oxidizing side of photosystem 2 and DCIP as electron acceptor showed that toxaphene inhibited electron transport in this region. In these experiments the chloroplasts were washed in high concentrations of Tris, a treatment which abolished electron flow from the physiological donor [19]. An experiment to locate the site of inhibition of toxaphene exploiting previous observations that the site of interaction of DPC is dependent on pH [12, 13] is

Table 1. Susceptibility of oat varieties to toxaphene as assessed by inhibition of the Hill reaction in chloroplasts from treated seedlings

Susceptible		Intermediate reaction
Algerian	Pinto	Moregrain
Banner	Saladin	New Nortex
Blyth	Silensia	
Fyne	Trafalgar	
Gentry HS 14160	CD 3994	
Illinois Hull-less	PI 203450	
Koelz W9478	PI 234842	
Leanda	PI 266827	
Maris Oberon	PI 355005	
Maris Tabard	PI 361866	
Orlando	PI 361912	

PI numbers are those designating unnamed varieties in the Small Grains Collection. For susceptible varieties the inhibition for DCIP photoreductions was 51 ± 9%, and for ferricyanide photoreduction was 48 ± 9%.

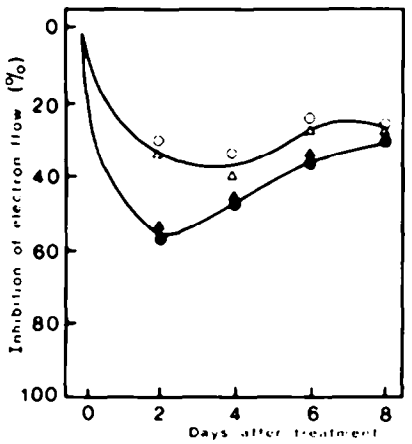

Fig. 2. Susceptibility of oat to toxaphene. Seedlings were treated with 1% v/v toxaphene (open symbols) or 2% v/v toxaphene (closed symbols) at day 0. At intervals up to 8 days chloroplasts were isolated from toxaphene-treated seedlings and rates of DCIP (○, ●) and ferricyanide (△, ▲) photoreduction compared with those for chloroplasts from untreated seedlings (*ca* 150 μmol dye reduced/hr/mg chlorophyll).

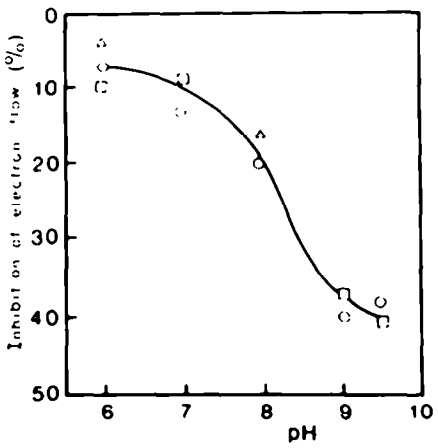

Fig. 3. Effect of pH on the site of electron donation by DPC in relation to a site of inhibition by toxaphene. Rates are given as per cent inhibition by toxaphene compared with data for DCIP photoreduction using DPC as donor at the same pH in chloroplasts from untreated plants. In controls not subjected to Tris-washing, electron transport from H_2O was inhibited some 70% in chloroplasts from toxaphene-treated seedlings. The symbols represent results from three separate experiments. For chloroplasts from untreated seedlings the rates of electron transport (μmol DCIP reduced/hr/mg chlorophyll) for H_2O as donor were about 110 at pH 6 and 100 at pH 9, and for DPC as donor were about 130 at pH 6 and 80 at pH 9.

shown in Fig. 3. At pH 9 there was a 40% inhibition of electron flow from DPC to DPIC in chloroplasts from toxaphene-treated susceptible oat. This was comparable to inhibitions of about 75% for electron flow from the physiological donor before Tris-washing. At pH 6, however, there was only a slight inhibition of electron flow when DPC was used as the electron donor. In the control experiments there was an apparent increase in inhibition by toxaphene of electron flow from water as the pH was increased from 60% at pH 6 to some 80% at pH 9.5. Since rates of electron flow in untreated chloroplasts were similar over this pH range the increased inhibition at the higher pH values may be due to some adverse effect on photosystem 2 by toxaphene not related to the specific mode of action of the pesticide. Increased inhibition at high pH values was not observed in comparable experiments with chloroplasts from DDT-treated susceptible rye [13]. Nevertheless, these data identify clearly a site of inhibition by toxaphene on the oxidizing side of photosystem 2. The site of inhibition is similar to that characterizing the action of another organochlorine insecticide, DDT, on susceptible barley [12] and rye [13], which suggests that a similar lamellar component may be involved. However, oat varieties susceptible to toxaphene are resistant to DDT. Whether the site on the oxidizing side of photosystem 2 is on a pathway of photosystem 2 cyclic electron transport involving cyt b559 [20], and involves a site for photophosphorylation [21] would be of interest. Although a successful assay system for this activity was not established for oat chloroplasts, an inhibition by toxaphene of cyclic photosystem 1 phosphorylation could be demonstrated clearly, as could the inhibition of non-cyclic photophosphorylation coupled to ferricyanide photoreduction. These data are given in Table 2. While electron transport was inhibited some 35% in these experiments, the concomitant non-cyclic photophosphorylation was inhibited more severely. This suggests that toxaphene has an uncoupling effect in addition to its action as an inhibitor of electron transport, and could therefore be classed as an inhibitory uncoupler [22]. However, toxaphene is complex in terms of chemical composition and different components may contribute differently to these two effects. PMS-catalysed photosystem 1 cyclic photophosphorylation was also inhibited, but only by 30%; this corresponds to the uncoupling attributed to toxaphene in non-cyclic photophosphorylation. However, this inhibition of photosystem 1 cyclic photophosphorylation might also be due to a second site of inhibition by toxaphene of electron flow. This possibility was investigated by using DPC as an electron donor at pH 6.5, so by-passing the inhibition site on the oxidizing side of photosystem 2, using $NADP^+$ as electron acceptor on the reducing side of photosystem 1. These data (Table 3) showed that electron transport from DPC to $NADP^+$ was inhibited by some 20% and suggest that a site of inhibition associated with photosystem 1 may be present. However, the inhibition is less than that seen for the site before photosystem 2, shown by the accompanying study of electron transport from water to DCIP.

Confirmation of this second inhibition site came from studies using DCIP, TMPD or DAD, in the presence of ascorbate as the secondary reductant, as electron donors to photosystem 1 and MV or $NADP^+$ as electron acceptor. Assays were in the presence of DCMU to inhibit photosystem 2. With $NADP^+$ as acceptor, electron transport from both TMPD and DAD were inhibited by about 30%, whereas no inhibition in electron flow from DCIP to $NADP^+$ was evident (Table 4). Results with MV

Table 2. Photophosphorylation by chloroplasts isolated from toxaphene-treated susceptible oat (var. Blyth)

	Non-cyclic photophosphorylation						Cyclic photophosphorylation		
	$Fe(CN)_6$ photoreduction			Pi esterification			Pi esterification		
Days after treatment	UT	T	% inhibition	UT	T	% inhibition	UT	T	% inhibition
2	260	86	67	600	395	34	670	450	33
4	240	85	65	730	460	37	790	550	30

Chloroplasts were isolated 2 or 4 days after treating seedlings with toxaphene (T) or a control spray (UT). Rates are μmol Pi esterified or acceptor reduced/hr/mg chlorophyll.

Table 3. Electron transport from DPC to NADP⁺ or DCIP in Tris-washed chloroplasts from susceptible oat

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	$NADP^+$	$NADP^+$
Toxaphene-treated	47	12	7	125	4	16
Untreated	100	24	10	135	4	20
% inhibition	53	50	—	7	—	20

Rates are expressed as μmol DCIP or $NADP^+$ reduced/hr/mg chlorophyll.

Table 4. Effect of toxaphene on photosystem 1 activity in susceptible oat with $DCIPH_2$, TMPD and DAD as electron donors and MV or $NADP^+$ as electron acceptor

Electron donor	$DCIPH_2$			TMPD			DAD		
Electron acceptor	MV	MV	$NADP^+$	MV	MV	$NADP^+$	MV	MV	$NADP^+$
pH	7.5	8	8	7.5	8	8	7.5	8	8
Toxaphene-treated	165	160	10	435	375	9	515	450	12
Untreated	175	180	9	480	535	13	605	735	18
% inhibition	6	11	—	9	30	31	15	39	33

Rates are expressed as μmol acceptor reduced/hr/mg chlorophyll. In the chloroplasts used there was a 40% inhibition of photosystem 2 activity as determined by DCIP photoreduction.

as electron acceptor at the same pH value (pH 8) were in good agreement. These data suggest that TMPD and DAD both supply electrons to a carrier on the oxidizing side of a site of inhibition by toxaphene in the intermediate electron transport chain, whereas electron donation from DCIP is on the reducing side of this site. At a somewhat lower pH, electron donation from TMPD or DAD is only inhibited by an amount arguably within experimental variation. Under these conditions, therefore, all three donors by-pass the second site of toxaphene inhibition.

There is striking similarity between these data and those reported for the effect of DDT on photosynthetic electron flow [12, 13]. In both cases two sites of inhibition with similar characteristics are present. Two differences are, however, evident. The site of inhibition by toxaphene in the intermediate electron transport chain in oat is to a significant extent by-passed by electron donations from TMPD and DAD at pH 7 (data not shown) and pH 7.5 (Table 4). This site of inhibition by DDT in susceptible rye [13] is seen at pH 7 as well as at pH 8. Studies of cytochrome responses suggested that the site of DDT inhibition in barley [22] was on the oxidizing side of cytochrome *f*. This may suggest that the site of inhibition in susceptible oat is further from cytochrome *f* than that in susceptible rye, possibly at the DBMIB site. The conclusions from studies of photosynthetic electron flow regarding sites of inhibition by toxaphene are given in Fig. 4. The second difference is that in the case of DDT the inhibition at the two sites was comparable, whereas for toxaphene the inhibition at the site in the intermediate electron transport chain in the various assays, including photosystem 1 cyclic photophosphorylation, only exceeded 30% on one occasion, compared with significantly higher inhibition for the site on the oxidizing side of photosystem 2. The sites may therefore differ in accessibility to the active constituent(s) of toxaphene. A single major gene is involved in both the reactions of barley and rye to DDT, and of oat to toxaphene. The idea that the two sites involve the same membrane component would be consistent with the available genetic and biochemical information. However, in responses to the

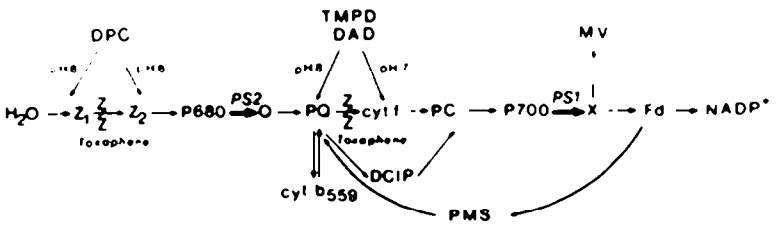

Fig. 4. Sites of inhibition of photosynthetic electron flow by toxaphene in relation to interaction of natural and artificial electron donor and acceptor systems. P_{680} and P_{700} are the reaction centres for photosystem 2 (PS2) and photosystem 1 (PS1), respectively, with primary electron acceptors Q and X. Z_1 and Z_2 are hypothetical sites; PQ, plastoquinone; PC, plastocyanin; Fd, ferredoxin.

two pesticides, different membrane components or different sites on the same component are involved since no oat varieties studied in a recent survey (data not presented) was susceptible to DDT. In contrast, responses of barley varieties to the two pesticides were very similar. The unusual nature of these sites of inhibition compared to other compounds acting on photosynthetic electron flow has been discussed for DDT in an earlier paper [13].

Instances of inhibition at two sites in photosynthetic electron flow, first reported for DDT [12], are still uncommon. They include sulphydryl reagents [23] and a calcium chelator [24] where DPC (pH 8) → DCIP electron transport is inhibited and also TMPD → MV. For sulphydryl reagents electron flow from DPC to DCIP at pH 6 was not inhibited. There are evident similarities in the interaction of these reagents and the organochlorine pesticides with the electron transport pathway. Stigmatellin, an antibiotic from a myxobacterium, inhibits photosynthetic electron transport at two sites in the intermediate electron transport chain [25]. These interactions appear to be at the Q_B protein region (DCMU site) and the cyt *f*–cyt b_{559} complex (DBMIB site); this latter may correspond to the site of interaction of toxaphene.

Most of the herbicides which affect photosynthetic electron transport act either at the DCMU site or the DBMIB site [26]. However, recent studies [27, 28] have shown that the phenolic herbicides in addition to their well-documented interaction at the DCMU site also inhibit on the donor side of photosystem 2. This latter site is characterized in Tris-washed chloroplasts by formation of a carotenoid radical cation [28]. Phenolic herbicides are thought to interact with a 41 kD polypeptide associated with the photosystem 2 reaction centre, and it is possible that toxaphene may also interact with this or a related polypeptide in the thylakoid membrane.

Toxaphene is derived by chlorination of camphene, which is manufactured from pinene, the major monoterpene in pine needles. Pinene itself has been shown to inhibit electron transport in spinach chloroplasts between photosystem 2 and photosystem 1, and also to have an uncoupling action [29]. Action at, or near, the plastoquinone site was suggested, since the site of inhibition was before the site of electron donation from DCIP or TMPD. However, the pH for these experiments was not quoted and so comparison with the data for toxaphene is not possible. Silicomolybdate photoreduction in the presence of DCMU with water as electron donor was not inhibited, indicating no site of inhibition on the oxidizing side of photosystem 2. These experiments involved adding pinene directly to isolated spinach chloroplasts, and under these conditions it may not readily penetrate to the photolytic side on the inside of the thylakoid membranes. It would be of interest to see if those cereal varieties susceptible to toxaphene are also susceptible to pinene, and if the mode of action of chloroplasts isolated from pinene-treated seedlings is similar. Pinene also inhibited respiratory electron flow in spinach mitochondria [30]. It is likely that toxaphene has an effect on other biological membranes but this has not been investigated.

EXPERIMENTAL

Treatment of plants with toxaphene. Seeds of *Avena* spp. were sown in John Innes No. 2 compost and grown in an environmental growth chamber under a 16 hr day regime (day temp. 23°, night temp. 17°) with fluorescent and tungsten lighting giving a photosynthetic photon flux density of 200 $\mu E/m^2/sec$ at the leaf surface. *Ca* 10 days after germination, when seedlings were at the two to three leaf stage of growth, they were sprayed with a 2% (v/v) emulsion of toxaphene in 0.1% (v/v) Tween 60. Toxaphene was supplied as Toxaphene 6E, an emulsifiable concentrate containing 6 lb of toxaphene per US gal (72% w/v formulation with 7.5% nonionic and anionic surfactants in a mixture of isomeric forms of $C_6H_4Me_2$. Seedlings used as controls were treated with 0.1% Tween 60–$C_6H_4Me_2$ (99:1 by vol.).

Assay of photosynthetic activities. Chloroplasts were isolated as in ref. [13]. Assays of DCIP or ferricyanide photoreduction, and of cyclic and non-cyclic photophosphorylation, at saturating light intensity were as in ref. [31]. Preparation of Tris-washed chloroplasts was as in ref. [12], using 0.5 M Tris in the washing medium. Photoreductions of $NADP^+$ involving photosystem 1 alone or both photosystems were as in ref. [12] except *Porphyra umbilicalis* ferredoxin was used. Assays of photooxidations of DCIP, DAD and TMPD by the oxygen electrode technique were as in ref. [12]. Assay of electron donation from DPC to DCIP over the pH range 6.0–9.5 was as in ref. [13].

Acknowledgements—We thank the Crops Research Division, US Department of Agriculture, Beltsville, Maryland, U.S.A. for supplying seed samples, and FBC Limited, Chesterford Park Research Station, Saffron Walden, Essex for toxaphene. S.A. was in receipt of a Government of Pakistan Scholarship.

REFERENCES

1. Saleh, M. A. (1983) *J. Agric. Food Chem.* **31**, 748.
2. Holmstead, R. L., Khalifa, S. and Casida, J. E. (1974) *J. Agric. Food Chem.* **22**, 939.
3. Saleh, M. A. and Casida, J. E. (1979) *Adv. Pestic. Sci.* **3**, 562.
4. Turner, W. V., Khalifa, S. and Casida, J. E. (1975) *J. Agric. Food Chem.* **23**, 991.
5. Turner, W. V., Engel, J. L. and Casida, J. E. (1977) *J. Agric. Food Chem.* **25**, 1394.
6. Nelson, J. O. and Matsumura, F. (1975) *J. Agric. Food Chem.* **23**, 984.
7. Nash, R. G., Harris, W. G., Ensor, P. D. and Woolson, E. A. (1973) *J. Assoc. Off. Anal. Chem.* **56**, 728.
8. National Cancer Institute Carcinogenesis Technical Report Series 37 (1979). Department of Health, Education and Welfare, Washington, DC, Publ. No. 79-837.
9. Ribick, M. A., Dubay, G. R., Petty, J. D., Stalling, D. L. and Schmitt, C. J. (1982) *Environ. Sci. Technol.* **16**, 310.
10. Gardenhire, J. H. and MacDaniel, M. E. (1970) *Crop Sci.* **10**, 299.
11. Coleman, O. H. and Dean, J. L. (1964) *Crop Sci.* **4**, 371.
12. Owen, W. J., Delaney, M. E. and Rogers, L. J. (1977) *J. Exp. Botany* **28**, 986.
13. Akbar, S. and Rogers, L. J. (1985) *Phytochemistry* **24**, 2791.
14. Koeppe, D. E., Cox, J. K. and Malone, C. P. (1978) *Science* **201**, 1227.
15. Saleh, M. A., Skinner, R. F. and Casida, J. E. (1979) *J. Agric. Food Chem.* **27**, 731.
16. Chandurkar, P. S. and Matsumura, F. (1979) *Arch. Environ. Contam. Toxicol.* **8**, 1.
17. Clark, J. M. and Matsumura, F. (1979) *Arch. Environ. Contam. Toxicol.* **8**, 285.
18. Dodge, A. D. (1983) in *Progress in Pesticide Biochemistry and Toxicology* (Hutson, D. H. and Roberts, T. R., eds) Vol. 3, pp. 163–197. John Wiley, Chichester.
19. Vernon, L. P. and Shaw, E. R. (1969) *Plant Physiol.* **44**, 1645.
20. Mende, D. (1980) *Plant Sci. Letters* **17**, 215.

21. Yocum, C. F. (1977) *Plant Physiol.* **60**, 592.
22. Delaney, M. E., Jones, M. and Rogers, L. J. (1978) *J. Exp. Botany* **29**, 25.
23. Barr, R. and Crane, F. L. (1982) *Biochim. Biophys. Acta* **681**, 139.
24. Barr, R., Troxel, K. S. and Crane, F. L. (1980) *Biochem. Biophys. Res. Commun.* **92**, 206.
25. Oettmeier, W., Godde, D., Kunze, B. and Hofle, G. (1985) *Biochim. Biophys. Acta* **807**, 216.
26. Moreland, D. E. and Novitzky, W. (1984) *Z. Naturforsch.* **39c**, 329.
27. Pfister, K. and Schreiber, U. (1984) *Z. Naturforsch.* **39c**, 389.
28. Mathis, P. and Rutherford, A. W. (1984) *Biochim. Biophys. Acta* **767**, 217.
29. Pauly, G., Douce, R. and Carde, J.-P. (1981) *Z. Pflanzenphysiol.* **104S**, 199.
30. Douce, R., Neuberger, M., Bligny, R. and Pauly, G. (1978) in *Plant Mitochondria* (Ducet, G. and Lance, C., eds) pp. 207–214. Elsevier/North Holland Biomedical Press, Amsterdam.
31. Owen, W. J., Rogers, L. J. and Hayes, J. D. (1975) *J. Exp. Botany* **26**, 692.