EFFECTS OF INHIBITORS AND DIVERTERS OF PHOTOSYNTHETIC ELECTRON TRANSPORT ON HERBICIDE RESISTANT AND SUSCEPTIBLE WEED BIOTYPES

By

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ABSTRACT

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Herbicide cross resistance was evaluated in triazine resistant biotypes of four species. Triazine resistant smooth pigweed, common lambsquarters, common groundsel, and rapeseed were also resistant to bromacil and pyrazon, moderately cross resistant to buthidazole, and more susceptible to dinoseb. Atrazine resistant smooth pigweed was also resistant to cyanazine and metribuzin, moderately cross resistant to linuron and desmedipham, and not resistant to diuron, bromoxynil, bentazon or dicamba. Resistance at the whole plant level was highly correlated with resistance at the level of photosynthetic electron transport.

The physiological basis for the increased susceptibility to dinoseb in triazine resistant smooth pigweed was studied. The increase in susceptibility to dinoseb could not be attributed to either a photosynthetic mechanism or to differences in carbohydrate levels in the two biotypes.

Numerous herbicide treatments were evaluated for control of triazine resistant common lambsquarters and pigweed (Amaranthus spp.) infestations. Satisfactory control of triazine resistant common lambsquarters was obtained with preemergence treatments of pendimethalin or postemergence treatments of dicamba, bromoxynil, or bentazon.

Satisfactory control of pigweed was obtained with preemergence treatments of alachlor or postemergence treatments of dicamba, bromoxynil, or 2,4-D.

The basis for alachlor protection of corn from buthidazole injury was studied. Alachlor protection was attributed to reduced uptake of buthidazole.

Two sites of inhibition of photosynthetic electron transport were characterized for buthidazole, buthidazole metabolites, and ioxynil. Buthidazole and ioxynil inhibited primarily at a site similar to atrazine or diuron. The secondary site of action of both herbicides is on the oxidizing side of photosystem II. Most buthidazole metabolites inhibited photosynthetic electron transport, but all were less active than buthidazole.

The basis for paraquat resistance in _Conyza linefolia_ was studied. Resistance was not due to an altered site of action. _In vivo_ chlorophyll fluorescence measurements indicated that paraquat was excluded from the site of action. Exclusion from the site of action was not due to differences in cuticular penetration. Autoradiograms of leaves fed ^{14}C-paraquat through the petiole suggested that resistance may be due to adsorption to the extracellular matrix and thus exclusion from the protoplast.

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CHAPTER 1

Herbicide Cross Resistance in Triazine Resistant Biotypes of Four Species

ABSTRACT

The cross resistance of triazine resistant biotypes of smooth pigweed (Amaranthus hybridus L.), common lambsquarters (Chenopodium album L.), common groundsel (Senecio vulgaris L.), and rapeseed (Brassica napus L.) to a selection of herbicides was evaluated in greenhouse studies. The triazine resistant biotypes of all four species showed a similar pattern of cross resistance. The four triazine-resistant biotypes showed resistance to injury from atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine], bromacil (5-bromo-3-sec-butyl-6-methyluracil), and pyrazon [5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone] and showed a slight resistance to buthidazole {3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxyl-1-methyl-2-imidazolidinone}. The triazine-resistant biotypes were distinctly more susceptible to dinoseb (2-sec-butyl-4,6-dinitrophenol). The triazine resistant smooth pigweed was studied in greater detail, and showed resistance to cyanazine {2-[(4-chloro-6-(ethylamino)-s-triazin-2-yl)amino]-2-methylpropionitrile} and metribuzin [4-amino-6-tert-butyl-3-(methylthio)-as-triazin-5(4H)-one], with slight resistance to linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea] and desmedipham (ethyl-m-hydroxycarbanilate carbanilate). There was little or no resistance to diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea], bromoxynil (3,5-dibromo-4-hydroxybenzonitrile), bentazon

[3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide], or dicamba (3,6-dichloro-o-anisic acid). Resistance was highly correlated whether evaluated as herbicidal injury or as inhibition of photosynthetic electron transport. This observation supports the widely held viewpoint that atrazine, cyanazine, metribuzin, pyrazon, bromacil, linuron, desmedipham, and buthidazole cause plant injury by inhibiting photosynthesis. This observation also supports the widely held viewpoint that *in vivo* triazine resistance and cross resistance is due to a decreased sensitivity at the level of photosynthetic electron transport. Subsequent studies indicated that dinoseb does not inhibit photosynthesis *in vivo* (Chapter 3). No conclusion can be made, based on these observations, concerning the cause of injury from diuron, bromoxynil, bentazon, or dicamba.

INTRODUCTION

Biological organisms which develop resistance to one biocide may simultaneously show resistance to other biocides to which they have never been exposed. This phenomenon, cross resistance, is well documented for insecticides (Brown, 1971) and fungicides (Dekker, 1976). It is reasonable to propose that the triazine resistant weeds may be cross resistant to herbicides, since so many herbicides inhibit photosynthesis at a similar site (Moreland, 1980). Cross resistance to herbicides has been measured in isolated chloroplast membranes of triazine resistant weed biotypes (Arntzen *et al.*, 1982; Pfister and Arntzen, unpublished data). In these studies, resistance was reported in members of the triazine, triazinone, pyridazinone, quinazoline, and uracil families. There was little resistance to certain members of the phenylurea and thiadiazolyl families. There was an increase in susceptibility to certain members of the nitrophenol, nitrile, benzothiadiazinone, and benzoxazinone families. (Such negatively correlated cross resistance has been reported previously in cases of insecticide resistance (Brown, 1971) and fungicide resistance (van Tuyl, 1977)). Moreover, the pattern of cross resistance was strikingly similar across several species, suggesting that the genetic basis for resistance was very similar in all species tested.

Resistance to herbicides in plants is a relatively recent development (Ryan, 1970). Numerous species have shown resistance to triazines, and this resistance has been attributed to the alteration of the triazine binding site (Arntzen _et al._, 1982). A chloroplast membrane localized polypeptide of about 32 kilodaltons (kD) has been shown to contain the atrazine binding site (Pfister _et al._, 1981; Steinback _et al._, 1981). A change in a single amino acid, from serine to glycine, is the mutation conferring resistance (Hirschberg and McIntosh, 1984) in both _A. hybridus_ and _Solanum nigrum_. Corn (_Zea mays_ L.) contains this 32 kD polypeptide and exhibits a natural and extreme tolerance to atrazine. Detoxification is the basis for this tolerance (Shimabukuro _et al._, 1971; Shimabukuro _et al._, 1970) in corn but detoxification has not yet been shown to be a primary basis for resistance in triazine resistant weeds.

The terms "resistance" and "tolerance" should be defined in a manner similar to the definitions used and accepted with reference to insecticide resistance (Brown, 1958). Thus, biocide or pesticide resistance is the capacity of a biological or pest organism to survive doses of toxicants which would be lethal to the original or normal population of the same species. "Resistance", as currently used in the entomology and phytopathology literature, refers to a stable and heritable trait. "Tolerance" is characteristic of species in which the original or normal population can survive large doses of toxicants which prove lethal to other species (e.g. corn) (Shimabukuro *et al.*, 1970). "Tolerance" also refers to cases of phenotypic adaptation, where an increased ability to survive toxicants may be acquired through environmental conditioning. For example, exposure to sublethal herbicide concentrations has been shown to increase the tolerance of corn to atrazine (Jachetta and Radosevich, 1981).

One objective of this investigation was to determine the extent of herbicide cross resistance in four triazine resistant species at the whole plant level. Herbicides were selected to represent a diversity in herbicide chemistry. A second objective was to determine whether the resistance measured in isolated chloroplast membranes is correlated with resistance *in vivo*.

MATERIALS AND METHODS

In vivo studies. Seeds of resistant and susceptible biotypes of smooth pigweed and common groundsel were obtained from Washington, common lambsquarter from Michigan, and rapeseed from Ontario, Canada. Seeds were sown in 170 ml cups in greenhouse mix soil (sand:sandy loam:peat moss, 1:1:1, pH 6.5). Commerical formulations of atrazine, bromacil, buthidazole, cyanazine, dicamba, diuron, linuron, metribuzin, and pyrazon were applied preemergence in a spray volume of 150 L/ha and pots were gently watered from the top with approximately 1 cm of water after application. Pots were subirrigated individually and plants were thinned to five per pot (or four per pot for rapeseed) after emergence. Plants were grown in a greenhouse at 26 ± 4°C with supplemental lighting 14/10h, light/dark, supplied by sodium vapor lamps with a photosynthetic photon flux density of 100-200 $\mu E \cdot m^{-2} \cdot sec^{-1}$. Shoot fresh weights were determined at the two to three-leaf stage of the control treatment. Bentazon, bromoxynil, desmedipham, and dinoseb were applied postemergence in a spray volume of 150 L/ha with 0.5% v/v alkylaryl polyoxyethyleneglycol-free fatty acid-isopropanol surfactant (X-77 Spreader) at the two leaf plant growth stage. Dinoseb was applied no more than 2 h after the onset of illumination. Shoot fresh weights were determined 2 days after application. For each herbicide, at least five herbicide rates were applied; these rates were on a logarithmic scale. Rates were selected based upon preliminary experiments. There were six replications of each treatment. Herbicide rates required for 50% injury (I_{50}, measured as reduction in fresh weight) were estimated using linear regression of logit-transformed observations (Finney, 1979). The sigmoid dose-response curves shown (Figure 1) were modeled by this logit regression method. Resistance ratios were then estimated by dividing the I_{50} for the resistant biotype by the I_{50} of the susceptible biotype. Thus, the resistance ratio is a measure of the degree of resistance. Each experiment was repeated; the values are reported with two significant digits in Tables 1 and 2. Combined analysis across species (Figure 3) was done with log-transformed data, using each species as one replication.

<u>In vitro studies</u>. Chloroplast thylakoid membranes were isolated from susceptible and resistant biotypes and rates of photosynthetic electron transport determined over a wide range of herbicide concentrations by monitoring photoreduction of dichlorophenol-indophenol (DCPIP) (Paterson and Arntzen, 1982). The assay mixture was preincubated with the herbicide for 10 minutes prior to assay, since some herbicides achieve maximal activity slowly. Herbicide concentrations which caused 50% inhibition

(I_{50}) and resistance ratios were computed as previously described and are reported with two significant digits in Tables 3 and 4.

RESULTS AND DISCUSSION

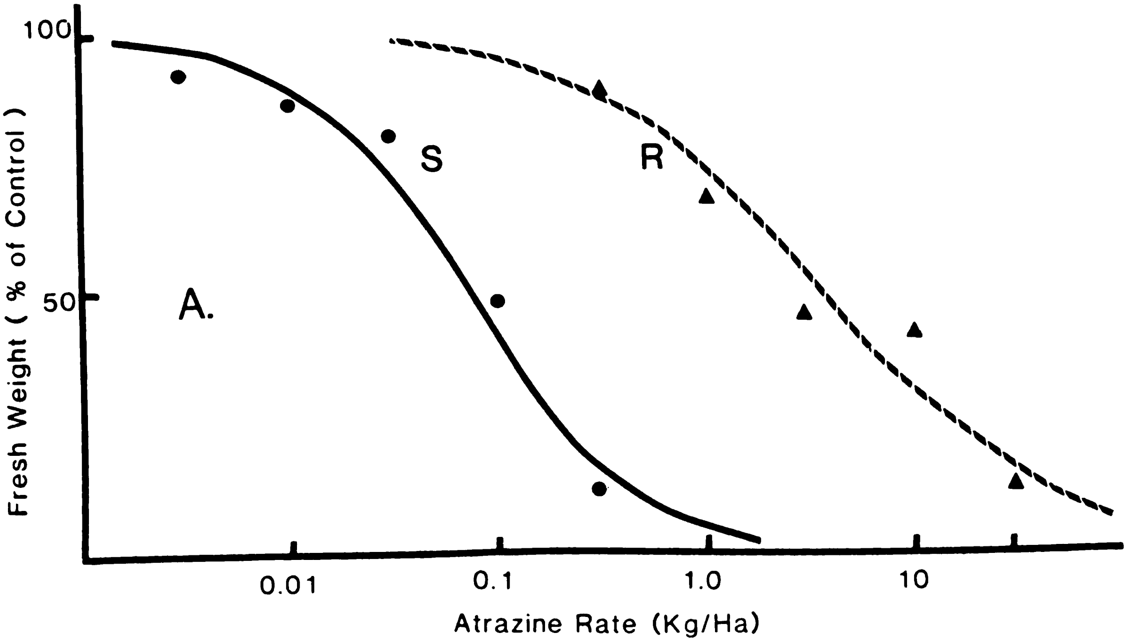
In vivo resistance. The four triazine resistant species were extremely resistant to atrazine and showed cross resistance to bromacil, pyrazon, and buthidazole (Figure 2). In vivo resistance ratios for pyrazon and bromacil were smaller than the resistance ratios for atrazine, but larger than the resistance ratios for buthidazole (except in rapeseed). Increased susceptibility to dinoseb (negatively correlated cross resistance) was observed in all four species (Figure 2). Cross resistance to cyanazine, metribuzin, linuron, and desmedipham was observed in triazine resistant smooth pigweed (Figure 4). The resistance ratios for linuron and desmedipham were much smaller than resistance ratios for cyanazine or metribuzin. Resistance to cyanazine and metribuzin was expected since they are members of the triazine and triazinone families, respectively, and are structurally related to atrazine. I_{50} values for the herbicides evaluated (Table 1) were generally far lower than rates which provide weed control in the field. It would be misleading to assume that injury to these species would occur at equivalent rates under field conditions.

The relatively large degree of resistance to pyrazon and bromacil (Figure 2) suggests that weed control problems may be anticipated if these herbicides are used where the triazine resistant weed species are present. Also, it seems reasonable to test the value of these herbicides as alternatives to the highly persistent triazines in the newly developing triazine resistant crops, such as the rapeseed studied here.

The increased susceptibility to dinoseb implies that this compound would be ideal for control of triazine resistant weeds if sufficient crop tolerance existed. Field studies have examined this potential in common lambsquarters and two pigweed species (Chapter 3). However, problems with using dinoseb for control of triazine resistant weeds include high mammalian toxicity (Anonymous, 1979) and limited label clearances for selective weed control in corn (Anonymous, 1981). Also, the efficacy of dinoseb on triazine resistant smooth pigweed (and presumably other species as well) was greatest when applied in the early morning (Chapter 2) on sunny days (personal observation). However, dinoseb and other herbicides showing negatively correlated cross resistance (Arntzen _et al._, 1982; Pfister and Arntzen, unpublished data) may provide as useful models for the future development of safe, highly selective new herbicides targeted at the triazine resistant weeds.

Correlation in vivo and in vitro resistance ratios. There was generally a close correlation of resistance ratios observed _in vivo_ and _in vitro_ (Figures 2-4). This correlation indicates that inhibition of photosynthetic electron transport is probably the primary biochemical basis for herbicidal injury from atrazine, cyanazine, metribuzin, pyrazon, bromacil, linuron, desmedipham, and buthidazole. Another mechanism appears to be involved in the case of dinoseb (Chapter 2). This correlation also indicates that resistance to these herbicides is due to a decreased sensitivity at the level of photosynthetic electron transport. However, this correlation alone is not direct proof that inhibition of photosynthesis is the primary or sole site of action. The resistance ratios observed _in vivo_ were consistently closer to 1 than the the resistance ratios observed _in vitro_ and were significantly smaller in the case

Figure 1. Response of common lambsquarters to atrazine: A. growth reduction, measured as fresh weight, as a preemergence treatment in seedlings; B. inhibition of photosynthetic electron transport, measured as photoreduction of DCPIP.


100
50
Fresh Weight (% of Control)
S
R
A.
0.01
0.1
1.0
10
Atrazine Rate (Kg/Ha)

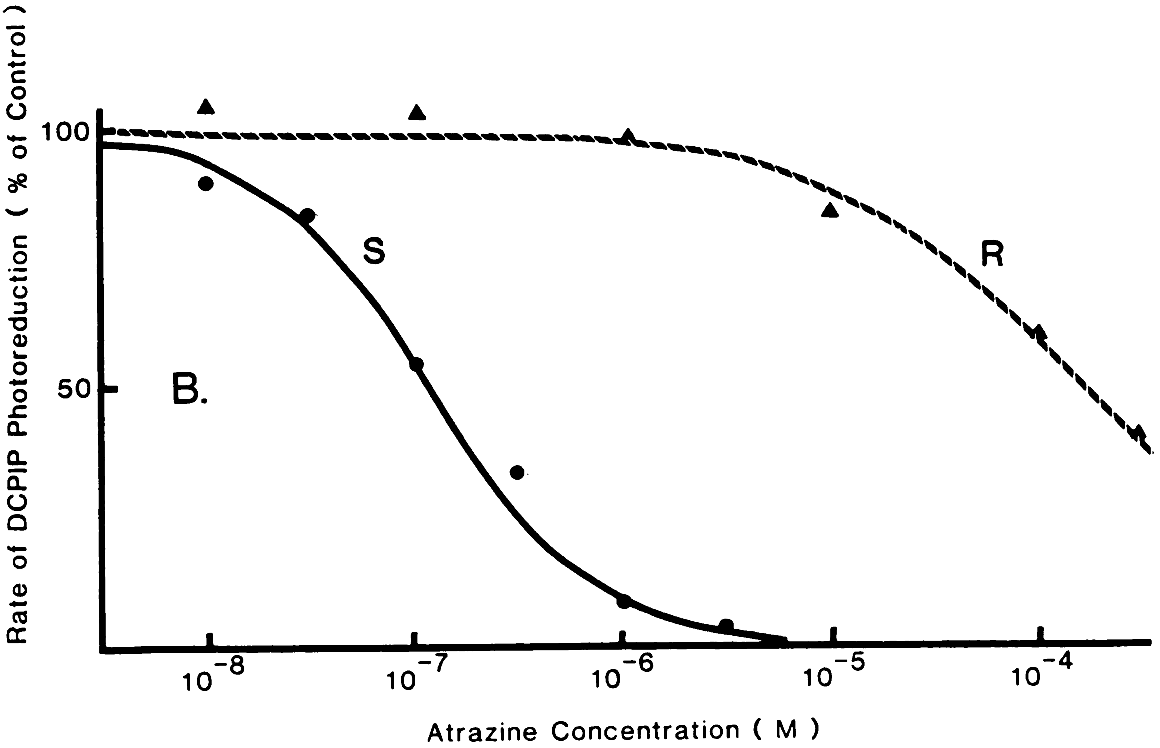

Rate of DCPIP Photoreduction (% of Control)
100
50
S
R
B.
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
Atrazine Concentration (M)

Table 1. Whole plant resistance ratios and I_{50} values (kg/ha) for atrazine, bromacil, pyrazon, buthidazole, and dinoseb.

		Amaranthus hybridus L.			*Chenopodium album* L.			*Brassica napus* L.			*Senecio vulgaris* L.		
Herbicide	Experiment	Resistant Biotype	Susceptible Biotype	Resistance Ratio	Resistant Biotype	Susceptible Biotype	Resistance Ratio	Resistant Biotype	Susceptible Biotype	Resistance Ratio	Resistant Biotype	Susceptible Biotype	Resistance Ratio
		---(I_{50} kg/ha)--			---(I_{50} kg/ha)--			---(I_{50} kg/ha)--			---(I_{50} kg/ha)--		
Atrazine	1	5.3	0.010	530	2.7	0.055	49	310	0.35	890	35	0.024	1,500
	2	11	0.025	440	3.7	0.075	50	42	0.25	170	310	0.200	1,600
Pyrazon	1	3.9	0.46	8.4	3.9	0.26	15	9.6	1.2	8.1	2.7	0.61	4.4
	2	3.2	0.11	28	20	1.7	12	20	4.1	4.8	12	0.50	24
Bromacil	1	0.64	0.024	28	0.095	0.021	4.5	0.45	0.044	10	0.37	0.047	7.8
	2	0.28	0.015	19	0.97	0.28	3.5	0.88	0.22	3.9	1.1	0.089	12
Buthidazole	1	0.12	0.057	2.0	0.028	0.020	1.4	0.18	0.029	6.1	0.067	0.027	2.6
	2	0.040	0.025	1.6	0.15	0.12	1.3	0.95	0.12	8.2	0.13	0.062	2.1
Dinoseb	1	0.032	0.083	0.39	0.056	0.36	0.16	0.25	0.42	0.60	0.054	0.42	0.13
	2	0.12	0.87	0.14	0.073	0.19	0.39	0.046	0.064	0.71	0.61	2.1	0.29

Table 2. Whole plant resistance ratios and I_{50} values (kg/ha) in smooth pigweed for the herbicides indicated.

		Amaranthus hybridus L.		
Herbicide	Experiment	Resistant Biotype	Susceptible Biotype	Resistance Ratio
		-------(I_{50} kg/ha)------		
Cyanazine	1	3.4	0.054	63
	2	4.3	0.028	160
Metribuzin	1	0.62	0.016	39
	2	0.55	0.042	13
Linuron	1	0.26	0.062	4.1
	2	0.026	0.010	2.4
Desmedipham	1	0.12	0.079	1.51
	2	0.19	0.044	4.32
Dicamba	1	0.029	0.021	1.4
	2	0.042	0.041	1.0
Bromoxynil	1	0.10	0.097	1.1
	2	0.29	0.27	1.1
Diuron	1	0.081	0.097	0.84
	2	0.030	0.029	1.0
Bentazon	1	0.39	0.44	0.90
	2	0.21	0.23	0.88

Table 3. Chloroplast I_{50} values (M) for atrazine, bromacil, pyrazon, buthidazole, and dinoseb in the four species studied.

	Amaranthus hybridus L.		*Chenopodium album* L.		*Brassica napus* L.		*Senecio vulgaris* L.	
Herbicide	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible
	--------(I_{50} M)--------		--------(I_{50} M)--------		--------(I_{50} M)--------		--------(I_{50} M)--------	
Atrazine	$>10^{-4}$[a]	3.6×10^{-7}[a]	1.5×10^{-4}	1.2×10^{-7}	3.1×10^{-7}[b]	2.0×10^{-4}[b]	4.5×10^{-7}[b]	4.0×10^{-4}[b]
Pyrazon	1.4×10^{-3}	4.9×10^{-6}	6.5×10^{-4}	2.0×10^{-6}	7.4×10^{-4}	1.3×10^{-5}	3.5×10^{-4}	2.3×10^{-5}
Bromacil	5.0×10^{-6}[a]	2.5×10^{-7}[a]	5.6×10^{-6}	6.3×10^{-8}	1.8×10^{-7}[b]	5.5×10^{-6}[b]	1.4×10^{-7}[b]	1.0×10^{-5}[b]
Buthidazole	1.5×10^{-6}	6.1×10^{-7}	10×10^{-8}	5.7×10^{-8}	8.5×10^{-7}	9.3×10^{-8}	1.6×10^{-6}	2.7×10^{-7}
Dinoseb	1.1×10^{-7}	1.0×10^{-6}	8.1×10^{-8}	5.0×10^{-7}	6.9×10^{-8}	1.0×10^{-6}	4.0×10^{-8}	3.6×10^{-7}

[a] Pfister and Arntzen, unpublished.

[b] Data supplied by C.J. Arntzen, corresponding to report by Arntzen *et al.*, 1982.

Table 4. Chloroplast I_{50} values (M) in smooth pigweed for the herbicides indicated.

	Amaranthus hybridus L.	
Herbicide	Resistant	Susceptible
	---------------(I_{50} M)---------------	
Cyanazine	$3.4\text{x}10^{-5}$	$7.3\text{x}10^{-8}$
Metribuzin	$5.4\text{x}10^{-5a}$	$2.1\text{x}10^{-7a}$
Linuron	$2.7\text{x}10^{-7}$	$7.8\text{x}10^{-8}$
Desmedipham	$2.5\text{x}10^{-7}$	$6.5\text{x}10^{-8}$
Dicamba	$>10^{-3}$	$>10^{-3}$
Bromoxynil	$5.1\text{x}10^{-6}$	$8.7\text{x}10^{-6}$
Diuron	$8.1\text{x}10^{-8a}$	$6.0\text{x}10^{-8a}$
Bentazon	$3.4\text{x}10^{-5a}$	$5.0\text{x}10^{-5a}$

[a] Pfister and Arntzen, unpublished.

Figure 2. Comparison of resistance ratios measured as whole plant injury (solid bars) or inhibition of photosynthetic electron transport (hollow bars) for atrazine, pyrazon, bromacil, buthidazole, and dinoseb. A = *Amaranthus hybridus*; C = *Chenopodium album*; B = *Brassica napus*; S = *Senecio vulgaris*.[a]

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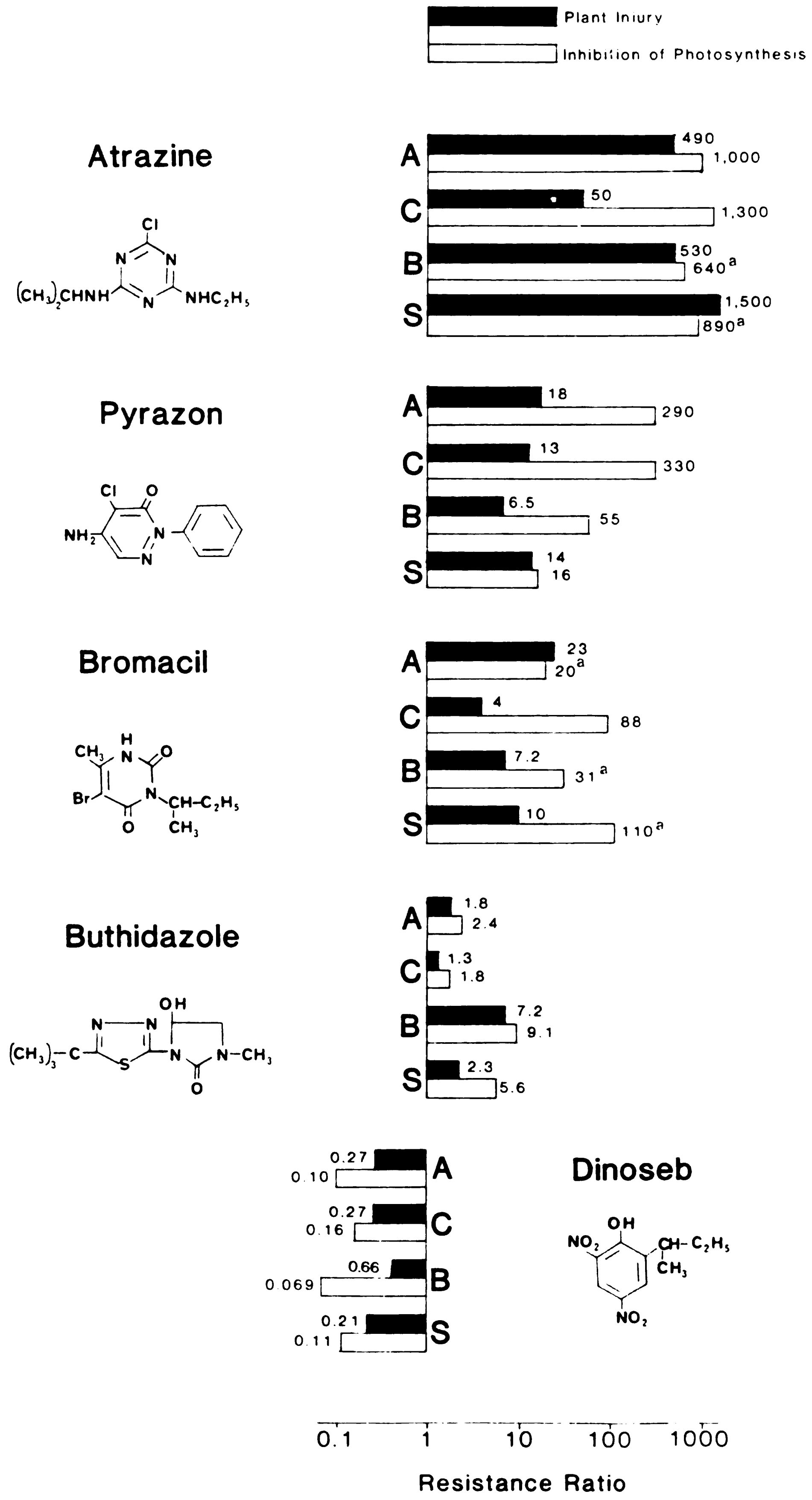
[a] Determined by Pfister and Arntzen, unpublished.



Plant Injury
Inhibition of Photosynthesis
Atrazine
A 490 1,000
C 50 1,300
B 530 640a
S 1,500 890a
Pyrazon
A 18 290
C 13 330
B 6.5 55
S 14 16
Bromacil
A 23 20a
C 4 88
B 7.2 31a
S 10 110a
Buthidazole
A 1.8 2.4
C 1.3 1.8
B 7.2 9.1
S 2.3 5.6
Dinoseb
A 0.27 0.10
C 0.27 0.16
B 0.66 0.069
S 0.21 0.11
0.1
1
10
100
1000
Resistance Ratio

Figure 3. Comparison of resistance ratios measured as whole plant injury (solid bars) or inhibition of photosynthetic electron transport (hollow bars) averaged across species. Means followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

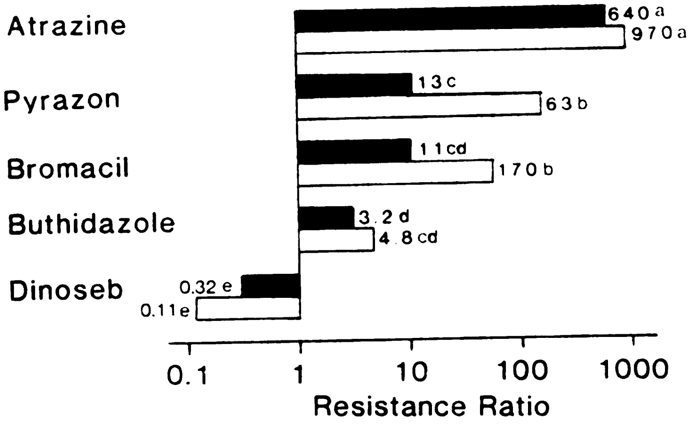

Atrazine
Pyrazon
Bromacil
Buthidazole
Dinoseb
640 a
970 a
13 c
63 b
11 cd
170 b
3.2 d
4.8 cd
0.32 e
0.11 e
0.1
1
10
100
1000
Resistance Ratio

Figure 4. Comparison of resistance ratios measured as whole plant injury (solid bars) or inhibition of photosynthetic electron transport (hollow bars) in smooth pigweed.

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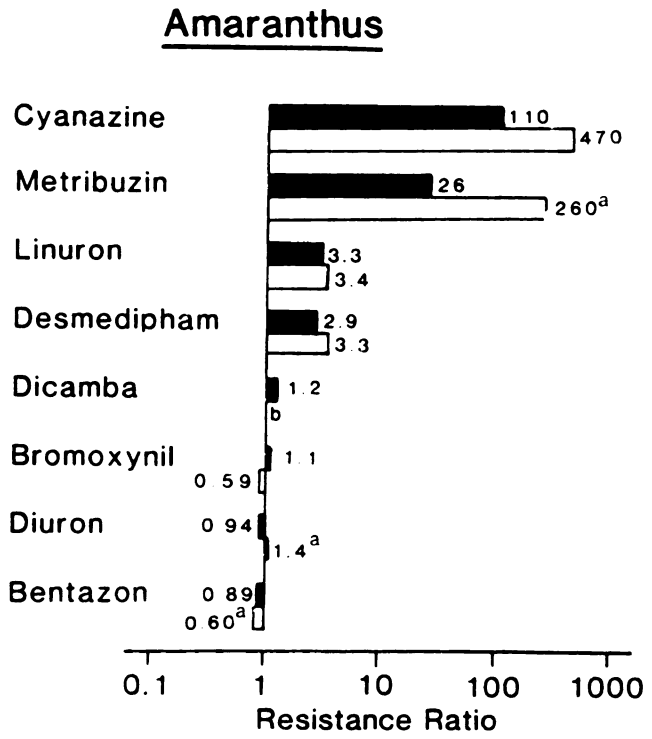
[a] Determined by Pfister and Arntzen, unpublished.

[b] Dicamba did not inhibit photosynthetic electron transport.


Amaranthus
Cyanazine
110
470
Metribuzin
26
260^a
Linuron
3.3
3.4
Desmedipham
2.9
3.3
Dicamba
1.2
b
Bromoxynil
1.1
0.59
Diuron
0.94
1.4^a
Bentazon
0.89
0.60^a
0.1
1
10
100
1000
Resistance Ratio

of bromacil and pyrazon (Figure 3). This suggests that these compounds act at additional sites other than inhibition of photosynthesis, or that the resistant biotypes exhibit a generalized increase in susceptibility to herbicides, or that herbicide detoxification rates, relative to the amount of pesticide applied, are lower in the resistant biotypes. That is, if the rates of detoxification in both biotypes are similar, the rate in the resistant biotype may be of relatively little consequence due to the much higher herbicide rates applied. Pyrazon caused a greater degree of chlorosis and a more delayed necrosis than other herbicides, particularly at high rates in the resistant biotype (personal observation). This, together with the large discrepancy between *in vivo* and *in vitro* resistance ratios for pyrazon (Figure 3) suggests that there may be a second site of action for pyrazon. Inhibition of carotenoid biosynthesis is a possible second site since this appears to be the primary site of action by the closely related compound, norflurazon [4-chloro-5-(methylamino)-2-(α,α,α-trifluoro-*m*-tolyl)-3(2H)-pyridazinone] (Bartels and Watson, 1978; Vaisberg and Schiff, 1976). However, our observations agree with previous reports (Eshel, 1969; Hilton *et al.*,1969) that inhibition of photosynthesis is the primary site of action in the susceptible biotypes.

Resistance ratios were close to one for dicamba, bromoxynil, diuron, and bentazon (Figure 4). *In vivo* resistance ratios for dalapon and acifluorfen were 0.81 and 1.07 respectively (data not shown). Since resistance ratios were close to one, no statement can be made regarding the cause of injury from these herbicides, based on our observations. However, inhibition of photosynthesis is a mechanism of action for diuron (Izawa, 1977), bentazon (Suwanketnikom *et al.*, 1982) and bromoxynil (Table 1) but not dicamba. There was no inhibition of electron transport by dicamba (Table 4) and there was no in vivo resistance to this compound. This was expected since the mechanism of action of dicamba is interference of growth regulation (Ashton and Crafts, 1981). This observation indicates that there is no cross resistance to compounds which do not affect photosynthesis.

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CHAPTER 2

Studies on the Basis for Differential Tolerance to Dinoseb in Triazine Resistant and Susceptible Smooth Pigweed (*Amaranthus hybridus*)

ABSTRACT

A triazine resistant biotype of smooth pigweed (*Amaranthus hybridus* L.) showed less tolerance than the susceptible biotype to dinoseb (2-*sec*-butyl-4,6-dinitrophenol) applied early in the morning. This same decrease in tolerance was observed in isolated chloroplasts, suggesting that dinoseb injury may be related to the inhibition of photosynthesis. However, dinoseb was actually slightly more active in the dark than in the light, indicating that dinoseb did not cause injury by inhibition of photosynthesis. Furthermore, the reduced tolerance of the triazine resistant biotype to dinoseb was also observed in the dark. To evaluate whether carbohydrate levels in the plant might be related to dinoseb injury, the levels of glucose, sucrose, reducing sugars, and starch were determined in leaves of the two biotypes in the morning and afternoon. Levels of glucose and sucrose were low in all cases. Starch levels were higher in the afternoon than in the morning in both biotypes. This is consistent with an hypothesis that reducing power obtained from hydrolysis and oxidation of starch may protect plants from dinoseb injury. However, starch and total carbohydrate levels were not lower in the triazine resistant biotype than in the triazine susceptible biotype in the morning, indicating that carbohydrate levels cannot explain the decreased tolerance of the triazine resistant biotype. Carbohydrate

levels were lower in the triazine resistant biotype than the triazine susceptible biotype in the afternoon. This was consistent with previous reports that the triazine resistant biotype had a lower photosynthetic efficiency. Finally, exogenous sucrose supplied to excised leaves via the petiole did not protect the leaves from dinoseb injury. Thus carbohydrate levels do not appear to be the basis for the differences in dinoseb tolerance observed.

INTRODUCTION

Dinoseb may affect many physiological process including ribonucleic acid synthesis, protein synthesis, photosynthesis, lipid synthesis and respiration (Ashton *et al.*, 1977). A single biochemical mechanism for herbicidal injury from dinoseb has not been shown, but effects on ATP synthesis have been studied (Aston and Crafts, 1981). The symptoms of injury include rapid desiccation which is more active in the dark than the light (Meggitt *et al.*, 1956; Mellor and Salisbury, 1965). The triazines and phenylureas are active almost solely in the light, and cause reduced growth and gradual chlorosis, followed by necrosis. Thus, symptoms of injury from the classical photosynthetic inhibitors are distinct from those of dinoseb. It was surprising to observe that resistance ratios for dinoseb were positively correlated in whole plant and isolated chloroplast studies in 4 species (Chapter 1) since this suggested a photosynthetic site of action. This cannot be explained by a general increase in susceptibility to herbicides, since resistance ratios for dicamba, dalapon and acifluorfen were very close to 1.0 (Chapter 1). These herbicides do not inhibit at the triazine site of action.

The differential response of the two biotypes at the whole plant level to dinoseb was clearly seen when applied in the morning on sunny days, but not when applied in the afternoon (personal observation). One objective of this study was to verify this under controlled conditions. A second objective was to examine a possible photosynthetic site of action by determining the efficacy of dinoseb on the two biotypes in dark vs. light. Several lines of evidence from this experiment suggested that carbohydrate levels would be correlated with dinoseb efficacy. Therefore carbohydrate levels were assayed and the effects of exogenous sucrose was tested.

MATERIALS AND METHODS

Injury response to dinoseb. Smooth pigweed was grown and thinned to a uniform stand as described previously (Chapter 1) but in a growth room. Plants were grown at constant 25^{o}C and 90% relative humidity, with 450 μE m^{-2} s^{-1} photosynthetic photon flux density with 14h/10h light/dark cycle. Dinoseb was applied at the two leaf stage either prior to illumination ("morning") or after 8 h of illumination ("afternoon") and plants were returned to the same chamber in light or dark conditions. Dinoseb was applied at the two leaf stage at several rates on a logarithmic scale as in Chapter 1, with 0.5% v/v alkylaryl polyoxyethyleneglycol-free fatty acid-isopropanol surfactant (X-77 Spreader). Shoot fresh weights were determined 24 h after treatment. The data reported are the means of two experiments with six replications per experiment. Resistance ratios were calculated by dividing the herbicide rate that caused 50% injury (I_{50}) in the triazine resistant biotype by the I_{50} in the triazine susceptible biotype. Due to the nature of these data, analysis was conducted using each experiment as one replicate of a completely randomized design.

Carbohydrate analyses. Details of the methods used were previously described by Haissig (1982a, 1982b) except that a different starch hydrolysis enzyme was used (Mylase-100; GB Fermentation Industries, Inc.; Charlotte, NC 28224). Plants were grown as in the previous experiment and harvested either prior to illumination ("morning") or after 8 h of illumination ("afternoon"). Dinoseb treatments were made to whole plants at these times to verify that differences in tolerance were as reported in Table 1 (data not shown). Leaf material was frozen as it was harvested, lyophilized, and ground in a Wiley mill to pass a 40-mesh screen. A 25 mg sample was analyzed. Soluble sugars were extracted three times in methanol-chloroform-water (12/5/3, v/v/v) and combined. Pigments and lipids were removed from the chloroform phase after the addition of water. The insoluble material was saved for starch analysis. Glucose was assayed spectrophotometrically by the glucose oxidase/horseradish peroxidase assay using o-dianisidine as the color reagent (Sigma Chemical Co. Technical Bulletin No. 510, 1982) using glucose standards. Sucrose was hydrolyzed at 100°C in HCl, neutralized, and assayed by the same method as glucose. Initial glucose levels were subtracted to obtain true estimates for sucrose. Sucrose standards were hydrolyzed and assayed in the same manner. Reducing sugars were assayed spectrophotometrically by the dinitrosalicylic acid method (Clark, 1964) and initial glucose levels were again subtracted. Glucose was used as the standard. Starch was enzymatically hydrolyzed for 48 h with 10 $mg \cdot ml^{-1}$ Mylase-100 in 100 mM acetate (pH 5.0) using potato starch as the standard. The hydrolyzed product was assayed by the same method as glucose. Mylase-100 is a mixture of α-amylase and other glucosidases, and is free of cellulase and hemicellulase activity (B.E. Haissig, personal communication). This enzyme was dialyzed against distilled water for 24 h to remove soluble sugars before use. Data reported are the means of two experiments with six replications per experiment.

Effect of exogenous sucrose. Triazine resistant smooth pigweed plants were grown as described above. Leaves were excised and placed with petioles in 0, 0.5, 1, 2, or 5% sucrose. Leaves were placed in the dark for 10 h to allow uptake of sucrose and then sprayed with 0.5 kg/ha dinoseb. Leaves were then placed in the illuminated growth room described above, and fresh and dry weights were determined 24 h later.

RESULTS AND DISCUSSION

Injury response to dinoseb. Both triazine resistant and susceptible biotypes had lower I_{50} values in the dark than in the light when treated in the morning (Table 1). This confirms previous reports of enhanced activity of dinitrophenolic compounds in the dark (Meggitt et al., 1956; Mellor and Salisbury, 1965), and contradicts a hypothesis that dinoseb affects photosynthetic electron transport. Resistance ratios observed for morning treatments were close to the value of 0.27 previously reported for smooth pigweed (Chapter 1). The resistance ratio of less than 1.0 in the morning treatments cannot be attributed to effects on photosynthetic electron transport since a similar and slightly lower ratio was also observed in the dark treatment.

I_{50} values were higher for both biotypes in the light in the afternoon than in the morning (Table 1). Furthermore, there was little difference in the I_{50} values for the two biotypes as shown in the resistance ratio. This documents previous unpublished observations with greenhouse experiments. Based on this observation, dinoseb may be more effective in the field if applied early in the morning; this is especially true if dinoseb is used for control of triazine resistant weed species.

Table 1. Plant tolerance to dinoseb in triazine resistant and susceptible biotypes of smooth pigweed under various light conditions described in the text.[a]

	Morning		Afternoon
	Dark	Light	Light
I_{50} Resistant Biotype (kg/ha)	0.045 b	0.091 b	1.05 a
I_{50} Susceptible Biotype (kg/ha)	0.19 b	0.32 b	1.08 a
Resistance Ratio	0.26 b	0.35 ab	0.98 a

[a] Means among the I_{50} values or among the resistance ratio values followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

The hypothesis that endogenous carbohydrates are responsible for the observed differences in dinoseb efficacy can be proposed to explain the differences in dinoseb efficacy reported in Table 1. Carbohydrates may supply ATP and reducing power from glycolysis and oxidative phosphorylation (if active in the presence of dinoseb) to maintain glutathione, carotenoids, ascorbate, α-tocopherol or other cellular protective mechanisms in a reduced state. Several arguments support this hypothesis: 1) The triazine resistant biotype, which was more susceptible in both morning treatments, fixes CO_2 more slowly than the susceptible biotype (Ort et al., 1983; Ahrens and Stoller, 1983); lower levels of carbohydrate may persist in the resistant biotype even after the dark period; 2) Dinoseb was more active in the dark than in the light; thus photosynthetic electron transport and CO_2 fixation may have provided protection; and 3) Both biotypes showed much greater tolerance to dinoseb in afternoon treatments. The accumulation of photosynthetic products such as starch may have provided protection from injury. It can be proposed that the reason that the two biotypes did not differ in their tolerances in the afternoon was that there is some maximal level of protection by carbohydrate and that carbohydrate levels in both biotypes exceeded the capacity of the protective mechanism. Therefore, according to this hypothesis, it can be predicted that carbohydrate levels would be lower in the resistant biotype than the susceptible biotype in the morning, and that that carbohydrate levels would be much higher in both biotypes in the afternoon.

Carbohydrate analyses. Glucose and sucrose levels were generally much lower than reducing sugars or starch (Table 2). In no instances were carbohydrate levels lower in the resistant biotype than the susceptible biotype in the morning. This contradicts the hypothesis stated. Several carbohydrate levels, most notably starch, were higher in the afternoon than in the morning. While this correlative evidence is consistent with the stated hypothesis, it was also obviously anticipated. It is of interest to note that total carbohydrate levels were significantly higher in the susceptible biotype than the resistant biotype in the afternoon. This is consistent with previous reports of lower CO_2 fixation rates in the resistant biotype (Ort, 1983; Ahrens and Stoller, 1983) and ultrastructural studies on starch quantities in several species (Burke et al., 1982; Vaughn and Duke, 1983).

Effect of exogenous sucrose. Sucrose or glucose has been shown to protect plants from injury by photosynthetic inhibitors (e.g., Shimabukuro et al., 1976). However, sucrose did not protect triazine resistant smooth pigweed from dinoseb injury (Table 3). At higher sucrose levels, desiccation by dinoseb was actually enhanced. Therefore it is difficult to support the hypothesis that dinoseb tolerance is related to carbohydrate levels.

The differential response of the two smooth pigweed biotypes cannot be attributed to differences in the sensitivity of the photosynthetic membranes, nor can it be attributed to differences in photosynthetic efficiency. However, it is known that chloroplast lipids are less saturated in the resistant biotype (Pillai and St. John, 1981; Burke et al., 1982). The possibility remains that dinoseb is more active in the resistant biotype for this reason. However, the increased tolerance of both biotypes to dinoseb in the afternoon remains unexplained. Finally, our observations do not contradict the hypothesis that dinoseb acts primarily

Table 2. Glucose, sucrose, reducing sugar, starch, and total carbohydrate (sucrose + reducing sugar + starch) levels in triazine resistant and susceptible smooth pigweed prior to illumination ("morning") or after 8h of illumination ("afternoon").

Carbohydrate assayed	Biotype	Carbohydrate level[a] (μmol/g dry weight) Morning	Carbohydrate level[a] (μmol/g dry weight) Afternoon
Glucose	Resistant	6.5 bc	7.8 b
	Susceptible	5.4 c	11.0 a
Sucrose	Resistant	11.1 a	6.4 b
	Susceptible	9.2 a	9.7 a
Reducing sugar	Resistant	97.8 b	100.0 b
	Susceptible	92.2 b	137.8 a
Starch	Resistant	17.3 b	142.6 a
	Susceptible	12.2 b	187.0 a
Total carbohydrate	Resistant	132.7 c	256.9 b
	Susceptible	119.0 c	345.5 a

[a] Means for each type of carbohydrate with the same letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

Table 3. Effect of exogenous sucrose on desiccation by dinoseb in excised leaves of triazine resistant smooth pigweed.

Dinoseb (kg/ha)	Sucrose (%)	Leaf Moisture[a] (%)
0	0	87.4 a
1	0	68.4 ab
1	0.5	63.1 bc
1	1	42.5 bc
1	2	52.7 cd
1	5[b]	26.8 d

[a] Means with same letters are not significantly different at the 5% level by D.M.R.T.

[b] Leaves showed injury, due to plasmolysis, before dinoseb treatment.

within the mitochondria, as has been discussed by others (Mellor and Salisbury, 1965).

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CHAPTER 3

Chemical Control of Triazine Resistant Common Lambsquarters (*Chenopodium album*) and Two Pigweed Species (*Amaranthus* spp.)

ABSTRACT

Various chemical treatments were evaluated over two growing seasons for control of triazine resistant common lambsquarters (*Chenopodium album* L.) and for control of a triazine resistant infestation containing both redroot pigweed (*Amaranthus retroflexus* L.) and Powell amaranth (*A. powellii* S. Wats.). Triazines, atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine], cyanazine {2-[[4-chloro-6-(ethylamino)-*s*-triazin-2-yl]amino]-2-methylpropionitrile}, and metribuzin [4-amino-6-*tert*-butyl-3-(methylthio)-*as*-triazin-5(4*H*)-one], provided unsatisfactory control of these species. Satisfactory control of lambsquarters was obtained with preemergence applications of pendimethalin [*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine] or dicamba (3-6-dichloro-*o*-anisic acid), postemergence applications of dicamba, bromoxynil (3,5-dibromo-4-hydroxybenzonitrile), or bentazon [3-isoproyl-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide], or various directed late postemergence treatments. Satisfactory control of pigweed was obtained with preemergence applications of alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide] or postemergence treatments of dicamba, bromoxynil, or 2,4-D [(2,4-dichlorophenoxy)acetic acid].

INTRODUCTION

LeBaron (1984) has recently reviewed the extent and implications of herbicide resistance in weeds. Resistance to six chemical classes of herbicides occurs in 46 species. Resistance to triazine herbicides occurs in 37 species in 12 countries. Infestations are most often serious but localized. Herbicide resistance is likely to become a more serious problem in the future since trends toward decreased tillage and closer row spacings require greater dependence on herbicides. Fortunately, in most cases, alternative cropping and/or weed control systems are available to provide control of the resistant biotypes.

Triazine resistant common lambsquarters was observed in Huron County, Michigan in 1980 on land which had been in continuous corn for at least 7 years and treated solely with atrazine over this period. Two triazine resistant pigweed species (Powell amaranth and redroot pigweed) were observed in Oceana County, Michigan in 1981 on land which had been in continuous corn for 5 years and treated with atrazine and butylate (S-ethyl diisobutylthiocarbamate) plus R-25788 (N,N-diallyl-2,2-dichloroacetamide). The two species were distinguished by the criteria of Ahrens et al. (1981). The objective of this study was to determine chemical methods for control of these triazine resistant weeds where crop rotation was not desired. Treatments were selected based on chemicals known to control these species (Barrett and Meggitt, 1983), on a knowledge of cross resistance (Chapter 1), and on reports on control of triazine resistant species (Anonymous, 1981c; Ritter, 1982; Parochetti et al., 1982). Buthidazole has been shown to inhibit photosynthesis at two sites (Hatzios et. al., 1980; York et al., 1981). Cross resistance to buthidazole is low (Chapter 1), perhaps for this reason. Therefore this compound was evaluated with and without acetanilide herbicides which have been shown to protect corn from buthidazole injury (York and Slife, 1981).

MATERIALS AND METHODS

All experiments were performed on plots 3.05m by 9.14m arranged as a randomized block with 0.76m row spacing. Treatments were applied with 90 L/ha water at a pressure of 210 kPa. Data were arcsine-transformed before analysis. Control of 80% or more of the weeds was considered "satisfactory."

Triazine resistant pigweed. Preemergence treatments were applied 1 day after planting (DAP), on May 15, 1982 and May 14, 1983. Postemergence treatments were applied when the pigweed was 1.5 to 2.5 cm tall; this was 22 DAP in 1982 and 38 DAP in 1983. Corn was at the three to four-leaf stage, approximately 15 cm tall, in 1982 and was at the four-leaf stage, approximately 15 cm tall, in 1983. Treatments were visually evaluated 71 DAP during tasseling in 1982, and 62 DAP, prior to tasseling, in 1983. The two pigweed species were not evaluated separately. The pigweed population was 150/m^2 at 62 DAP in 1983 and was similar in 1982. There were four replications. The soil was a sandy loam, 5.9% organic matter, pH 6.5. The corn variety was Pioneer 3901 in 1982 and Supercross 1940 in 1983.

Triazine resistant common lambsquarters. Preplant treatments were incorporated with two passes of a field cultivator set to a 13 cm depth. Preemergence treatments were applied on the same day (May 22, 1981; May 25, 1983), following planting. Postemergence treatments were applied when the common lambsquarters was 1.5 to 2.5 cm tall. This was 19 DAP in

1981 and 29 DAP in 1983. Corn was at the two to three-leaf stage, approximately 10 cm tall, in 1981 and was at the six-leaf stage, approximately 17 cm tall, in 1983. Directed late postemergence treatments were applied 44 DAP both years when the common lambsquarters was 20 to 30 cm tall. Drop nozzles directed sprays at the base of the corn plants. Corn was 45 to 60 cm tall in 1981 and 60 cm tall in 1983. Treatments were visually evaluated 62 DAP in 1981 and 66 DAP in 1983 during the tasseling stage of corn. The lambsquarters population was $200/m^2$ at 66 DAP in 1983 and was similar in 1981. There were three replications. The soil was a sandy clay loam, 3.7% organic matter, pH 5.7. The corn variety was Payco 342 both years.

RESULTS AND DISCUSSION

Triazine resistant-pigweed. The triazines, atrazine, cyanazine, or metribuzin, provided unsatisfactory control of pigweed as preemergence treatments (Table 1). Alachlor was the most effective preemergence herbicide and provided significantly higher levels of control than metolachlor (Table 1). Control with metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide] was unsatisfactory. Alachlor and metolachlor have previously been reported to be equally effective on redroot pigweed (Barrett and Meggitt, 1983) and on triazine resistant smooth pigweed (*A. hybridus* L.) (Ritter, 1982). The relative efficacy of alachlor and metolachlor is dependent upon tillage system and soil type (Strek and Weber, 1981; 1982).

Alachlor plus atrazine applied preemergence in sequence with dicamba or bromoxynil applied postemergence gave satisfactory control in 1983 (Table 1). However, control was not significantly better than dicamba or bromoxynil alone postemergence.

Table 1. Triazine resistant pigweed control with various chemical treatments.

Treatment	Rate (kg/ha)	% Control[a] 1982	1983
Preemergence			
Atrazine	1.1	6 i	4 m
Atrazine	11	-	15 klm
Cyanazine	1.1	0 i	8 m
Metribuzin + atrazine	0.28 + 1.1	0 i	10 lm
Alachlor	2.8	85 abcde	78 efg
Alachlor + atrazine	2.8 + 1.1	91 abc	86 def
Metolachlor + atrazine	2.8 + 1.1	77 def	56 ghij
Pendimethalin + atrazine	1.7 + 1.1	45 h	42 ijkl
Pre- and postemergence			
Alachlor + atrazine (preemergence) and dicamba (postemergence)	2.8 + 1.1 0.28	-	100 a
Alachlor + atrazine (preemergence) and bromoxynil (postemergence)	2.8 + 1.1 0.33	-	98 abc
Postemergence			
Atrazine + C.O.C.[b]	2.2 + 2.3 L/Ha	16 i	16 klm
Dicamba	0.28	94 a	97 abcd
Dicamba	0.56	95 a	99 ab
2,4-D	0.56	88 abcde	91 bcde
Dicamba + 2,4-D	0.28 + 0.56	95 a	100 a
Bromoxynil	0.28	69 fg	92 bcde
Bromoxynil	0.33	81 bcdef	88 cdef
Bromoxynil	0.56	77 ef	92 bcde
Dinoseb	1.1	69 fg	38 jklm
Dinoseb	2.2	82 bcdef	45 hijk
Dinoseb	4.5	88 abcde	71 fgh
Buthidazole	0.14	58 gh	31 jklm
Buthidazole	0.28	76 ef	51 hij
Buthidazole + dicamba	0.14 + 0.28	94 a	99 ab

[a] Means within columns with same letters are not significantly different at the 5% level by Duncan's multiple range test.

[b] C.O.C. = crop oil concentrate.

Satisfactory pigweed control was obtained with postemergence dicamba, 2,4-D, and the two combined (Table 1). Bromoxynil provided significantly less control than dicamba in 1982 but not in 1983. Control with dinoseb (2-sec-butyl-4,6-dinitrophenol) tended to increase as rates increased from 1.1 to 4.5 kg/ha. The highest dinoseb rate caused slight corn injury (5%) in 1983 (data not shown). It is possible that more effective pigweed control would have been obtained with morning treatments of dinoseb (Chapter 2), since our treatments were made near midday. Buthidazole {3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2yl]-4-hydroxy-1-methyl-2-imidazolidinone} did not provide satisfactory postemergence control at the rates tested and showed slight injury (5%) at the higher rate in 1983 (data not shown). Buthidazole in combination with dicamba gave satisfactory control, but not significantly more effective than dicamba alone.

Triazine resistant common lambsquarters. The triazines, atrazine, cyanazine, or metribuzin, gave unsatisfactory control of this common lambsquarters biotype in various preplant, preemergence, and postemergence treatments (Table 2).

EPTC (S-ethyl dipropylthiocarbamate) plus R-25788 provided the highest level of control of the preplant treatments both years, but not significantly greater than butylate plus R-25788 (Table 2). Decreased control in 1983 by the two treatments can be attributed to desorption and leaching of the herbicide as a result of a prolonged cool wet period after treatment. Buthidazole preplant treatments in 1983 provided 100% control, but crop injury was unacceptable. However, crop injury was somewhat less in the presence of the acetanilides alachlor or metolachlor as was previously reported (York and Slife, 1981).

Table 2. Triazine resistant common lambsquarter control and corn injury with various chemical treatments.

Treatment	Rate (kg/ha)	% Control[a] 1981	% Control[a] 1983	% Injury[a] 1981	% Injury[a] 1983
Preplant[a]					
Atrazine	2.2	19 d	7 de	0	0
Cyanazine	2.2	53 c	7 de	0	0
EPTC + R-25788 + atrazine	4.5 + 0.37 + 2.2	94 ab	65 b	0	0
Butylate + R-25788 + atrazine	4.5 + 0.19 + 2.2	88 b	27 cde	0	0
Buthidazole	1.1	-	100 a	-	75
Alachlor	2.2	-	14 de	-	0
Buthidazoel + alachlor	1.1 + 2.2	-	100 a	-	55
Buthidazole + metolachlor	1.1 + 2.2	-	100 a	-	63
Preemergence					
Atrazine	11.2	10 no	22 mnopq	0	0
Cyanazine + metolachlor	1.7 + 2.2	30 mno	47 jklmnop	0	0
Metribuzin + atrazine + metolachlor	0.28 + 1.1 + 2.2	0 o	55 ijklmno	0	0
Metolachlor	2.2	-	0 q	0	0
Metolachlor + atrazine	2.2 + 1.1	10 no	34 mnopq	0	0
Alachlor + atrazine	2.2 + 1.1	13 no	59 hijklmn	0	0
Pendimethalin + atrazine	1.7 + 1.1	82 efhij	99 abc	0	0
Dicamba + metolachlor	0.42 + 2.2	96 abcdef	88 bcdefgh	0	0
Dicamba + metolachlor + atrazine	0.42 + 2.2 + 1.1	95 abcdefg	82 defghij	0	0

Table 2. Continued.

Treatment	Rate (kg/ha)	% Control[a] 1981	% Control[a] 1983	% Injury[a] 1981	% Injury[a] 1983
Dicamba + alachlor	0.42 + 2.2	98 abcd	92 abcdefg	0	0
Dicamba + pendimethalin	0.42 + 2.2	98 abcd	99 abcd	0	0
Dicamba + pendamethalin + atrazine	0.42 + 1.7 + 1.1	95 abcdefg	100 a	0	0
Dinoseb + metolachlor	2.5 + 2.2	97 abcde	63 hijklm	0	0
Dinoseb + metolachlor	5.0 + 2.2	99 abc	97 abcdef	0	0
Dinoseb + metolachlor	10.1 + 2.2	98 abcd	99 abcd	0	0
Buthidazole	0.34	97 abcdef	99 abcd	0	3
Buthidazole	1.1	100 a	100 a	88	47
Buthidazole + metolachlor	0.34 + 2.2	85 defghi	94 abcdefg	0	0
Buthidazole + metolachlor	1.1 + 2.2	100 ab	100 ab	17	17
Buthidazole + dicamba + metolachlor	0.34 + 0.42 + 2.2	100 a	97 abcdef	0	0
<u>Pre- and Postemergence</u>					
Pendimethalin (preemergence) and dicamba (postemergence)	1.7 0.28	97 abcdef	100 a	0	0
Pendamethalin + atrazine (preemergence) and dicamba (postemergence)	1.7 + 1.1 0.28	99 abcd	100 a	0	5

Table 2. Continued.

Treatment	Rate (kg/ha)	% Control[a] 1981	% Control[a] 1983	% Injury[a] 1981	% Injury[a] 1983
Postemergence[b]					
Atrazine + C.O.C.[c]	2.2 + 2.3 L/Ha	41 lmn	5 pq	0	0
Cyanazine	2.2	30 mno	15 opq		
Dicamba	0.28	98 abcd	79 efghij	0	0
Dicamba	0.42	-	97 abcdef	0	0
Dicamba	0.56	100 ab	98 abcde	0	0
2,4-D	0.56	100 ab	59 hijklmn	0	3
Dicamba + 2,4-D	0.28 + 0.28	-	99 abcd	0	5
Dicamba + 2,4-D	0.28 + 0.56	99 abcd	100 ab	0	0
Bromoxynil	0.28	94 abcdefg	99 abc	0	0
Bromoxynil	0.42	-	98 abcde	0	0
Bromoxynil	0.56	94 abcdefg	98 abcde	0	0
Bentazon + C.O.C.	1.1 + 2.3 L/Ha	98 abcde	100 a	0	0
Dinoseb	1.1	92 bcdefg	99 abcd	0	3
Dinoseb	2.2	94 abcdefg	100 a	0	8
Dinoseb	4.5	97 abcde	100 abc	0	17
Buthidazole	0.14	94 abcdefg	17 nopq	0	0
Buthidazole	0.28	100 a	60 hijklm	0	0
Buthidazole + dicamba	0.14 + 0.28	100 ab	100 ab	7	0
Directed Late Postemergence[b,d]					
Atrazine + C.O.C.	2.2 + 2.3 L/Ha	35 lmno	13 opq	0	0
Dicamba	0.28	47 klmn	76 fghijk	0	0
Dicamba	0.56	64 ijklm	89 abcdefgh	0	0

Table 2. Continued.

Treatment	Rate (kg/ha)	% Control[a] 1981	% Control[a] 1983	% Injury[a] 1981	% Injury[a] 1983
2,4-D	0.56	64 ijklm	98 abcde	0	0
Dicamba + 2,4-D	0.28 + 0.28	-	73 ghijkl	0	0
Dicamba + 2,4-D	0.28 + 0.56	76 ghijk	85 efghi	0	0
Bromoxynil	0.42	55 jklm	98 abcde	0	0
Bromoxynil	0.56	-	76 fghijk	0	0
Bromoxynil + 2,4-D	0.42 + 0.56	87 cdefghi	100 a	0	0
Bromoxynil + dicamba	0.42 + 0.56	77 ghijk	97 abcdef	0	0
Bentazon + C.O.C.	1.1 + 2.3 L/Ha	58 jklm	97 abcdef	0	0
Linuron	0.56	37 lmno	99 abc	0	0
Buthidazole	0.28	67 hijkl	49 jklmnop	0	7
Buthidazole + dicamba	0.28 + 0.28	65 hijkl	93 abcdefg	0	13

[a] Means within columns with same letters are not significantly different at the 5% level by Duncan's multiple range test. Preplant treatments analyzed as a separate experiment.

[b] All postemergence and directed late postemergence treatments received metolachlor 2.2 kg/ha preemergence.

[c] C.O.C. = crop oil concentrate.

[d] Approximately 0.5 - 1.0 cm rain received 1 h after application in 1981.

Preemergence treatments of the acetanilides, metolachlor and alachlor, provided unsatisfactory control (Table 2). The dinitroaniline, pendimethalin provided satisfactory levels of control. Dicamba applied in various combinations provided satisfactory control. The preemergence efficacy of dicamba is dependent on soil type (Anonymous, 1981b). Dinoseb provided satisfactory control at the recommended rate of 10.1 kg/ha. Significant levels of control at 0.25 times the recommended rate may be due to the increased susceptibility to dinoseb of this biotype (Chapter 1). Buthidazole at 0.34 kg/ha provided satisfactory control with low levels of crop injury. However, severe injury to corn occurred at 1.1 kg/ha buthidazole. Insufficient protection was obtained by addition of metolachlor.

Preemergence pendimethalin in combination with postemergence dicamba gave satisfactory weed control, but control was not significantly greater than when this same combination was applied preemergence, or when dicamba was applied alone postemergence (except 0.28 kg/ha dicamba in 1983) (Table 2).

Postemergence applications of dicamba, 2,4-D, or the combination provided satisfactory control except in 1983 at 0.28 kg/ha dicamba or 0.56 kg/ha 2,4-D (Table 2). Bromoxynil provided satisfactory control but higher rates did not improve efficacy as was also true with control of pigweed with this herbicide (Table 1). Bentazon also provided satisfactory weed control. Control with bentazon was greater than expected (Barrett and Meggitt, 1983). This may be due to the greater susceptibility of this triazine-resistant biotype to bentazon (Arntzen *et al*., 1982). Dinoseb provided satisfactory control at all rates tested; control at the lowest rate (0.25 times the recommended rate) may again be due to the increased susceptibility of this biotype to dinoseb. Corn injury occurred due to misapplication at all dinoseb rates tested in 1983. Dinoseb application was late with respect to the labelled corn growth stage (Anonymous, 1981a). Buthidazole alone provided inconsistent levels of control, whereas it provided 100% control in combination with dicamba.

A rainfall of 0.5 to 1.0 cm 1 h following treatments apparently reduced the efficacy of most directed late postemergence treatments in 1981 (Table 2). This complicates the interpretation of the 1981 data. Bromoxynil plus 2,4-D was the only treatment which provided satisfactory control in 1981. Further discussion relates to 1983 results. Satisfactory control was obtained with the following treatments: dicamba at 0.56 kg/ha, 2,4-D, dicamba at 0.28 kg/ha plus 2,4-D at 0.56 kg/ha, bromoxynil at 0.42 kg/ha, bromoxynil plus 2,4-D, bromoxynil plus dicamba, bentazon, linuron, and buthidazole plus dicamba. Effective options are available for control of large weeds late in the growing season. However, considerable interference of crop growth would have occurred by this time.

Both weed infestations showed resistance to atrazine, cyanazine, and metribuzin. Thus it is appropriate to consider them to be "triazine" resistant. Resistance to metribuzin at the levels used in these experiments indicates that problems controlling such weeds can be expected if the land is rotated to soybeans (Glycine max L.) and if control of these species relies solely on metribuzin. Fortunately, fair to good control of common lambsquarters and pigweed is expected with certain acetanilide, dinitroaniline, and thiocarbamate compounds which are widely used in combination with metribuzin (Barrett and Meggitt, 1983). Therefore, the problem anticipated here is more likely to occur with other triazine resistant species. Tridiphane [2-(3,5-dichlorophenyl)-2-(2,2,2-trichloroethyl) oxinane], an atrazine synergist, has recently been developed to enhance postemergence grass control in corn. Since enhancement of atrazine uptake and detoxification is the mechanism of tridiphane action (Burroughs and Slife, 1983), and since an altered site of action is the mechanism for resistance in the weeds discussed here (Arntzen *et al*., 1982), enhanced control of these weeds is not expected.

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CHAPTER 4

Protection of Corn (*Zea mays*) From Buthidazole Injury with Alachlor

ABSTRACT

Less corn (*Zea mays* L.) injury was observed, and less ^{14}C-buthidazole {3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidinone} was present in the shoots of corn, when buthidazole was applied with alachlor [2-chloro-2',6'-diethyl-*N*-(methoxymethyl)acetanilide] than when buthidazole was applied alone. These results indicate that the basis for alachlor protection is reduced uptake.

INTRODUCTION

Protection from buthidazole injury in corn by acetanilide herbicides in field and greenhouse conditions was reported by York and Slife (1981) but not by Hatzios and Penner (1980). We have confirmed that some protection occurs in the field (Chapter 3). It was proposed that the basis for this protection was reduced root uptake (York and Slife, 1981). The purpose of this experiment was to test this hypothesis.

MATERIALS AND METHODS

Pots 14 cm tall with a 1.0 L volume were filled with soil in three bands: a 5 cm "root zone" band with greenhouse mix soil, a 2 cm middle band with 10% activated charcoal in greenhouse mix soil, and a 5 cm "shoot zone" band with greenhouse mix soil. The purpose of the activated charcoal was to provide a barrier to the movement of the herbicides. Five seeds of corn variety Pioneer 3901 were planted 1 cm deep in the middle zone. Alachlor at 4 kg/ha and buthidazole at 1 kg/ha were incorporated separately or together in the root or shoot zone. Buthidazole treatments included 1.0 μCi ^{14}C-buthidazole, also incorporated in the soil. Pots were placed in a greenhouse with supplemental lighting of 100-200 $\mu E{\cdot}m^{-2}s^{-1}$ in a 14h/10h light/dark cycle. Pots were watered once and then covered with paper to minimize evaporation prior to corn emergence. The treatments were assigned in a completely randomized design with four replications. Corn shoots were at the four leaf stage, 20 cm height, when harvested. Fresh and dry weights were determined. Lyophilized shoot material from the buthidazole treatments was then combusted and $^{14}CO_2$ trapped and counted by liquid scintillation spectrometry. The liquid scintillation data were log-transformed prior to analysis. The data reported are a combined analysis for two experiments.

RESULTS AND DISCUSSION

Alachlor alone did not significantly affect fresh or dry weight except when applied in the shoot zone, where dry weight was reduced by 13% (Table 1). Buthidazole alone caused significant fresh and dry weight reduction when applied in the shoot or root zones. Buthidazole caused significantly more weight reduction when applied in the root zone. The combination of alachlor plus buthidazole caused less weight reduction than buthidazole alone. Therefore alachlor provided protection from buthidazole injury as previously reported (York and Slife, 1981). Alachlor may not protect corn from buthidazole injury when it is applied preemergence and is not incorporated (Hatzios and Penner, 1980; personal observation) although some protection was observed in preemergence field

Table 1. Shoot fresh and dry weight response and ^{14}C-buthidazole present in corn shoots after various root zone and shoot zone treatments.[a]

Herbicde treatment		Fresh weight	Dry weight	^{14}C Recovery		
Shoot zone	Root zone	(% of Control)	(% of Control)	(Dpm/5 plants)	(Dpm per g fresh weight)	(Dpm per g dry weight)
-	-	100 a	100 a	-	-	-
Alachlor	-	89 ab	87 b	-	-	-
Buthidazole[b]	-	71 c	57 d	58,000 a	5,000 b	51,000 a
Alachlor + buthidazole[b]	-	79 bc	74 c	31,000 b	2,300 c	20,000 b
-	Alachlor	90 ab	99 a	-	-	-
-	Buthidazole[b]	44 d	39 e	56,000 a	7,700 a	67,000 a
-	Alachlor + buthidazole[b]	74 c	67 cd	35,000 b	2,800 c	26,000 b

[a] Means within columns followed by the same letters are not significantly different at the 5% level according to Duncan's multiple range test.

[b] Buthidazole treatments included 1.0 μCi ^{14}C buthidazole.

treatments (Chapter 2). More ^{14}C was present in treatments with buthidazole alone than with buthidazole plus alachlor. Therefore alachlor provides protection from buthidazole by reducing the amount reaching the leaves, and this is probably due to reduced root uptake. Bucholtz and Lang (1978) reported that alachlor caused reduced uptake of the photosynthetic inhibitors atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-_s_-triazine], cyanazine {2-[[4-chloro-6-(ethylamino)-_s_-triazin-2-yl]amino]-2-methylpropionitrile} and diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea]. The reduced uptake was attributed to root pruning. However, these experiments were done on a susceptible species, oats (_Avena sativa_ L.), and alachlor was used at rates which caused 46% decrease in dry weight and more than 70% decrease in root length. However, our experiment involved the use of corn, a tolerant species, and the rates of alachlor used caused only slight decreases in corn shoot weight. York and Slife (1981) used alachlor at similar rates and corn was protected from buthidazole injury. However, there was no effect on corn root weights. Therefore, the decreased root uptake may not be due to root pruning; it is possible that an altered uptake mechanism may be involved.

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CHAPTER 5

Characterization of Two Sites of Action for Buthidazole, Buthidazole Metabolites, and Ioxynil

ABSTRACT

Buthidazole {3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidinone} and ioxynil (4-hydroxy-3,5,-diiodobenzonitrile) inhibit photosynthetic electron transport at two sites in chloroplast thylakoid membranes isolated from pea (*Pisum sativum* L.). The primary site of inhibition by both herbicides is at the secondary quinone acceptor, Q_B, the site of action of atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] or diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea]. The secondary site of action was measured as inhibition of silicomolybdate (SiMo) photoreduction. Onset of inhibition at the secondary site of action was relatively rapid for ioxynil but slow for buthidazole. This inhibition was reversible for ioxynil but not buthidazole. Buthidazole did not quench *in vitro* chlorophyll fluorescence although ioxynil did. The secondary site of action of buthidazole and ioxynil is on the oxidizing side of photosystem II. Buthidazole may have reduced the rate constant of an electron carrier. Ioxynil inhibited at an electron carrier which was required for electron donation by NH_2OH or diphenylcarbazole.

Most buthidazole metabolites had potential herbicidal activity *in vitro*, but were less active than buthidazole. Therefore, metabolism of

buthidazole is a detoxification process. The potential contribution of buthidazole metabolites to plant injury is discussed.

INTRODUCTION

Buthidazole inhibits photosynthetic electron transport at two sites including a primary site similar to atrazine or diuron, and a secondary site on the water oxidizing side of photosystem II (Hatzios *et al*., 1980; York *et al*., 1981). Ioxynil inhibits at the triazine binding site (Vermaas and Arntzen, 1983) as well as on the water oxidizing side of photosystem II as measured by delayed luminescence (Van Assche, 1982).

The objectives of this study were: 1) to determine the relative importance of the two sites of action of buthidazole and ioxynil; 2) to determine the herbicidal potential at the secondary site by a large number of herbicides; 3) characterize the reversibility of inhibition, delay of inhibition and localization of the secondary site of action; and 4) evaluate the herbicidal activity of buthidazole metabolites.

MATERIALS AND METHODS

Photosystem II partial reactions. Chloroplast thylakoid membranes were isolated from 2-3 week old pea seedlings and rates of photosynthetic electron transport were determined by monitoring the photoreduction of dichlorophenol-indophenol (DCPIP) as previously described (Paterson and Arntzen, 1982). SiMo accepts electrons from Q_A, the primary quinone acceptor of photosystem II (Izawa, 1980; Girault and Galmiche, 1974). Rates of photosystem II electron transport to SiMo were measured under saturating light by continuous recording of oxygen evolution using a water-jacketed Clark-type oxygen electrode maintained at 20°C. The assay buffer contained 25 μg chlorophyll·ml^{-1}, 20 mM Tricine-NaOH (pH 7.8), 100 mM sorbitol, 5 mM NaCl, 10 μM diuron, 0.5 mM FeCN, and 200 μM SiMo.

Herbicides were preincubated in the assay buffer for 15 min and SiMo was added immediately prior to assay. Rates in control samples were approximately 50 μmol O_2· mg chl^{-1}·h^{-1} and were stable for at least 10 min. All data reported are the means of two replications. Partial reactions using diphenylcarbazole (DPC) or NH_2OH as artificial donors to photosystem II were unsuccessful because these compounds directly reduced SiMo in the dark (personal observation).

Reversibility of inhibition. Thylakoid membranes (500 mg chlorophyll·5 ml^{-1}) were incubated in 1 mM buthidazole, 300 μM ioxynil or 10 μM diuron (control) in a buffered solution with 10 mM Tricine NaOH (pH 7.8), 10mM NaCl, 5 mM $MgCl_2$, and 100 mM sorbitol for 15 min. Aliquots were removed to measure inhibition of electron transport to SiMo. The thylakoid suspensions were diluted in 30 ml of the same buffer which included 5 mg·ml^{-1} bovine serum albumin (BSA). Thylakoid membranes were centrifuged at 1000 g for 5 min and resuspended in the buffer with BSA. This centrifugation/washing process was repeated three more times, with a 15 min incubation in the buffer each time. Aliquots were then removed to measure electron transport to SiMo. All steps except the assay were carried out at 4°C.

Localization of the secondary site of action. *In vitro* chlorophyll fluorescence transients were recorded with a Nicolet Explorer digital oscilloscope with assay conditions as previously described (Paterson and Arntzen, 1982). Herbicides were preincubated with the assay buffer 15 min prior to assay. Various chemical treatments were added to modulate the fluorescence maximum, including 500 μM DPC, 20 mM NH_2OH, or 1 mg·ml^{-1} dithionite. Tris washing or heat treatment inactivates water oxidation. Tris washing was done by incubating 200-400 μg chlorophyll·ml^{-1} in 0.8 M tris buffer pH 8.0 at 0°C for 20 min. Heat treatment was done by heating a thylakoid suspension in a preheated tube for 3 min. at 50°C and then cooling immediately to 4°C. The initial fluorescence value (F_0) and the maximal fluorescence value (F_M) were determined and the variable fluorescence value, $(F_M - F_0)/F_0$, is reported.

The sites of action of the various compounds and treatments used in these experiments are shown in Figure 1. All data reported are the means of two replications.

RESULTS AND DISCUSSION

Buthidazole and ioxynil inhibited DCPIP photoreduction at lower concentration than SiMo photoreduction (Table 1). The activities of buthidazole and ioxynil were compared over time at this secondary site (Figure 2). Concentrations of the herbicides were used which previously caused 50% inhibition after a 15 min incubation (Table 1). Buthidazole had no initial activity and 30-60 min. were required to reach maximal activity. In contrast, ioxynil had relatively high initial activity and reached maximal activity by 7 min. Therefore the action of these two herbicides at the secondary site differs with respect to the degree of delay in action.

The reversibility of inhibition at the secondary site was examined (Table 2). Inhibition by buthidazole decreased slightly but not significantly after washing. In contrast, inhibition by ioxynil was reversible to a significant extent. Therefore, the reversibility of inhibition was greatest for the herbicide for which the delay in action was least (Figure 1). Thus, the action of these two compounds can also be distinguished with respect to the reversibility of inhibition.

Figure 1. Simplified scheme of photosynthetic electron transport. Sites of reduction, oxidation and inhibition by various compounds are indicated. P680 is the photosystem II reaction center. P700 is the photosystem I reaction center.

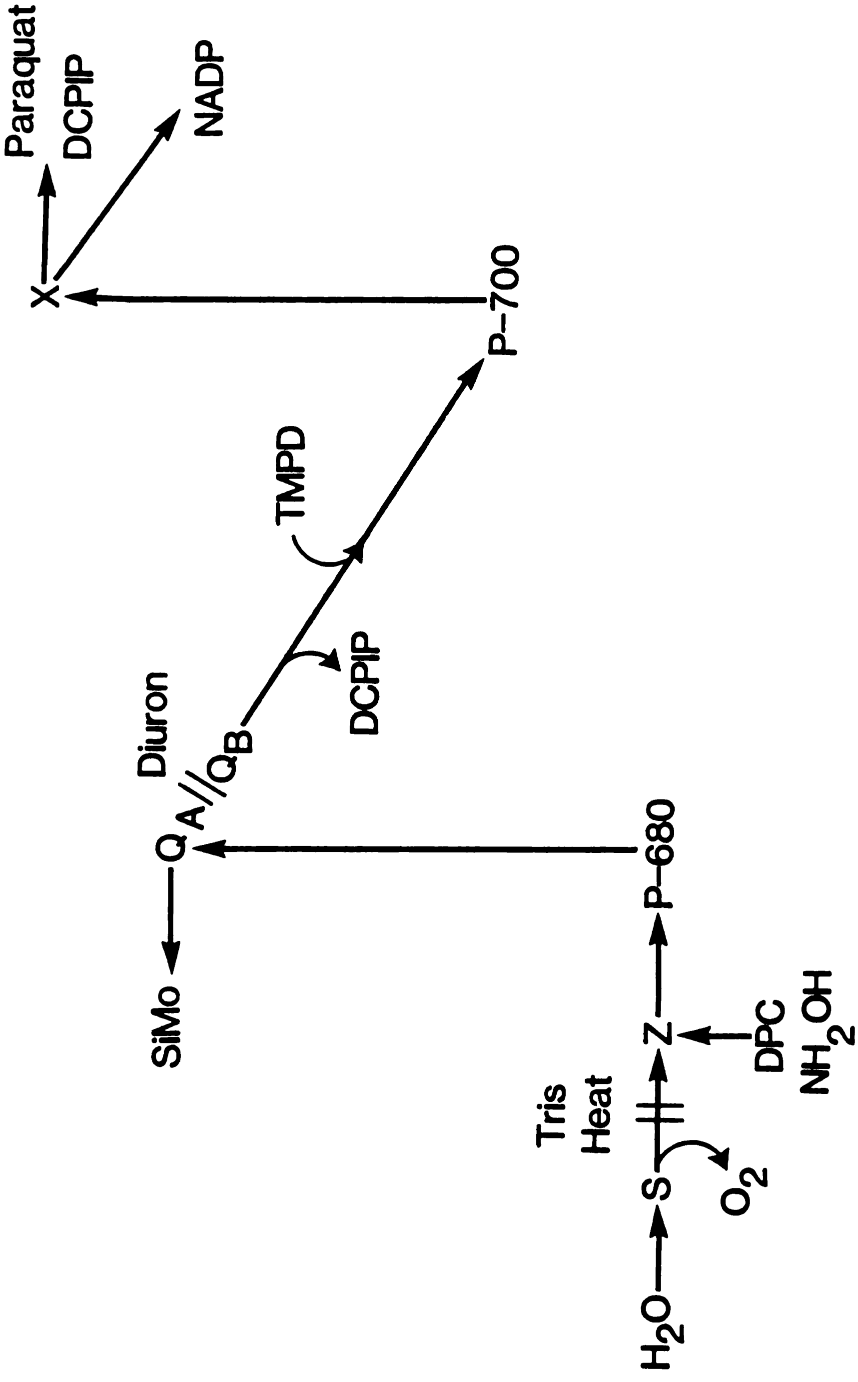
H_2O
S
O_2
Tris
Heat
Z
DPC
NH_2OH
P-680
Q_A
Q_B
Diuron
SiMo
DCPIP
TMPD
P-700
X
Paraquat
DCPIP
NADP

Figure 2. Effect of length of incubation on inhibition of SiMo photoreduction by buthidazole and ioxynil.

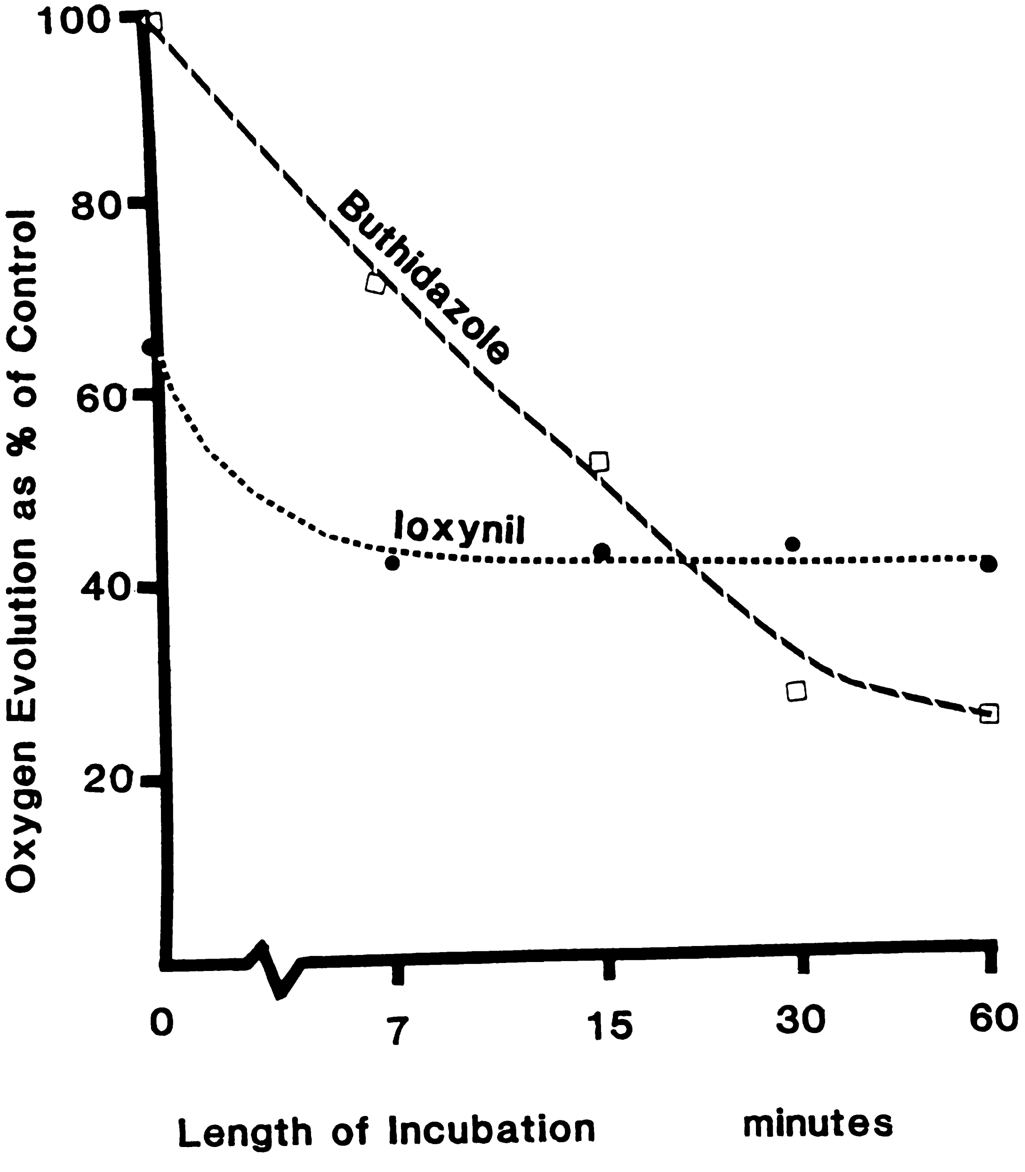
Oxygen Evolution as % of Control
100
80
60
40
20
Buthidazole
Ioxynil
0
7
15
30
60
Length of Incubation
minutes

Table 1. Concentrations of buthidazole and ioxynil which cause 50% inhibition (I_{50}) of photosynthetic electron transport by two assays.

	I_{50} Values (μM)	
Herbicide	DCPIP Photoreduction	SiMo Photoreduction
Buthidazole	0.11	150
Ioxynil	0.70	43

Table 2. Reversibility of inhibition of SiMo photoreduction. (Activities are as a % of a 10 μM diuron control treatment.)

	% Inhibition[a]	
Herbicide	Before Washing	After Washing
Buthidazole	75b	68b
Ioxynil	82b	32a

[a] Means followed by the same letters are not significantly different at the 5% level according to Duncan's multiple range test.

A wide range of herbicides were evaluated at 300 μM concentration for their ability to inhibit SiMo photoreduction (Table 3). Nitrofluorfen [2-chloro-1-(4-nitrophenoxy)-4-(trifluoromethyl)benzene], swep [methyl-N-(3,4-dichlorophenyl)-carbamate], karsil (2-methyl-valeric-3,4-dichloroanilide), and JNP-867 (a 2-cyanoacrylic acid ester derivative) were more active than ioxynil or buthidazole. Nitrofluorfen and JNP-867 caused 100% inhibition at 30 μM (data not shown). These observations suggest that there is herbicidal potential at this secondary site. Diuron was a very active inhibitor.

The response of variable fluorescence to various herbicide concentrations was also evaluated (Table 4). The concentrations of ioxynil and buthidazole tested previously caused inhibition at the secondary site (Table 1). Treatments which inhibit photosynthetic electron transport on the water-oxidizing side of photosystem II, such as Tris-washing or heat treatment, cause a quenching of variable fluorescence (Table 4). Diuron at 10 μM is assumed to inhibit solely on the reducing side of photosystem II. Diuron at 1 mM and buthidazole at 300 μM or 1 mM did not quench variable fluorescence compared to diuron at 10 μM. This indicates that no inhibition was occurring at the immediate electron donor to P_{680}. The secondary site of buthidazole action cannot be at Q_A, since variable fluorescence would not be observed if inhibition occurred at that site. It is possible that buthidazole is reducing the rate constant of a rate limiting electron carrier on the oxidizing side. The reason that variable fluorescence is not quenched may be that charge separation at P_{680} may be rate limiting under the nonsaturating light conditions used for the fluorescence experiments.

Ioxynil quenched variable fluorescence, in contrast to buthidazole. This suggests that it inhibits on the water oxidizing side of photosystem

Table 3. Inhibition of SiMo photoreduction by a wide range of herbicides at a concentration of 300 μM.[a]

Herbicide	% Inhibition
Nitrofluorfen	100 a
Swep	100 a
Karsil	100 a
JNP-867	100 a
Ioxynil	94 a
Buthidazole	83 b
Diuron	61 c
Metribuzin[b]	49 d
Tebuthiuron[c]	35 e
Propanil[d]	29 ef
Bromoxynil[e]	28 ef
Bromacil[f]	27 ef
Desmedipham[g]	25 fg
Dinoseb[h]	18 gh
Atrazine	15 h
Pyrazon[i]	-2 i
Bentazon[j]	-7 i

[a] Means followed by the same letters are not significantly different at the 5% level by Duncan's multiple range test.

[b] 4-amino-6-tert-butyl-3-(methylthio)-as-triazin-5(4H)-one.

[c] N-[5-(1,1-dimethylethyl)-1,3,4-thidiazol-2-yl]-N-N'-dimethylurea.

[d] 3',4'-dichloropropionanilide.

[e] 3,5-dibromo-4-hydroxybenzonitrile.

[f] 5-bromo-3-sec-butyl-6-methyluracil.

[g] ethyl m-hydroxycarbinilate carbanilate (ester).

[h] 2-sec-butyl-4,6-dinitrophenol.

[i] 5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone.

[j] 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide.

Table 4. Variable fluorescence $(F_M-F_0)/F_0$, after various herbicide and chemical treatments.[a]

			$(F_M-F_0)/F_0$	
			Subsequent additions:[b]	
Pretreatment		DPC	NH_2OH	Dithionite
Experiment 1:				
Diuron 10 μM	1.99 d	1.96 d	-	-
Diuron 1 mM	2.03 d	1.99 d	-	-
Ioxynil 30 μM[c]	0.98 f	0.95 f	-	-
Ioxynil 100 μM[c]	0.76 g	0.74 g	-	-
Buthidazole 300 μM	2.29 b	2.27 b	-	-
Buthidazole 1 mM	2.12 bcd	2.08 cd	-	-
Diuron 10 μM	2.25 bc	-	2.28 b	2.53 a
Ioxynil 30 μM[c]	1.26 e	-	1.24 e	1.37 e
Experiment 2:				
Diuron 10 μM	1.31 b	-	1.42 b	-
Ioxynil 100 μM[c]	0.30 d	-	0.26 d	-
Buthidazole 300 μM	1.28 b	-	1.38 b	-
Tris-wash	0.31 d	-	1.62 a	-
Heat	0.23 d	-	0.77 c	-

[a] Means within an experiment followed by the same letters are not significantly different at the 5% level by Duncan's multiple range test.

[b] Additions are listed in the order in which they were used.

[c] Ioxynil decreased the variable fluorescence. However, unlike Tris-washing or heat treatment, this was partially due to an increase in F_0.

II. However, variable fluorescence was not restored by the addition of NH_2OH or DPC. This was unlike the tris-washed or heat-treated thylakoids. DPC did not restore variable fluorescence either. This suggests that the site of inhibition of ioxynil is on the oxidizing side of photosystem II, but at an electron carrier which is required for electron donation by NH_2OH or DPC.

The herbicidal activity of buthidazole metabolites was also evaluated. All of the buthidazole metabolites had I_{50} values greater than those of buthidazole (Table 5). All of the buthidazole metabolites, except for the amine and urea, had I_{50} values less than 10 μM and therefore have potential physiological significance as *in vivo* inhibitors. The amine and the urea metabolites, the proposed final products or buthidazole metabolism (Yu *et al*., 1980), have very low inhibitory activity. Metabolism of buthidazole is therefore a detoxification process. The buthidazole metabolites inhibited SiMo photoreduction less than buthidazole (Table 6). The slow onset of buthidazole activity has been reported previously (York *et al*., 1981). Buthidazole metabolites showed varying degrees of delay in activity (Table 7); however, the dehydrate showed no delay in activity.

Buthidazole was reported to be more abundant than any of its metabolites in pigweed (*Amaranthus retroflexus* L.) (Hatzios and Penner, 1982), quackgrass (*Agropyron repens* (L.) Beauv.) (Hatzios and Penner, 1980), and corn (*Zea mays* L.) (Yu *et al*., 1980) over a time course of 6, 6, or 25 days, respectively. A polar metabolite presumed to be a conjugate (Hatzios and Penner, 1980), and the amine (Yu *et al*., 1980) were reported to be rapidly formed in alfalfa (*Medicago sativa* L.). The amine has low herbicidal activity (Table 5) and the polar metabolite would not be expected to be active as a herbicide due to the hydrophobic

Table 5. I_{50} and I_{80} values for buthidazole and its metabolites measured by DCPIP photoreduction.

Metabolite	I_{50} (μM)	I_{80} (μM)
Buthidazole	0.11	0.32
Methylurea	0.36	1.4
Methylol	0.95	3.7
Dehydrate	1.2	5
Dihydroxy	1.5	3.5
Desmethyl	1.7	8.5
Desmethyl-dihydroxy	9.1	30
Amine	170	-
Urea	210	-

Table 6. Inhibition of SiMo photoreduction by buthidazole and its metabolites. All compounds were tested at 300 μM.

Metabolite	Inhibition (%)
Buthidazole	83 a
Dihydroxy	31 b
Methylurea	21 bc
Methylol	19 bc
Dehydrate	9 cd
Desmethyl-dihydroxy	1 d
Desmethyl	-1 d
Amine	-1 d
Urea	-3 d

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[a] Means followed by the same letters are not significantly different at the 5% level by Duncan's multiple range test.

Table 7. Time for 50% inhibition, using concentrations which caused 80% inhibition at 15 min. (Table 5) measured by DCPIP photoreduction.

Metabolite	Time for 50% inhibition (min.)
Dehydrate	0
Desmethyl	1.4
Methylurea	1.5
Methylol	3.0
Buthidazole	6.2
Desmethyl-dihydroxy	6.6
Dihydroxy	7.2

nature of the herbicide binding site (Hirschberg and McIntosh, 1984). The highly active metabolites reported in Table 5 were not abundant in any of the species tested (Hatzios and Penner, 1980; 1982; Yu *et al.*, 1980). Therefore the *in vivo* toxicity of the buthidazole metabolites is presumably low in the plant species evaluated.

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CHAPTER 6

Studies on the Mechanism of Paraquat Resistance in *Conyza linefolia*

ABSTRACT

A biotype of *Conyza linefolia** originating in Egypt is resistant to the herbicide, paraquat (1,1'-dimethyl-4,4'-bipyridinium ion). The resistant and susceptible (wild) types were indistinguishable by measuring *in vitro* photosystem I partial reactions using paraquat, diquat (6,7-dihydrodipyrido [1,2-α:2',1'-c] pyrazinediium ion), or triquat (7,8-dihydro-6H-dipyrido [1,2-α:2',1'-c] [1,4] diazepinediium ion) as electron acceptors. Therefore, an altered site of action is not the basis for resistance. Chlorophyll fluorescence measured *in vivo* is quenched in the susceptible biotype by leaf treatment with the bipyridinium herbicides. Resistance to quenching of *in vivo* chlorophyll fluorescence was observed in the resistant biotype, indicating that the herbicide was excluded from the active site. Penetration of the cuticle by ^{14}C-paraquat was greater in the resistant biotype than the susceptible biotype; therefore resistance was not due to differences in uptake. ^{14}C was localized in vascular regions of the resistant biotype when excised leaves were supplied ^{14}C paraquat through the petiole. It is proposed that the mechanism of

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*Genus and species were communicated to A. Dodge by M. Parham (ICI; Jealott's Hill, England). Taxonomists have used "*C. linifolia*" (not *C. linefolia*) in several instances to identify different plant species. At least two such species have been reclassified under a different genus. The true identify of the species studied here must therefore be questioned, and no botanical designation can be given.

resistance to paraquat is exclusion from the protoplast by adsorption to the extracellular matrix. However, direct evidence of selective adsorption to a cell wall fraction was not obtained by sucrose density gradient fractionation.

INTRODUCTION

Weed resistance to paraquat (Figure 1) has been reported in annual bluegrass (*Poa annua* L.), Philadelphia fleabane (*Erigeron philadelphicus* L.) and *Conyza linefolia* in England, Japan, and Egypt, respectively (Gressel *et. al.*, 1982). In every case, paraquat was applied several times per year for more than 5 years. Paraquat tolerant lines of perennial ryegrass [*Lolium perenne* L. (Causeway)] have also recently been developed (Harvey and Harper, 1982).

The resistant biotype of *C. linefolia* originated in the Tahrir irrigation area in Egypt. An intensive paraquat spraying program was undertaken in vine and citrus plantations in 1970 and difficulties in controlling this weed were first observed in the mid 1970's (Parham, 1982). The resistant biotype survived 2 kg/ha paraquat while the wild (susceptible) type was controlled by 0.5 kg/ha. The resistant biotype was controlled by 1 kg/ha diquat, but only 0.2 kg/ha was required for the wild type (Parham, 1983). Thus cross resistance to another bipyridinium herbicide was evident.

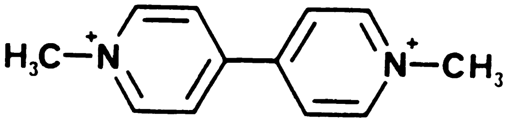
Members of the genus *Conyza* are common weeds in the U.S. and are commonly called horseweed or marestail. These weeds have become a locally serious agronomic problem in reduced tillage systems (Anonymous, 1983), including some areas of Michigan (J. Kells, personal communication). These weed species proliferate under conditions in which weed control often depends upon the use of paraquat. It is possible that paraquat resistance may become a problem in this genus in the U.S. due to the trend toward reduced tillage and heavier reliance upon paraquat.

Genetic engineering and conventional breeding techniques are being used to transfer triazine resistance into susceptible crop species (LeBaron, 1983). It is possible that resistance to paraquat can be exploited by the same means. The paraquat resistance trait in C. linefolia may not confer a yield loss since dry weight accumulation was equal in the two biotypes in greenhouse experiments (Parham, 1983).

The mechanism of paraquat action involves the photosystem I-mediated reduction of the paraquat di-cation. This results in the formation of the mono-cation radical. The mono-cation radical reduces O_2 to $O_2^{\cdot -}$, the superoxide anion radical, resulting in the regeneration of the paraquat di-cation. Subsequently, hydrogen peroxide and the hydroxyl radical ($OH^{\cdot}$) may be produced by a variety of reactions (Dodge, 1982; 1983). Hydroxyl radicals cause peroxidation of fatty acids. This is apparently a cause of the observed loss of membrane integrity (Harris and Dodge, 1972; Hutchison, 1979; Dodge 1983). Superoxide and hydrogen peroxide may not directly cause the paraquat-induced loss of membrane integrity (Hutchison, 1979). In addition to the formation of reactive forms of oxygen, the presence of paraquat causes the diversion of electrons which normally would reduce NADP. Cellular protective mechanisms which utilize reducing power, including carotenoids, α-tocopherol, glutathione, and ascorbate, are assumed not to be maintained in the presence of paraquat. The action of superoxide dismutase, catalase, and peroxidase would presumably remain unaffected, however.

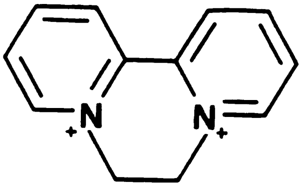
Several hypotheses were developed which could explain the mechanism of paraquat resistance in C. linefolia. These were: 1) detoxification of the superoxide anion radical or other reactive forms of oxygen produced in

Figure 1. Structures and redox potentials of the three bipyridinium herbicides tested.



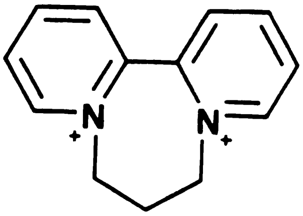
Paraquat

−446 mV



Diquat

−349 mV



Triquat

−538 mV

the presence of paraquat; 2) alteration in the redox potential of the photosystem I primary electron acceptor; 3) detoxification of paraquat; and 4) altered compartmentalization of paraquat, resulting in reduced localization of the herbicide at the active site.

There have been several studies on the possible mechanism(s) of paraquat resistance. A three-fold increase in superoxide dismutase activity in the resistant C. linefolia has been reported (Youngman and Dodge, 1981). Activities of superoxide dismutase, catalase, and peroxidase were 50%, 32%, and 35% higher, respectively, in paraquat resistant lines of perennial ryegrass (Harvey and Harper, 1982). Resistance in perennial ryegrass could not be attributed to differences in uptake, translocation or metabolism of paraquat. Therefore, resistance to paraquat may in part be due to dismutation of superoxide. Resistance to paraquat but not diquat has been observed when measuring CO_2 fixation and chlorophyll loss (Dodge, personal communication). The redox potential of paraquat is more negative than that of diquat (Figure 1). This is consistent with a hypothesis that the site of action of these herbicides is modified in the resistant biotype, such that the redox potential of the electron donor to these herbicides is less negative. The objective of this study was to examine the validity of the hypotheses discussed above.

METHODS AND MATERIALS

Photosystem I partial reaction. Chloroplast thylakoid membranes of resistant and susceptible biotypes of C. linefolia were isolated as previously described (Chapter 1). Rates of photosystem I-mediated electron flow from reduced TMPD (N,N,N',N'-tetramethyl-p-phenylenediamine) to paraquat (Figure 1, Chapter 5), diquat, and triquat were monitored as oxygen uptake using an oxygen electrode. The bipyridinium herbicides selected represent a wide range of redox potential (Figure 1). Rates of electron transport were measured under saturating light by continuous recording of O_2 uptake using a water-jacketed Clark-type oxygen electrode maintained at 20°C. The assay buffer contained 50 μg chlorophyll·ml^{-1}, 50 mM Tricine-NaOH (pH 7.8), 100 mM sorbitol, 1mM NH_4Cl, 0.1 μM gramicidin, 5 mM $MgCl_2$, 10 mM NaCl, 100 μM NaN_3, 10 μM diuron, 2.5 mM ascorbate, 25 μg/ml superoxide dismutase and 100 μM TMPD. Herbicide concentrations were varied as indicated in the results.

Dose-response effects on excised leaves. Leaves were excised under water and dipped in solutions containing a range of herbicide concentrations and 0.5% surfactant [alkylaryl polyoxyethylene glycol-free fatty acid-isopropanol (X-77 Spreader)]. Excised leaves were supported by a sponge, in a water-filled 18 by 150 mm test tube. There were four replications of each treatment and a different plant was used for each replicate. Leaves were placed in darkness for 4 h to allow uptake of the herbicides.

In vivo chlorophyll fluorescence was monitored with a model SF-10 fluorimeter (Richard Brancker Research Ltd.) as described previously (Ahrens et al., 1981). Transients were recorded with a Nicolet Explorer digital oscilloscope. The variable fluorescence values reported represent $(F_P - F_0)/F_0$, where F_P is the peak fluorescence value (recorded at about 2 sec. in this experiment; Figure 3) and F_0 is the fluorescence level at which the variable component of fluorescence begins to change (Figure 5). After recording the fluorescence transients, the leaves were moved to a chamber at 25°C with white light (450 $\mu E \cdot m^{-2} \cdot s^{-1}$) for 5 h. Leaves were then placed in darkness for 24 h to allow drying of injured tissue. Injury was evaluated by visually estimating the percent green leaf area. Percent moisture was determined by measuring fresh and dry weights.

Cuticular penetration. Leaves were excised under water and supported in test tubes as described earlier. A 50 μl solution, containing 0.16 μCi ^{14}C-paraquat (0.13 mCi·mmol^{-1}) and 0.5% X-77 surfactant, was applied uniformly to both surfaces of the leaf in 0.5 μl droplets. This dose of paraquat causes injury in the susceptible biotype but not the resistant biotype (data not shown). After 4 h, leaves were dipped in water for 2 min., blotted dry, dipped in chloroform 3 times and then wiped with a glass filter paper wetted with chloroform. The chloroform extract was allowed to dry. The purpose of the chloroform dips and wipes was to remove the cuticle. The amount of ^{14}C paraquat penetrating the cuticle was estimated by subtracting the amount of ^{14}C obtained in the washes from the amount applied. Liquid scintillation spectrometry was used to measure ^{14}C. Leaf areas were determined on a model LI-300 portable area meter (Lambda Instruments Corp.). There were three replications for each biotype, and a different plant was used for each replicate.

Autoradiography. Uniform leaves approximately 150 mg fresh weight were excised as previously described and placed in a 0.8 ml vial containing 50 1 of a solution with 0.064 μCi ^{14}C-paraquat (1.4 mCi·mmol^{-1}) in 10 mM pH 7 phosphate buffer. This dose of paraquat selectively desiccated the susceptible biotype when placed in sunlight (data not shown). In another experiment, leaves were placed in an unbuffered solution of pH 3 with the same amount of ^{14}C-paraquat. Distilled water was added to maintain the solution level over a 4 h period. In a second experiment, leaves were transferred after 4 h from the initial vial to a second vial containing either water or 24 mg·ml^{-1} nonlabelled paraquat (100X higher than the concentration in the original radioactive solution). Leaves were kept in dim light and showed no signs of paraquat injury except in leaves treated at pH 3, where 10% and 15% injury was visually estimated in the resistant and susceptible biotypes, respectively. Leaves were then lyophilized overnight and mounted. X-ray film was placed in contact with the leaves for 36 hr. All experiments contained at least three replications of each treatment for each biotype and utilized a different plant for each replicate. Representative autoradiograms are shown.

Sucrose density gradient. A linear sucrose density gradient, from 5 to 60% sucrose with a 1.5 ml 70% sucrose cushion was prepared in a 12 ml centrifuge tube. The gradient contained 50 mM tricine-NaOH (pH 7.8), 10 mM NaCl and 5 mM $MgCl_2$. One leaf was excised from each of three resistant and three susceptible plants. ^{14}C-paraquat was supplied to these leaves in the same manner as in the autoradiography experiments. Leaves were ground individually at 4°C in a mortar and pestle in 0.8 ml 5% sucrose, 50 mM Tricine-NaOH pH 7.8, 10 mM NaCl and 5mM $MgCl_2$. After grinding, 0.5 ml water was added, and 1.0 ml of the extract was loaded onto the gradient. The gradients were centrifuged at 55,000 g for 2.5 h at 2°C in a swinging bucket rotor. Fractions of 0.85 ml were removed from the gradient and the 1.5 ml cushion containing cellular debris was removed separately. The location of chloroplasts and other subcellular fractions were monitored by spectrophotometric determinations of chlorophyll (MacKinney, 1941) and absorbance at 280 nm (A_{280}). A 0.5 ml aliquot of each fraction was solubilized with 2.0 ml of NCS tissue solubilizer (Amersham Corp.) at 20°C for 24 hr and then bleached with 350 µl benzoyl peroxide (100 mg/ml) at 40°C for 4 h. ^{14}C was then determined by liquid scintillation spectrometry.

RESULTS AND DISCUSSION

Photosystem I partial reaction. Since the bipyridinium herbicides act as electron acceptors, rates of electron transport increased as herbicide concentration increased (Figure 2). The responses of the two biotypes were indistinguishable for all three herbicides. Therefore an altered site of action does not appear to be responsible for resistance. Triquat activity (Figure 2C) was less than that of paraquat (Figure 2A) or diquat (Figure 2B). This is presumably due to its highly negative redox potential. Paraquat and diquat had similar activities (Figures 2A and B).

Dose-response effects on excised leaves. Resistance to the bipyridinium herbicides was observed by desiccation and by visual estimation of % green leaf area (Figure 3). Since diquat and triquat are structurally quite similar but differ greatly in their redox potentials (Figure 1), a larger degree of resistance to triquat would be anticipated if resistance is due to an altered site of action, i.e. an altered redox potential of the photosystem I primary acceptor. However, the degree of resistance to triquat was quite similar to that of diquat (Figures 3 and 4; Table 1). This confirms previous discussion indicating that a modified site of action is not the basis for resistance.

Bipyridinium herbicides quench chlorophyll fluorescence by diverting electron flow from photosystem I. Quenching of *in vivo* chlorophyll fluorescence transients by paraquat is shown in Figure 5. Paraquat caused quenching in both biotypes, but much higher concentrations were required in the resistant biotype. All three bipyridinium compounds caused quenching of *in vivo* fluorescence (Figure 4). Higher concentrations of all herbicides were required to cause quenching in the

Figure 2. Effect of bipyridinium herbicide concentration on photosystem I-mediated electron transport in resistant (R) and susceptible (S) biotypes, using TMPD as the electron donor.

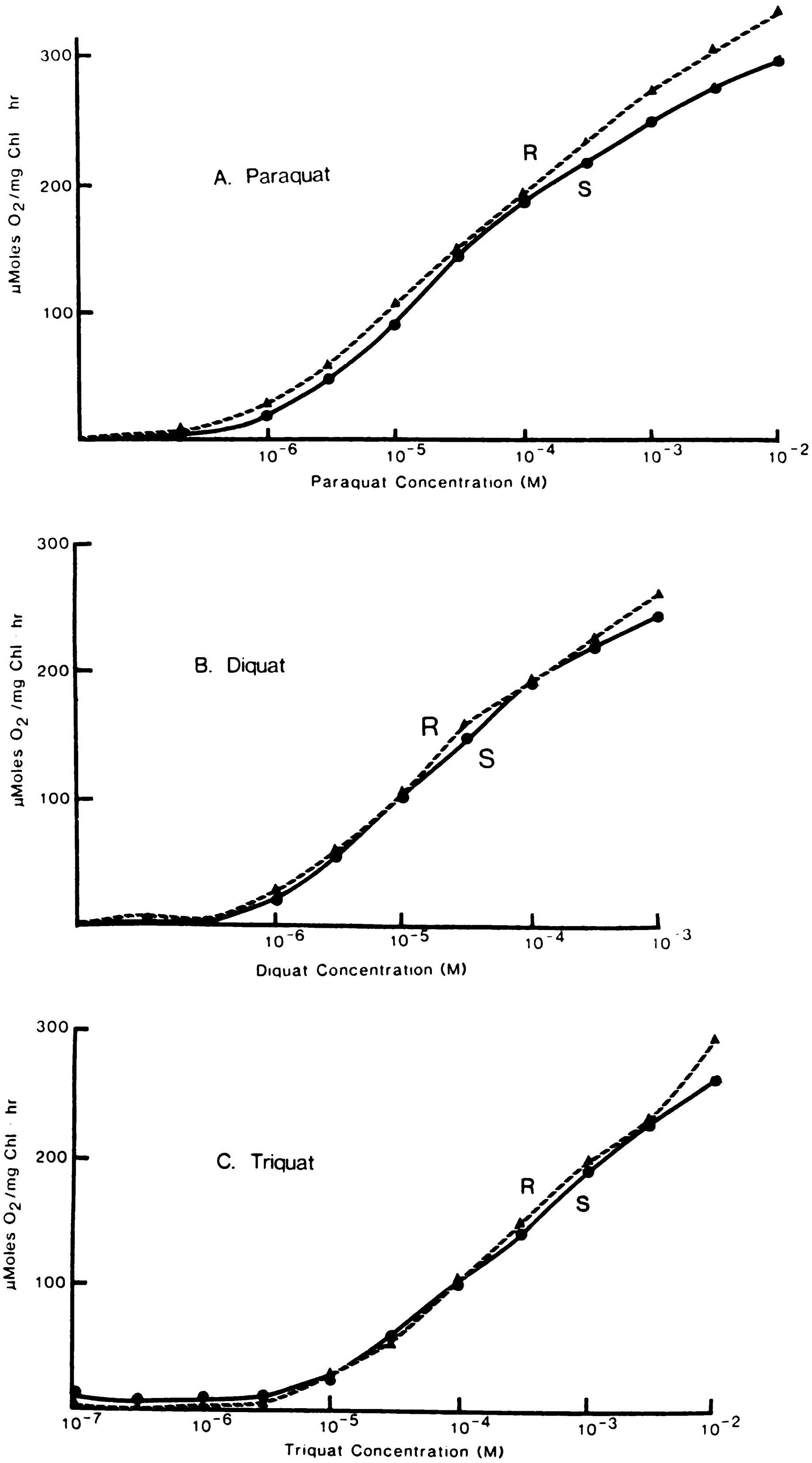

A. Paraquat
R
S
300
200
100
μMoles O_2/mg Chl · hr
10^{-6}
10^{-5}
10^{-4}
10^{-3}
10^{-2}
Paraquat Concentration (M)
B. Diquat
R
S
300
200
100
μMoles O_2 /mg Chl · hr
10^{-6}
10^{-5}
10^{-4}
10^{-3}
Diquat Concentration (M)
C. Triquat
R
S
300
200
100
μMoles O_2/mg Chl · hr
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
10^{-2}
Triquat Concentration (M)

Figure 3. Response of % leaf area remaining green (open symbols) and % moisture (closed symbols) to bipyridinium herbicide concentration in excised leaves of resistant (R) and susceptible (S) biotypes.

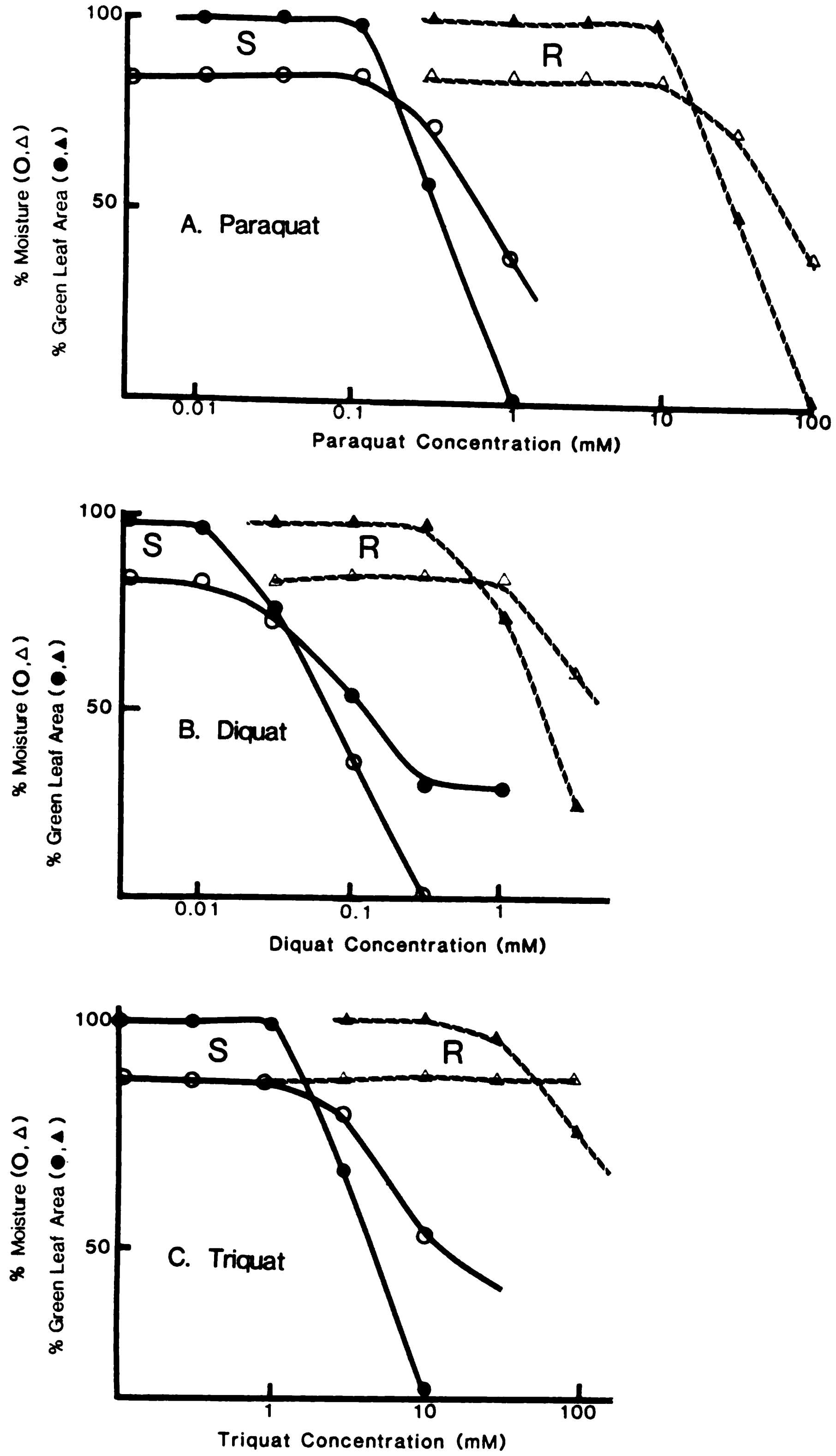
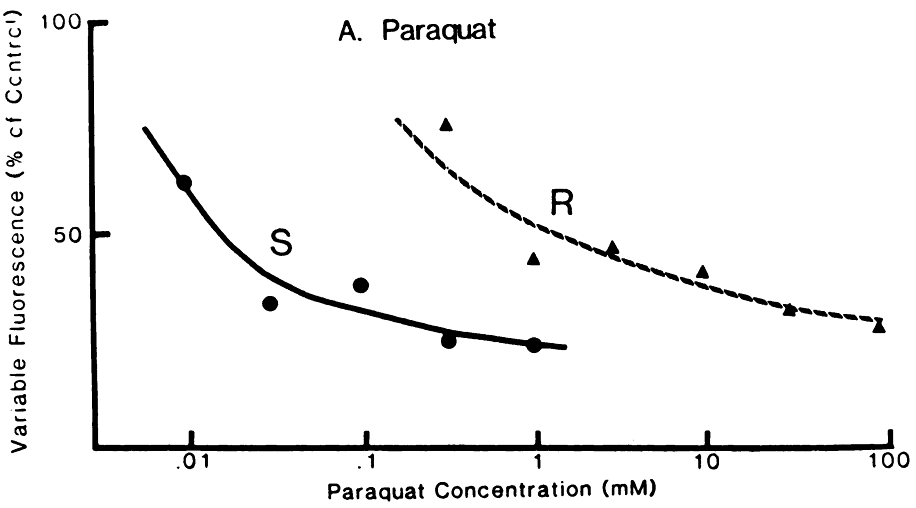
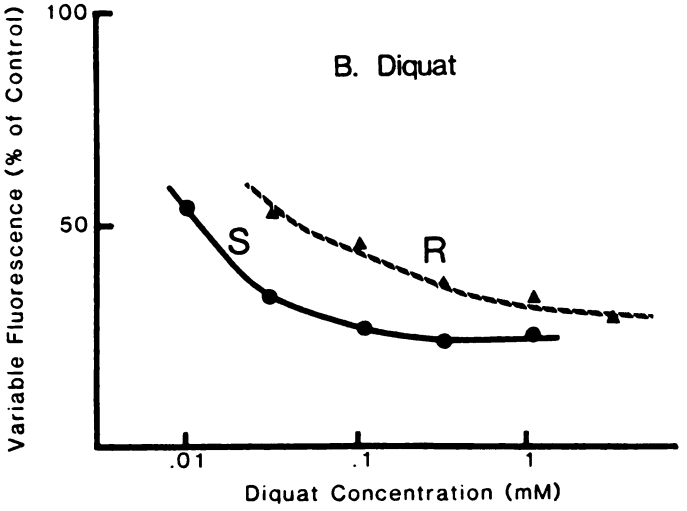

100
S
R
% Moisture (O,Δ)
% Green Leaf Area (●,▲)
50
A. Paraquat
0.01
0.1
1
10
100
Paraquat Concentration (mM)
100
S
R
% Moisture (O,Δ)
% Green Leaf Area (●,▲)
50
B. Diquat
0.01
0.1
1
Diquat Concentration (mM)
100
S
R
% Moisture (O,Δ)
% Green Leaf Area (●,▲)
50
C. Triquat
1
10
100
Triquat Concentration (mM)

Figure 4. Response of *in vivo* fluorescence to bipyridinium herbicide concentration in excised leaves of resistant (R) and susceptible (S) biotypes.


A. Paraquat


B. Diquat

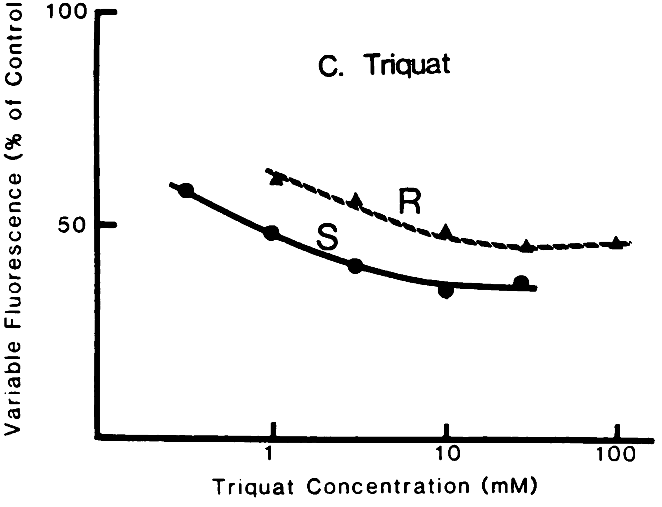

C. Triquat

Figure 5. Response of *in vivo* fluorescence transients to paraquat in excised leaves of resistant and susceptible biotypes.

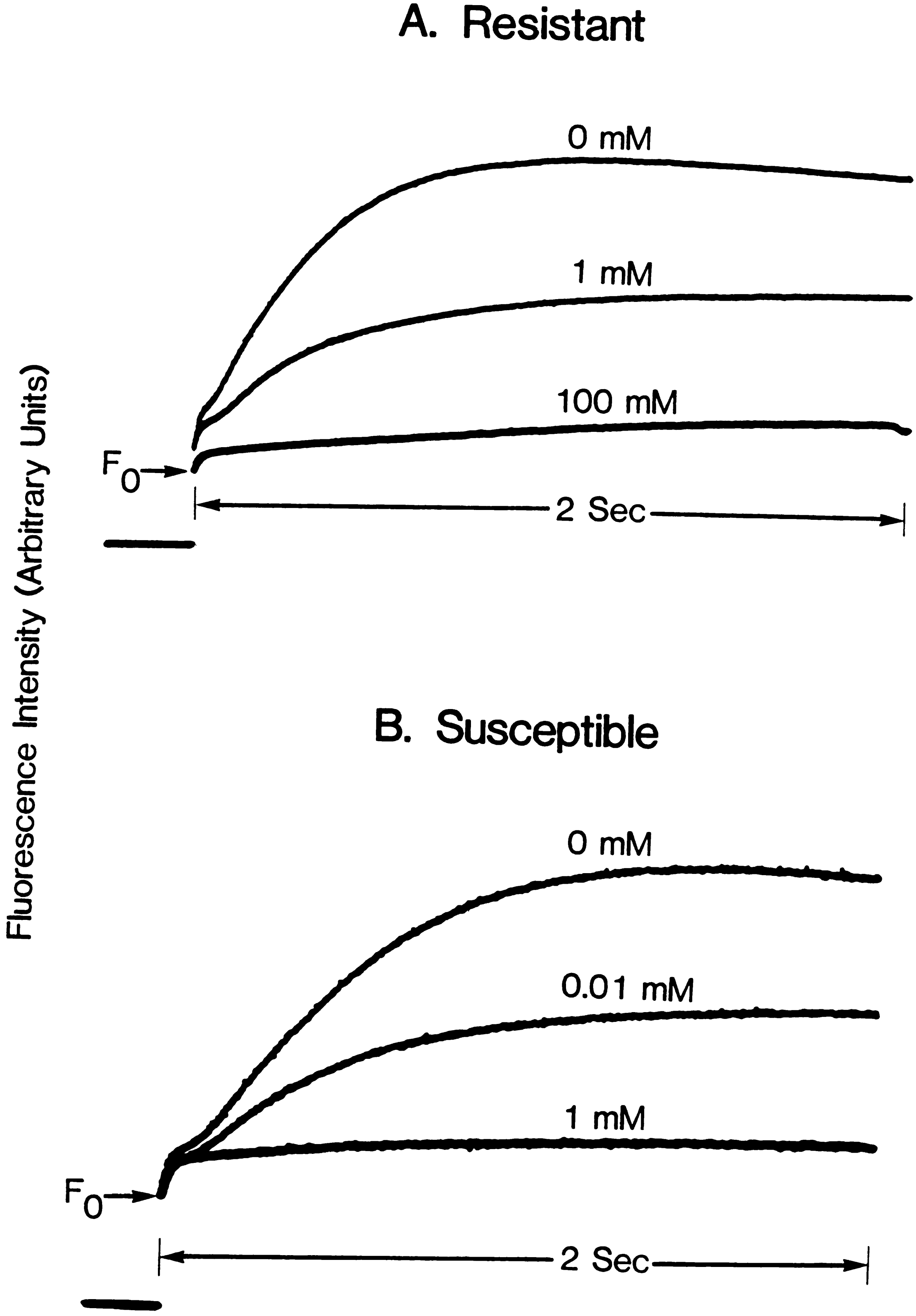

A. Resistant
0 mM
1 mM
100 mM
F0
2 Sec
Fluorescence Intensity (Arbitrary Units)
B. Susceptible
0 mM
0.01 mM
1 mM
F0
2 Sec

Table 1. Estimated concentrations which cause 50% injury by reduction of *in vivo* chlorophyll fluorescence, % moisture or % of leaf area remaining green. The resistance ratio was estimated by dividing the I_{50} for the resistant biotype by the I_{50} for the susceptible biotype.

Herbicide	Method of Estimation of Injury	I_{50} Resistant Biotype (mM)	I_{50} Susceptible Biotype (mM)	Resistance Ratio
Paraquat	Chlorophyll Fluorescence	2.3	0.015	150
	% Moisture	62	0.62	100
	% Green Leaf Area	30	0.32	94
Diquat	Chlorophyll Fluorescence	0.060	0.013	4.6
	% Moisture	4.5[a]	0.12	38
	% Green Leaf Area	1.7	0.064	27
Triquat	Chlorophyll Fluorescence	7.2	0.078	9.2
	% Moisture	(b)	7.3	-
	% Green Leaf Area	130[a]	3.6	35

[a] Projected value.

[b] No % moisture response to triquat at the concentrations tested.

Table 2. Cuticular penetration of ^{14}C paraquat.[a]

Biotype	Average leaf area (cm^2)	Aqueous wash (% of applied)	Chloroform wash (% of applied)	Amount penetrating cuticle (% of applied[b])
Resistant	7.8	70.0 b	0.05 a	33.0 a
Susceptible	9.6	78.8 a	0.07 a	21.4 b

[a] Means within columns followed by the same letters are not significantly different at the 5% level according to Duncan's multiple range test.

[b] Calculated by subtraction.

resistant biotype. Resistance to quenching of *in vivo* fluorescence by the bipyridinium herbicides must be attributed to exclusion of the herbicides from the active site, since the quenching of fluorescence is a result of the effects of the herbicides at their primary site of action. This exclusion might occur by rapid detoxification or by compartmentalization. A proposed resistance mechanism involving detoxification of superoxide (Youngman & Dodge, 1981) or other reactive forms of oxygen must be considered to be of secondary importance in providing resistance since the primary basis for resistance involves exclusion from the active site.

Resistance ratios and herbicide concentrations which caused 50% injury, as evaluated by the three techniques discussed are shown in Table 1. Resistance to paraquat and cross resistance to diquat were observed for all herbicides by all methods employed. However, the resistance ratios for paraquat were larger than those for diquat or triquat. The resistance mechanism is therefore somewhat specific for the herbicide which provided the selective force in the field. The resistance ratios determined for paraquat by the three different methods are in reasonably close agreement (Table 1). The similarity of the ratios is consistent with the generally accepted principle that paraquat causes plant injury by its effects on the chloroplast. However, the resistance ratios determined for diquat and triquat were lower by chlorophyll fluorescence than by the other method(s). It is possible that diquat or triquat moved from the chloroplast subsequent to the chlorophyll fluorescence determinations, since chlorophyll fluorescence was determined 4 h after treatment, and the other determinations were made 24 h later.

Diquat was the most active of the three herbicides. The ranking of I_{50} values within any biotype, for any method used, yields the order: I_{50} diquat < I_{50} paraquat < I_{50} triquat (Table 1). Therefore, herbicidal activity corresponds quite closely with the redox potential of these herbicides (Figure 1), with a less negative redox potential corresponding to a more active herbicide. High herbicidal activity in bipyridinium compounds is known to require a redox potential in the range of -350 to -450 mV (Summers, 1980). This explains the relatively low activity of triquat. It was previously noted that resistance was observed to paraquat but not to diquat when measuring CO_2 fixation and chlorophyll loss (Dodge, personal communication). It is possible to attribute the failure to observe resistance to diquat to the use of a single herbicide concentration which may have been too high and therefore nonselective with respect to the two biotypes.

Quenching of fluorescence, by all herbicides tested, begins at herbicide concentrations far below those that cause loss of moisture or chlorophyll (Figures 3 and 4). Also, the I_{50} for fluorescence quenching was much lower than the I_{50} for % moisture or % green leaf area (Table 1) for each herbicide and biotype combination. It is possible that sufficient reducing power is still generated to maintain protective mechanisms when fluorescence is only partially quenched, and therefore when electrons are only partially diverted from NADP. Only highly quenched fluorescence is therefore correlated with apparent tissue injury.

Cuticular penetration. Table 2 indicates that most of the ^{14}C-paraquat applied was removed in the aqueous wash and that more ^{14}C was removed in the aqueous washes of the susceptible biotype. Very little ^{14}C was removed in the chloroform wash in either biotype. Therefore, considerably more ^{14}C-paraquat penetrated the cuticle of the resistant biotype. The mean leaf areas of the two biotypes differed; however it is difficult to explain the difference in cuticular penetration by this alone. Parham previously reported by personal communication (Harvey and Harper, 1982) that paraquat adsorption on leaf tissue was greater in the resistant biotype of this species. This is consistent with our observation. Resistance to paraquat cannot be explained by differences in cuticular penetration.

Autoradiography. ^{14}C-Paraquat at pH 7 became uniformly distributed in the leaves of the susceptible biotype, but was localized in vascular regions especially in the lower petiole, in the resistant biotype (Figure 6). Therefore a compartmentalization mechanism may be responsible for resistance to paraquat. Compartmentalization may be due to adsorption to the extracellular matrix (cell wall). Further distribution of ^{14}C was observed in the resistant biotype when leaves fed ^{14}C-paraquat pH 7 were transferred to a high concentration of unlabelled paraquat (Figure 7). ^{14}C moved out of the petiole region and became somewhat more uniformly distributed in the leaf. Redistribution of ^{14}C was more restricted in the susceptible biotype. These observations are consistent with an hypothesis that ^{14}C paraquat is primarily localized extracellularly in the resistant biotype and primarily intracellularly in the susceptible biotype. ^{14}C was primarily localized in the peripheral parts of the leaves in both biotypes when a pH 3 solution was supplied to the excised leaves (Figure 8). It is possible that protons in this acidic solution were preventing adsorption of paraquat by adsorbtion to the cation exchange sites in the resistant biotype. Greater cuticular penetration of ^{14}C paraquat in the resistant biotype was previously noted. It is possible that the proposed adsorption mechanism provides the driving force for this uptake.

Figure 6. Autoradiograms (A and B) of excised leaves (C and D) fed a solution of ^{14}C paraquat pH 7.

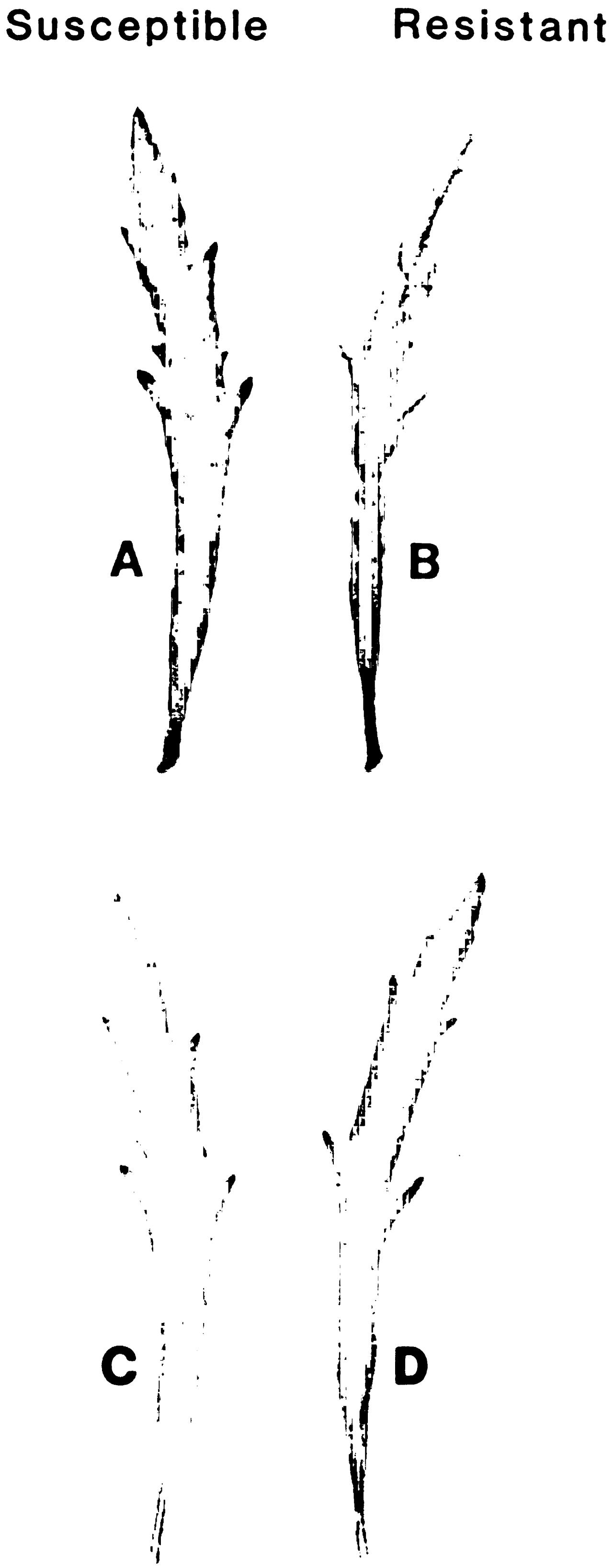

Susceptible
Resistant
A
B
C
D

Figure 7. Autoradiograms (A-D) of leaves (E-H) of leaves fed a solution of ^{14}C paraquat pH 7, and then transferred either to water (A,C,E,G) or to a solution of 24 mg/ml paraquat (B,D,F,H).

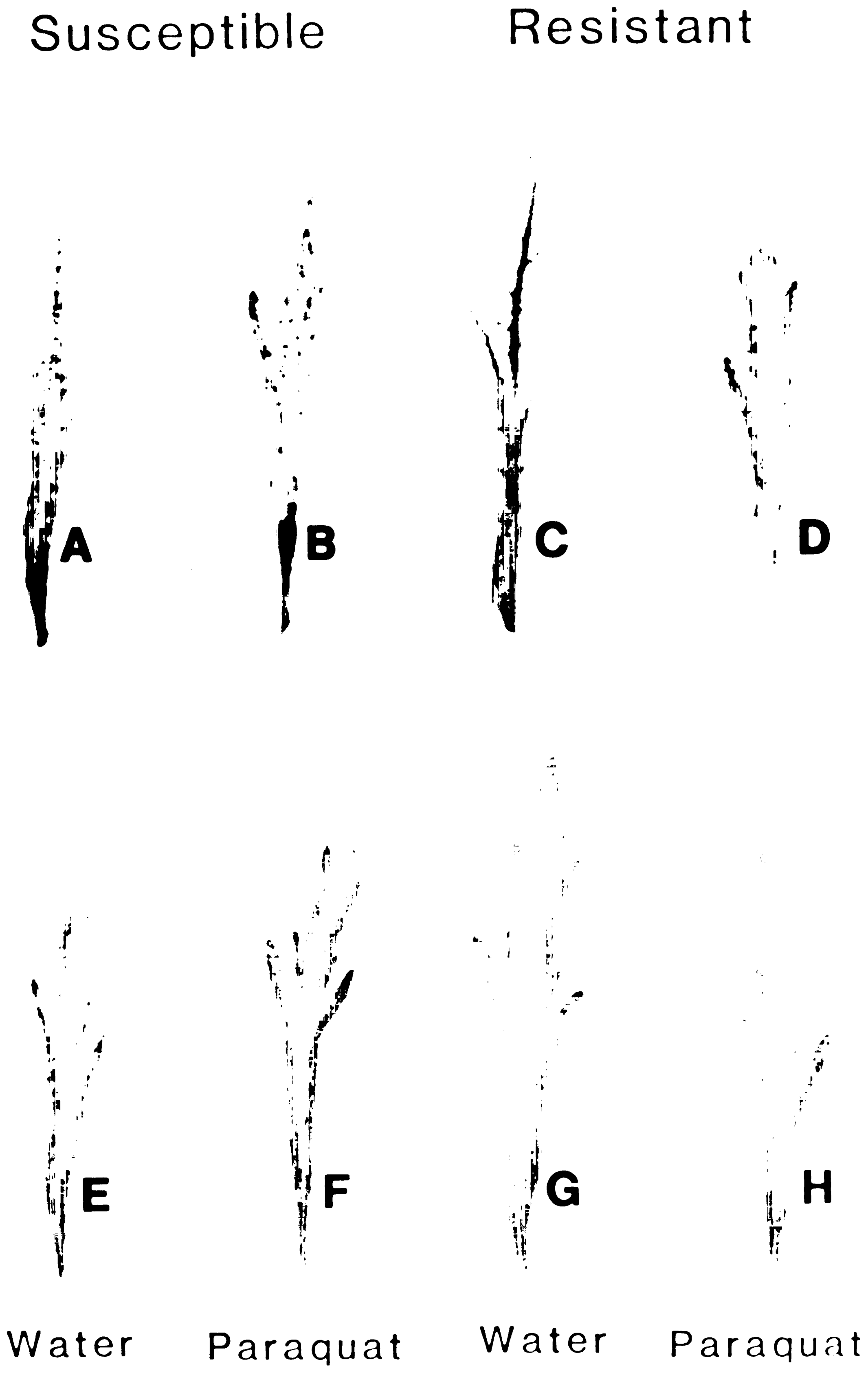

Susceptible
Resistant
A
B
C
D
E
F
G
H
Water
Paraquat
Water
Paraquat

Figure 8. Autoradiograms (A and B) of excised leaves (C and D) fed a solution of ^{14}C paraquat pH 3.


Susceptible
Resistant
A
B
C
D

The ion exchange properties of the cell wall are largely due to uronic acids, especially α 1,4-linked polygalacturonic acid (pectic acid) (Hall *et al.*, 1976). Divalent cations such as Ca^{++} and Mg^{++} bind noncovalently to pectic acid. Therefore paraquat may also bind to pectic acid. Many uronic acids are nonionic due to methylation (Hall *et al.*, 1976). The degree of methylation of uronic acids may be the basis for the difference in tolerance to paraquat in the two biotypes. Paraquat is inactivated in soils by adsorption to anionic soil particles (Akhavein and Linscott, 1968). This may be similar, in principle, to the basis for bio-inactivation in paraquat resistant *C. linefolia*. ^{14}C-atrazine was localized in the vascular regions of an atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] -tolerant line of cucumber (*Cucumis sativus* L.) (Werner and Putnam, 1980). However, since atrazine is not cationic, the basis for tolerance to atrazine in cucumber is probably different from the basis for paraquat resistance in *C. linefolia*.

Sucrose density gradient. The most dense sucrose fraction, containing cell wall materials, had similar amounts of ^{14}C in both biotypes (Table 3). Most of the ^{14}C was recovered in the least dense sucrose fractions (data not shown). It is possible that ^{14}C-paraquat was readily exchangeable under the experimental conditions since the cations present (10 mM Na^+ and 5 mM Mg^{++}) may have displaced paraquat from the cation adsorption sites.

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Table 3. ^{14}C-paraquat in the cushion of the sucrose density gradient.[a]

	Dpm		
Biotype	{As % of total ^{14}C recovered)	(As % of total ^{14}C recovered/A280 as % of total A280 measured)	(As % of total ^{14}C recovered/chlorophyll as % of total chlorophyll measured)
Resistant	12 a	0.49 a	0.66 a
Susceptible	14 a	0.60 a	1.03 a

[a] Means separated by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

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