WHEAT MICROSOMAL CYTOCHROME P450 MONOOXYGENASES: CHARACTERIZATION AND IMPORTANCE IN THE METABOLIC DETOXIFICATION AND SELECTIVITY OF WHEAT HERBICIDES[1]

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SUMMARY

This review describes the important role and significance of cytochrome P450 monooxygenases in the metabolism, detoxification and selectivity of a broad spectrum of herbicides used for weed control in wheat and other cereals. Recent research on the isolation, characterization and reconstitution of induced microsomal cytochrome P450 monooxygenases directly responsible for herbicide oxidation, selectivity and resistance in wheat is emphasized. Reported evidence for the selection of similar wheat-like cytochrome P450 monooxygenases in the evolution of metabolism-based herbicide resistance in grassy weed populations is also presented.

KEY WORDS

wheat, herbicide, metabolism, cytochrome P450, selectivity, resistance

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1. INTRODUCTION

Numerous reports and reviews of herbicide metabolism over the past 30 years have implicated the involvement of cytochrome P450 monooxygenases and Phase I oxidation reactions as important factors in the metabolism, selectivity and detoxification of herbicides in higher plants /1-5/. This is especially true in wheat where a number of chemically unrelated herbicides are metabolized and detoxified by sequential Phase I oxidation and Phase II conjugation reactions that compartmentalize or sequester the herbicides as polar metabolites or bound residues and limit their activity at different sensitive target sites in the plant /6/.

Evidence for the direct involvement of cytochrome P450 monooxygenases in the oxidative metabolism of wheat selective herbicides was first provided in reports of diclofop and chlortoluron oxidation by microsomes from etiolated wheat seedling shoot tissues and cell suspension cultures /7-9/. Since these initial reports in 1989 and 1990, interest in the isolation, purification and further characterization of cytochrome P450 monooxygenases from wheat has increased rapidly. Representative members from most of the major classes of herbicides currently used for selective weed control in wheat have now been shown to be oxidized by induced microsomal cytochrome P450 monooxygenases in wheat (Table 1).

TABLE 1

Cytochrome P450-dependent oxidations of herbicides by wheat microsomes

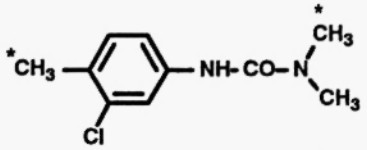
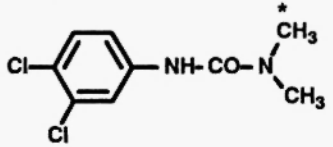
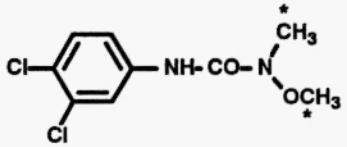
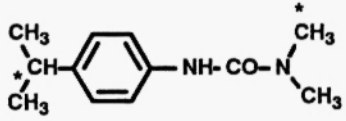
Herbicide		Reaction(s)	References
Phenylureas			
Chlortoluron		Alkyl hydroxylation and N-demethylation	(9), (10), (11), (12), (16)
Diuron[1]		N-demethylation	(10)
Linuron		N-dealkoxylation and N-demethylation	(13)
Isoproturon[1]		Alkyl hydroxylation and N-demethylation	(10)

TABLE 1 (continued)

Aryloxyphenoxypropanoates			
Diclofop (acid)		Aryl hydroxylation with NIH shift	(7), (8), (12), (13), (14)
Phenoxyalkanoic acids			
2,4-D[1]		Aryl hydroxylation with NIH shift	(12)
Diphenylethers			
Bifenox (acid)[1]		Aryl hydroxylation with NIH shift	(15)
Sulfonylureas			
Chlorsulfuron		Aryl hydroxylation	(12), (13), (16)

Triasulfuron		Aryl hydroxylation	(13), (16), (17)
Prosulfuron		Alkyl hydroxylation, aryl hydroxylation and O-demethylation	(16), (18)
Triazolopyrimidine sulfonanilides			
Flumetsulam		Aryl hydroxylation and alkyl hydroxylation	(19)
Chloroacetamides			
Metolachlor[1]		O-demethylation	(16)

*Oxidation sites.

[1]Tentative identification of oxidation products.

This review summarizes recent studies on microsomal cytochrome P450 monooxygenases responsible for herbicide detoxification in wheat and also examines evidence for the involvement of similar oxidative enzymes in grassy weed populations that have evolved metabolism-based cross resistance to many of the herbicides currently used for selective weed control in wheat /20,21/.

2. HERBICIDE METABOLISM AND CYTOCHROME P450 MONOOXYGENASES IN WHEAT

2.1. Phenylureas

Metabolism studies with a number of different substituted phenylurea herbicides in tolerant and sensitive plants have shown that Phase I oxidation reactions are important factors in the detoxification and selectivity of this broad class of photosystem II inhibitors /1-4,22-24/. Alternative pathways for the oxidative metabolism and detoxification of chlortoluron in wheat are shown in Figure 1. Chlortoluron tolerance in wheat depends primarily on an initial oxidation of the ring-methyl group followed by conjugation of the hydroxylated metabolite with glucose /25,26/. Limited detoxification by partial oxidative N-demethylation also occurs. In contrast to wheat, sensitive cereal varieties and grassy weeds have a reduced capacity for ring-methyl hydroxylation and chlortoluron detoxification is limited primarily to partial N-demethylation /26-28/.

Indirect evidence for cytochrome P450 involvement in the oxidative metabolism and detoxification of chlortoluron and isoproturon in wheat has been provided by *in vivo* metabolism studies with inhibitors and inducers of cytochrome P450 monooxygenase activity /29-34/. Cytochrome P450 inhibitors (synergists) that are known to block oxidative metabolism and increase the phytotoxicity of chlortoluron and isoproturon in wheat and other tolerant plants include aminobenzotriazole, piperonyl butoxide, paclobutrazol and tetcyclacis. Preferential inhibition of ring-methyl hydroxylation by aminobenzotriazole has suggested that different cytochrome P450-dependent monooxygenases are responsible for the alternative pathways of chlortoluron oxidation in wheat (Fig. 1). Inducers that stimulate the oxidative metabolism of chlortoluron in pretreated wheat cell suspension cultures include 2,4-D, cyometrinil and dichlormid /34/.

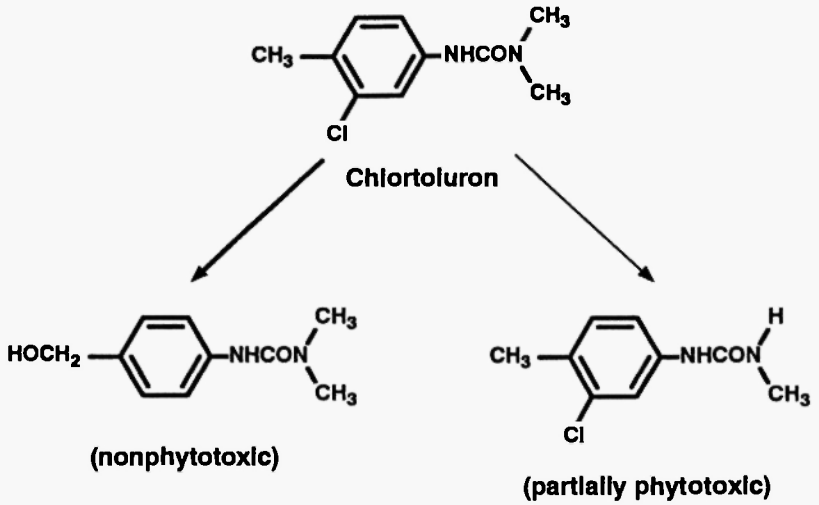


Fig. 1: Alternative oxidative pathways of chlortoluron metabolism and detoxification in wheat /25,26/.

Recent studies with microsomes from induced wheat cell suspension cultures and etiolated wheat seedlings have provided additional evidence for cytochrome P450 involvement in the oxidative metabolism of wheat selective phenylurea herbicides (Table 1). *In vitro* metabolism studies with chlortoluron showed for the first time that microsomal ring-methyl hydroxylase and N-demethylase activities both required NADPH and molecular oxygen and were inhibited by carbon monoxide /9-11/.

Microsomal reaction products were the same as those reported *in vivo* and were identified by co-chromatography (TLC) with reference standards as ring-methyl hydroxylated chlortoluron and N-monodemethylated chlortoluron /9/. A requirement for NADPH cytochrome P450 (cytochrome *c*) reductase in both oxidative reactions was shown by inhibition studies with 2′,5′-dichlorophenolindophenol (DCPIP), menadione and cytochrome *c*, by inhibition with analogues of NADPH and by inhibition with polyclonal antibodies raised against purified NADPH-cytochrome *c* (P450) reductase from Jerusalem artichoke /9,10/. Dependence of microsomal ring-methyl hydroxylase and N-demethylase activities on cytochrome P450 was indicated by inhibition studies with carbon monoxide, O-phenanthroline, n-

octylamine, tetcyclacis, paclobutrazol, procloraz and piperonyl butoxide and also by difference spectra of induced microsomes that showed typical reduced carbon monoxide binding spectra, type I substrate binding spectra with chlortoluron and type II binding spectra with the fungicide procloraz /9-11/.

Low levels of constitutive cytochrome P450, ring-methyl hydroxylase and N-demethylase activities in microsomes from untreated wheat cell cultures were increased several fold by induction in cell cultures pretreated with chlortoluron, the phenoxy herbicides 2,4-D and mecoprop, and the oxime ethers cyometrinil, oxabetrinil and CGA 133205 /11/. Differences in the response of ring-methyl hydroxylase and N-demethylase to induction again suggested that at least two different cytochrome P450 monooxygenases are responsible for the oxidative metabolism and detoxification of chlortoluron in wheat /10,11/. Differential responses of microsomal ω-1-lauric acid hydroxylase and cinnamic acid 4-hydroxylase to induction also suggested that these two constitutive cytochrome P450 monooxygenases were different from those responsible for chlortoluron oxidation /11/. Substrate specificity studies and inhibition studies with polyclonal antibodies raised against purified *trans*-cinnamic acid 4-hydroxylase from Jerusalem artichoke provided additional evidence that *trans*-cinnamic acid 4-hydroxylase activity was distinct from either chlortoluron ring-methyl hydroxylase or N-demethylase activities in wheat microsomes /10/. Microsomal chlortoluron ring-methyl hydroxylase and N-demethylase activities were not inhibited by *trans*-cinnamic acid or by *trans*-cinnamic acid 4-hydroxylase antibodies.

Preferential inhibition of ring-methyl hydroxylase with aminobenzotriazole and N-demethylase with the fungicide triazole triadimefon together with differences in the responses of both enzymes to carbon monoxide inhibition and cumene hydroperoxide as an alternate oxygen source also indicated that two distinct cytochrome P450 monooxygenases are responsible for chlortoluron oxidation in wheat /9-11/.

Microsomal oxidation of chlortoluron was inhibited by a number of phenylurea herbicide analogues, including linuron and diuron, and also by the triazine herbicides atrazine and terbutryne, but not by 2,4-D, chlorsulfuron and isoproturon /11/. The failure of 2,4-D, chlorsulfuron and isoproturon to inhibit the microsomal oxidation of chlortoluron has suggested that these herbicides are not oxidized by the same

cytochrome P450 isoforrus that are responsible for chortoluron hydroxylation and N-demethylation in wheat.

Microsomes isolated from wheat cell cultures pretreated with 2,4-D also catalyzed the *in vitro* oxidation of diuron and isoproturon in the presence of NADPH /10/. N-Monodemethylated diuron and isoproturon as well as ring-isopropyl hydroxylated isoproturon were tentatively identified by co-chromatography (TLC) as oxidation products. Microsomes from etiolated wheat seedling shoots pretreated with ethanol and/or naphthalic anhydride also oxidized linuron primarily by *N*-demethylation and to a limited extent by N-dealkoxylation /13/. Isolated linuron oxidation products were identified by co-chromatography (HPLC) with reference standards and by electron impact mass spectrometry (EI/MS).

2.2. Aryloxyphenoxypropanoates

Diclofop-methyl is a member of a group of aryloxyphenoxypropanoate herbicides that perturb the proton gradient across the plasma membrane and act as potent inhibitors of acetyl CoA carboxylase (EC 6.4.1.2) in long-chain fatty acid biosynthesis /35,36/. Diclofop-methyl is a selective postemergence herbicide used for the control of annual grassy weeds in wheat, barley and other crops. Phase I hydrolysis and oxidation reactions are key factors in diclofop-methyl selectivity. Foliarly absorbed diclofop-methyl is hydrolyzed rapidly to the phytotoxic acid (diclofop) in both tolerant wheat and sensitive oat or wild oat (Fig. 2). In wheat, diclofop is detoxified by aryl hydroxylation and conjugation of the oxidation products as O-glucosides. In oat and wild oat, aryl hydroxylation is limited and diclofop is conjugated primarily as a glucose ester intermediate that may be hydrolyzed to phytotoxic diclofop /35-41/.

The involvement of arene oxide intermediates and a NIH shift mechanism in the aryl hydroxylation of diclofop in wheat has been reported /42/. Three arylhydroxylated metabolites of diclofop were isolated and identified as the 2,3-dichloro-4-hydroxy, 2,5-dichloro-4-hydroxy and the 2,4-dichloro-5-hydroxy isomers. In addition to the identification of NIH shift metabolites, *in vivo* metabolism studies with tissue sections from etiolated wheat seedlings showed that diclofop metabohsm required molecular oxygen and was inhibited by carbon monoxide, tetcyclacis, aminobenzotriazole and tridiphane /7,43/.

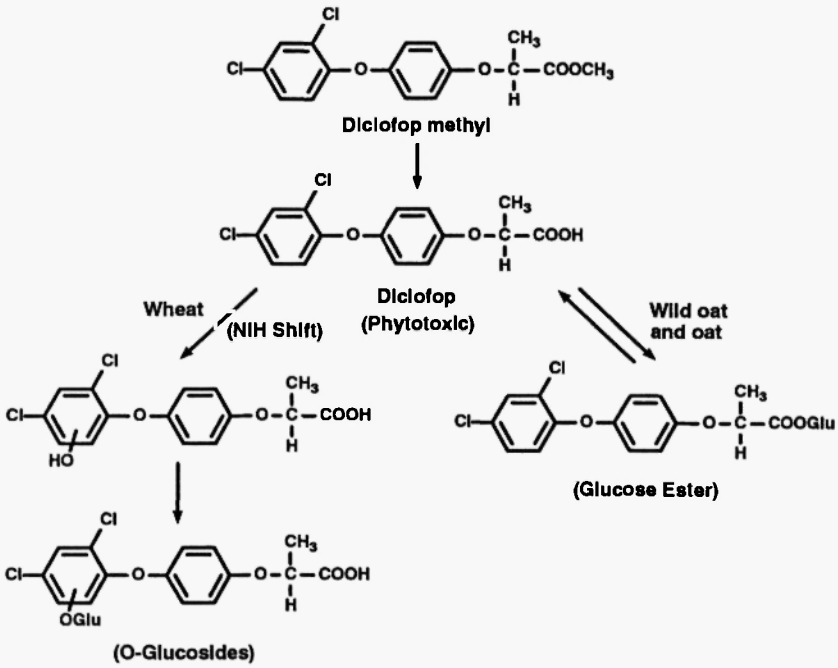


Fig. 2: Differential metabolism and detoxification of diclofop in tolerant wheat and sensitive oat and wild oat /35-40/.

Early reports of the *in vitro* metabolism of diclofop by microsomes from etiolated wheat seedling shoot tissues established that diclofop was hydroxylated by a cytochrome P450-dependent monooxygenase /7,8/. Diclofop hydroxylase activity was associated with a smooth endoplasmic reticulum fraction and required both molecular oxygen and NADPH as cofactors. Hydroxylase activity was inhibited by polyclonal antibodies raised against purified NADPH-cytochrome P450 reductase from Jerusalem artichoke, by NADPH-cytochrome P450 reductase inhibitors $NADP^+$, $AADP^+$ (3-aminonicotinamide adenine dinucleotide phosphate), 2′AMP, DCPIP and menadione, and by cytochrome P450 inhibitors tetcyclacis, aminobenzotriazole and carbon monoxide /7,8/. Carbon monoxide inhibition was reversed by light /8/.

Induction of microsomal enzyme activity by pretreatment of etiolated wheat seedlings with phenobarbital resulted in a more than

15-fold increase in the activities of diclofop hydroxylase and ω-1-lauric acid hydroxylase /8/. In contrast, microsomal *trans*-cinnamic acid 4-hydroxylase and NADPH-cytochrome *c* (P450) reductase activities were unaffected and cytochrome P450 levels increased only 1.2-fold /8/. These data suggest that diclofop hydroxylation is catalyzed by a minor cytochrome P450 isoform and that different cytochrome P450 monooxygenases are responsible for diclofop and *trans*-cinnamic acid hydroxylation in wheat microsomes. Weak inhibition of diclofop hydroxylation by *trans*-cinnamic acid also suggests that diclofop and *trans*-cinnamic acid hydroxylase activities are associated with different cytochrome P450 isoforms /7/.

Additional studies with etiolated wheat seedling microsomes have shown that diclofop hydroxylase activity is also induced with naphthalic anhydride, ethanol, and a combination of naphthalic anhydride with either phenobarbital or ethanol /12-14/. Ethanol was the most effective inducer of diclofop hydroxylase activity followed by phenobarbital and naphthalic anhydride /12,13/. Microsomal diclofop hydroxylase and ω-1-lauric acid hydroxylase activities were increased 20-fold by pretreatment of etiolated wheat seedlings with combinations of naphthalic anhydride and either phenobarbital or ethanol /12-14/. At the same time, *trans*-cinnamic 4-hydroxylase activity was decreased and cytochrome P450 levels were increased only 2- to 3-fold. Wheat seedling microsomes induced with naphthalic anhydride and phenobarbital also formed the same three isomeric NIH shift hydroxylated products that were isolated and identified as diclofop aglycones from *in vivo* metabolism studies /12/.

Similar enzyme kinetics and responses of both diclofop hydroxylase and ω-1-lauric acid hydroxylase to induction, inhibition and autocatalytic inactivation by 11-dodecenoic acid together with molecular modeling studies of substrate conformations have suggested that both hydroxylation reactions have narrow substrate specificities and are catalyzed by a single cytochrome P450 isozyme /12,14/. Both enzymes have similar K_m values for their respective substrates and both substrates act as competitive inhibitors of each other with nearly identical K_i values. This appears to be the first report of a physiological role for a microsomal cytochrome P450 monooxygenase that is also responsible for the oxidative metabolism and detoxification of a herbicide /12/.

2.3. Phenoxyalkanoic acids

The herbicide 2,4-D was first introduced in the 1940s and has been used extensively for the selective postemergence control of broadleaf weeds in wheat and other cereals /44/. As an important member of the group of phenoxyalkanoic acid herbicides, 2,4-D is an auxinic herbicide that mimics the action of the plant growth regulator IAA (indol-3-yl acetic acid) and exhibits a multiplicity of biochemical and physiological effects in plants /45/.

Pathways of 2,4-D metabolism and detoxification in wheat are illustrated in Figure 3. Characteristic cytochrome P450-dependent aryl hydroxylation and subsequent glucose conjugation of NIH shift oxidation products of 4-OH-2,5-D and 4-OH-2,3-D has been established as the principle route of 2,4-D detoxification in wheat /46,47/. Other metabolic pathways that modulate 2,4-D phytotoxicity in wheat include reversible conjugation of 2,4-D with glucose or amino acids and compartmentation or sequestration of 2,4-D as a bound residue /6,44-48/.

Attempts to demonstrate *in vitro* aryl hydroxylation of 2,4-D with induced wheat seedling microsome preparations have met with mixed results /12,13/. Microsomes from etiolated wheat seedlings that were induced with a combination of ethanol and naphthalic anhydride oxidized diclofop, triasulfuron and linuron, but failed to oxidize 2,4-D at detectable levels /13/. In other studies, wheat seedling microsomes induced with a combination of phenobarbital and naphthalic anhydride oxidized diclofop, lauric acid, *trans*-cinnamic acid, chlortoluron and 2,4-D /12/. However, the levels of microsomal 2,4-D hydroxylase activity in these latter studies were not indicated and the oxidation products were not identified. It has been speculated that lipophilic, ether-soluble amino acid amide conjugates of 2,4-D may function as substrates in the aryl hydroxylation of 2,4-D /3/.

Weak 2,4-D inhibition of chlortoluron ring-methyl hydroxylase, chlortoluron N-demethylase, diclofop hydroxylase and triasulfuron hydroxylase activities associated with wheat microsomes has suggested that a distinct cytochrome P450 isoform may be responsible for 2,4-D hydroxylation in wheat /7,11,13/. Additional *in vitro* studies are needed to further characterize the microsomal cytochrome P450 monooxygenase isoform responsible for the aryl hydroxylation of 2,4-D in wheat.

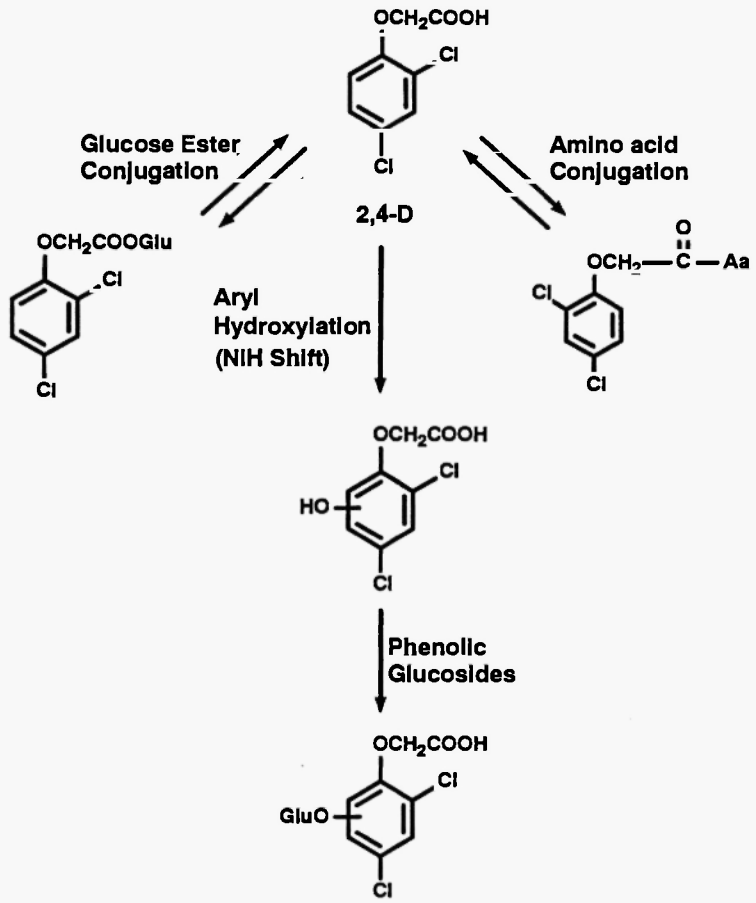


Fig. 3: Pathways of 2,4-D metabolism and detoxification in wheat /3,46-48/.

2.4. Diphenylethers

Bifenox is a member of a light activated *p*-nitrodiphenylether class of herbicides and is used as a postemergence herbicide for the selective control of a broad range of annual broadleaf and grass weeds in wheat and other cereals. The enzyme target site for *p*-nitrodiphenylether herbicides is located in the chloroplast and has been identified as protoporphyrinogen oxidase, the last enzyme in the common pathway of chlorophyll and heme biosynthesis /49,50/. Metabolism studies with

excised leaves from tolerant wheat and barley suggest that bifenox is metabolized and detoxified by a similar pathway to that reported for diclofop-methyl /35-37/. Initial rapid hydrolysis of the bifenox methyl ester to phytotoxic bifenox acid was followed by aryl hydroxylation of the 2,4-dichlorophenoxy moiety and the formation of compartmentalized non-phytotoxic O-glucoside conjugates /15/.

In vitro studies with induced microsome preparations from coleoptiles of etiolated wheat and barley seedlings also showed that bifenox acid was oxidized to yield two major reaction products and a third minor product /15/. Negative FAB/MS analysis of the two major oxidation products established that both products were isomeric and hydroxylated on the 2,4-dichlorophenoxy moiety. FAB/MS analysis of the minor oxidation product and proton NMR analysis of the major oxidation products were not obtained. However, the pattern of multiple bifenox acid oxidation products suggested that the major and minor metabolites may be NIH shift products similar to the *in vivo* and *in vitro* metabolites of diclofop in wheat /12,42/ and the *in vivo* metabolites of 2,4-D in wheat cell cultures /46,47/.

2.5. Sulfonylureas

The sulfonylureas are a group of highly active compounds developed as selective herbicides for use in a number of different crops /51-53/. Several sulfonylureas, including chlorsulfuron, triasulfuron and prosulfuron, have been developed as selective postemergence herbicides for the control of broadleaf weeds and the suppression of some grassy weeds in wheat and other cereals. The target site of action for sulfonylurea herbicides has been identified as acetolactate synthase (EC 4.1.3.18), the first enzyme common to the biosynthesis of the branched chain amino acids, valine, leucine and isoleucine /54,55/. The resistance of wheat to chlorsulfuron and other wheat-selective sulfonylurea herbicides is based on the ability of wheat to rapidly detoxify the herbicides and prevent lethal herbicide levels from reaching the sensitive target site /51-53,56/. In wheat, chlorsulfuron is detoxified by aryl hydroxylation at the 5-position on the chlorophenyl ring followed by rapid conjugation with glucose (Fig. 4). Only one atom of oxygen from $^{18}O_2$ was incorporated in the phenolic oxidation product /52/. Studies with excised wheat leaves also showed that the oxidative metabolism of chlorsulfuron was increased more than two-fold by pretreatment with naphthalic acid or cyometrinil /57/. Induction

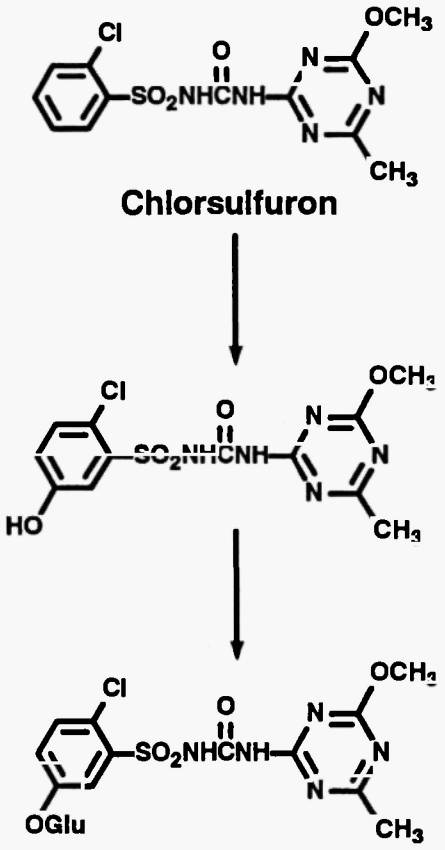


Fig. 4: Oxidative metabolism and detoxification of chlorsulfuron in wheat /51/.

of increased chlorsulfuron metabolism was inhibited by the protein synthesis inhibitor, cycloheximide.

Direct evidence for the involvement of cytochrome P450 in the oxidative metabolism and detoxification of wheat selective sulfonylurea herbicides was first reported in 1991 /13/. Microsomes from etiolated wheat seedling shoot tissues hydroxylated both chlorsulfuron and triasulfuron at the 5-position on the substituted phenyl ring. Hydroxylation of both herbicides was dependent on NADPH and molecular oxygen and was inhibited by carbon monoxide. Triasulfuron hydroxylase activity was also inhibited by a number of herbicides used

for selective weed control in wheat, including linuron, chlortoluron and bifenox, but was only negligibly inhibited by diclofop, diclofop-methyl, 2,4-D or dicamba /13/. Low constitutive levels of chlorsulfuron and triasulfuron hydroxylase activity in wheat microsomes were increased from 5- to 25-fold by pretreatment of seedlings with naphthalic anhydride, phenobarbital or ethanol and by a combination of naphthalic acid anhydride and ethanol /13,16/. In contrast to a 25-fold increase in induced triasulfuron hydroxylase activity, NADPH cytochrome *c* (P450) reductase activity was not increased and cytochrome P450 levels were increased only 1.5-fold /17/. Marked differences in the induction of triasulfuron hydroxylase activity and cytochrome P450 levels suggested that even though the cytochrome P450 isoform responsible for triasulfuron hydroxylation is highly inducible, it represents only a relatively small portion of the total cytochrome P450 pool. Studies have also shown that cytochrome P450 levels were increased only 1.5- to 3-fold while chlorsulfuron hydroxylase activities were increased 9- to 33-fold in wheat microsomes by induction with naphthalic anhydride or phenobarbital and by a combination of naphthalic anhydride and phenobarbital /12/. Recent studies have shown that induced wheat seedling microsomal triasulfuron hydroxylase activity can be solubilized and reconstituted and that microsomal hydroxylase activity is inhibited by piperonyl butoxide, tetcylcacis, paclobutrazol, cytochrome *c* and by polyclonal antibodies raised against a purified NADPH cytochrome *c* (P450) reductase from wheat /17/. Typical reduced cytochrome P450 carbon monoxide difference spectra, type I substrate binding spectra with triasulfuron and strong type II inhibitor binding spectra with tetcyclacis were also observed together with light-reversible carbon monoxide inhibition.

Recent *in vitro* metabolism studies with wheat seedling microsomes induced with naphthalic anhydride and ethanol have shown that prosulfuron (CGA 152005), a new sulfonylurea herbicide for selective postemergence control of broadleaf weeds in cereals, is also oxidized by cytochrome P450-dependent monooxygenases in wheat /16,18/. In addition to aryl hydroxylation at the 5-position on the phenyl ring as shown with chlorsulfuron and triasulfuron, prosulfuron was also subject to limited oxidative detoxification by alkyl hydroxylation and demethylation of the methyl and methoxy groups on the triazine ring (Fig. 5). One major (5-OH prosulfuron) and two minor (methyl and methoxy) microsomal oxidation products were tentatively identified by negative ion FAB/MS and co-chromatography (TLC and HPLC) with

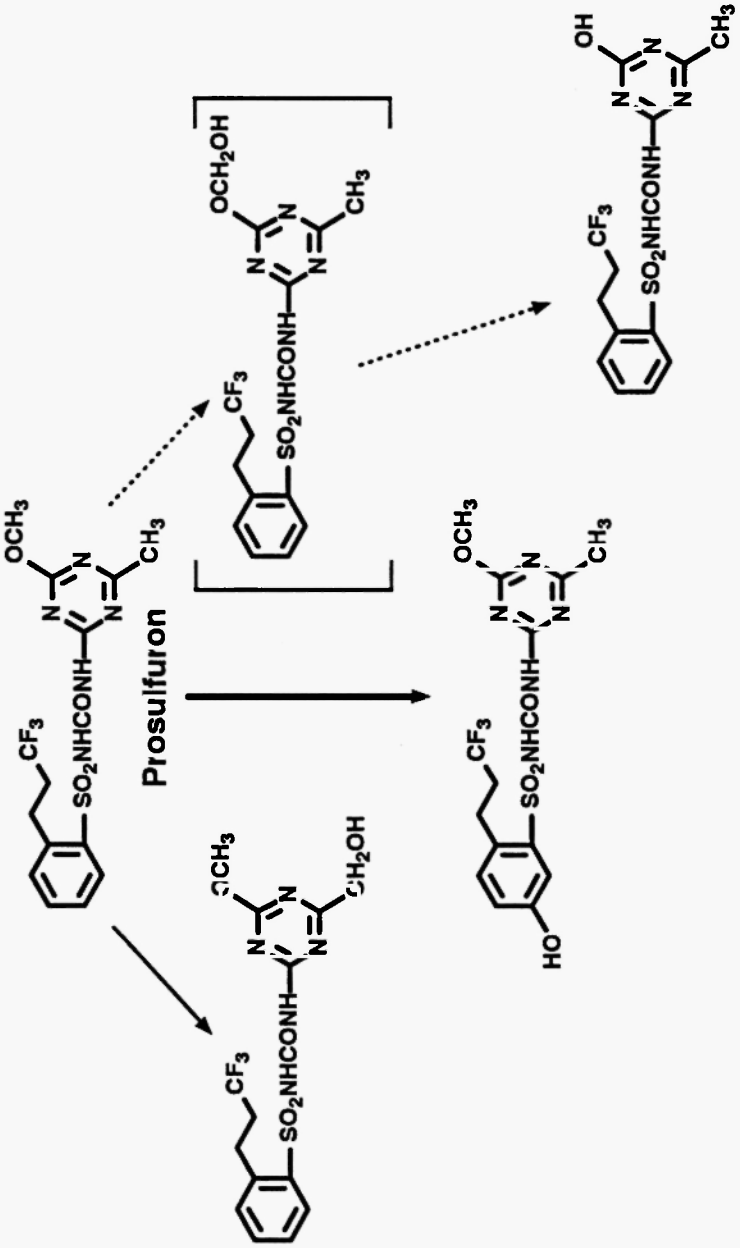


Fig. 5: Oxidation of prosulfuron by wheat seedling microsomes /18/.

reference standards /18/. Identification of the major aryl hydroxylated product as 5-OH prosulfuron was confirmed by proton NMR spectroscopy. Constitutive enzyme activity was increased 5- to 28-fold by induction with naphthalic anhydride, the herbicide safener CGA 185072 (5-chloro-8-quinolinoxyacetic acid-1-methylhexyl-ester), ethanol and by combinations of naphthalic anhydride or CGA 185072 with ethanol. Constitutive NADPH cytochrome *c* (P450) reductase activity was not increased and cytochrome P450 levels were increased less than two-fold. Microsomal prosulfuron hydroxylase activity required molecular oxygen and NADPH. Enzyme activity was inhibited by carbon monoxide, tetcyclacis, piperonyl butoxide, cytochrome *c* and by polyclonal antibodies raised against a purified NADPH cytochrome *c* (P450) reductase from wheat /18/. Carbon monoxide inhibition was light reversible.

2.6. Triazolopyrimidine sulfonanilides

Flumetsulam is a member of a new class of selective acetolactate synthase inhibiting herbicides developed for pre- or postemergence broadleaf weed control in wheat, corn, barley and soybeans /58-61/. Flumetsulam is metabolized *in vivo* in tolerant wheat, corn and barley by oxidation of the methyl group on the triazolopyrimidine ring and at the 4-position on the phenyl ring /19/. Both oxidation products are rapidly conjugated and detoxified as polar O-glucosides (Fig. 6). Monohydroxylated and dihydroxylated metabolites were isolated and identified by negative ion FAB/MS and proton NMR spectroscopy. The selective action of flumetsulam between tolerant crops and sensitive target weeds has been attributed to differences in the rates of oxidative metabolism and glucose conjugation /58/. Rates of metabolism and detoxification in tolerant monocots were 3-5 fold faster than in sensitive dicots.

In vitro metabolism studies with microsomes from etiolated wheat seedlings showed that flumetsulam hydroxylation at both reaction sites required NADPH and molecular oxygen and was inhibited by carbon monoxide /19/. Inhibition by carbon monoxide was reversed by light. A comparison of naphthalic anhydride and ethanol induced flumetsulam hydroxylase activities in corn, wheat and barley microsomal preparations showed that cytochrome P450 levels and NADPH cytochrome *c* (P450) reductase activities were similar in all three species and that both monohydroxylated products of flumetsulam were

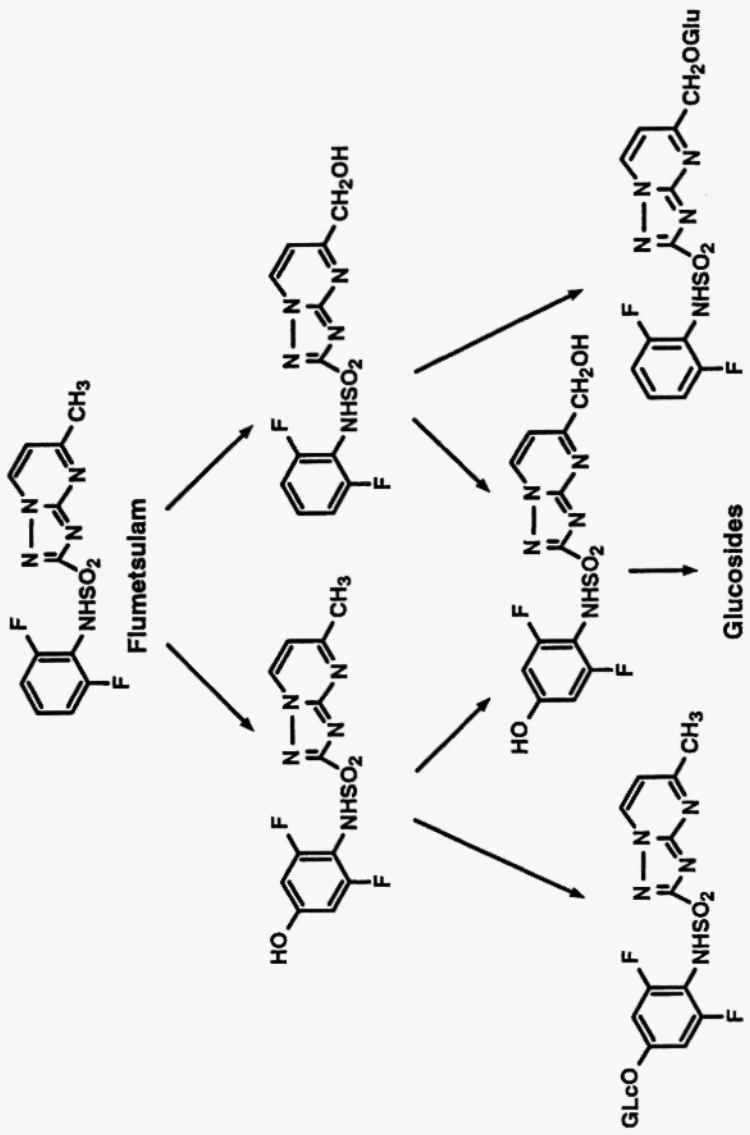


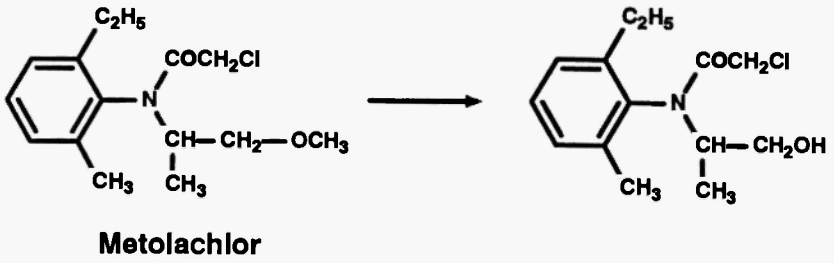
Fig. 6: Alternative oxidative pathways of flumetsulam metabolism and detoxification in wheat, corn and barley /19/.

formed in approximately equal amounts. However, differences in the expression of flumetsulam hydroxylase activity in induced corn, wheat and barley microsomes were noted. Flumetsulam hydroxylase activity in corn microsomes was nearly twice the activity in wheat microsomes and approximately four times greater than in barley microsomes /19/. Both microsomal hydroxylation reactions were induced by pretreatment of etiolated seedlings with naphthalic anhydride, ethanol or a combination of naphthalic anhydride and ethanol. Both reactions were also inhibited by cytochrome *c*, tetcyclacis, piperonyl butoxide and paclobutrazol. However, differences in the inhibition of aryl and alkyl hydroxylase activities were noted with piperonyl butoxide and paclobutrazol /19/. Piperonyl butoxide was a more effective inhibitor of alkyl hydroxylase activity while paclobutrozol was a stronger inhibitor of aryl hydroxylase activity. These differences suggested the involvement of different cytochrome P450 isoforms in the oxidative metabolism and detoxification of flumetsulam in wheat and other cereals. Marked differences in the apparent K_m values for the formation of aryl hydroxylated and alkyl hydroxylated reaction products also suggested the presence of inducible cytochrome P450 isoforms with different affinities for the common substrate, flumetsulam.

2.7. Chloroacetamides

Metolachlor is a widely used chloroacetamide herbicide developed for the selective preemergence control of annual grass and broadleaf weeds in corn, sorghum, cotton, soybeans, peanuts and other crops /62/. In resistant corn and sorghum, rapid glutathione conjugation by displacement of chlorine in the chloroacetyl side chain is the major metabolic pathway responsible for metolachlor detoxification. O-Demethylation of the metolachlor methoxypropyl side chain and subsequent conjugation of the O-demethylated metabolite with glucose has also been reported to occur in both plants and animals /62/.

Recent *in vitro* metabolism studies with microsomes from sorghum and corn seedlings established that the O-demethylation of the methoxypropyl side chain of metolachlor was catalyzed by a cytochrome P450-dependent O-demethylase (Fig. 7). Microsomal O-demethylase activity was dependent on NADPH and molecular oxygen and was subject to light reversible carbon monoxide inhibition /63,64/. Reduced carbon monoxide difference spectra, type I substrate binding



Metolachlor

Fig. 7: Cytochrome P450-dependent oxidation of metolachlor by wheat, sorghum and corn microsomes /16,63,64/.

spectra with metolachlor and type II inhibitor binding spectra with tetcyclacis were also observed. Microsomal O-demethylase activity was also induced with naphthalic anhydride and inhibited by tetcyclacis, piperonyl butoxide, aminobenzotriazole, SKF-525A and the herbicide synergist, tridiphane. Although metolachlor is not recommended as a selective herbicide for use in wheat, recent studies with naphthalic anhydride and ethanol induced microsomes from etiolated wheat seedlings have shown that wheat has the capacity to oxidize the methoxypropyl side chain of metolachlor /16/. Cytochrome P450-dependent metolachlor O-demethylase activity associated with induced wheat microsomes required NADPH and was also inhibited by tetcyclacis.

3. CYTOCHROME P450 MONOOXYGENASES AND METABOLIC CROSS RESISTANCE TO WHEAT HERBICIDES

Increasing evidence from *in vivo* and *in vitro* metabolism studies suggests that herbicide selectivity between resistant wheat and susceptible grassy weeds is largely based on marked differences in the expressed levels of cytochrome P450 isoforms and monooxygenases associated with herbicide oxidation and metabolic detoxification. Unfortunately, the repeated use of a single herbicide or a given class of herbicide for the selective control of annual ryegrass (*Lolium rigidum* Gaud.) and blackgrass (*Alopecurus myosuroides* Huds.) in wheat has resulted in the selection of biotypes from natural weed populations that mimic wheat and exhibit wheat-like cytochrome P450-dependent non-target site cross resistance to different classes of herbicides with different mechanisms of action /20,21/.

An annual ryegrass biotype SLR31 selected for resistance to diclofop-methyl is also cross resistant to chlorsulfuron and other sulfonylurea herbicides as well as to the wheat selective imidazolinone herbicide, imazamethabenz /21,56,65-67/. *In vivo* metabolism studies suggested that cross resistance to chlorsulfuron was due to increased oxidative metabolism by a cytochrome P450-dependent monooxygenase similar to that in wheat. The metabolism of chlorsulfuron in cross resistant biotype SLR 31 was twice the rate of metabolism in a susceptible biotype. Chlorsulfuron metabolism in both susceptible and resistant biotypes was inhibited by the insecticide malathion, a reported inhibitor of cytochrome P450-dependent monooxygenases responsible for the hydroxylation of the sulfonylurea herbicide primisulfuron in corn /68/. In contrast to biotype SLR31, biotype WLR2 was selected for resistance against amitrole and atrazine and was also cross resistant to several different classes of Photosystem II inhibitors, but was not resistant to diclofop-methyl or chlorsulfuron /24,69-71/. Another biotype VLR69 selected against several different classes of herbicides, including diuron and atrazine, was resistant to other Photosystem II inhibitors as well as to a number of herbicides that inhibit acetyl CoA carboxylase and acetolactate synthase, including diclofop-methyl and chlorsulfuron /24/. Resistance of annual ryegrass biotypes WLR2 and VLR69 to Photosystem II inhibitors was associated with increased oxidative metabolism and not to an alteration of the target site. Increased oxidative N-dealkylation of simazine and chlortoluron was inhibited by aminobenzotriazole and both resistant biotypes were more susceptible to herbicide injury when cytochrome P450-inhibitors aminobenzotriazole and piperonyl butoxide were applied with simazine or chlortoluron.

Similar metabolism-based cross resistance to herbicides with different target sites of action has also evolved in blackgrass populations treated repeatedly over several years with chlortoluron /21,24,72-74/. A "Peldon" biotype selected for chlortoluron and phenylurea herbicide resistance was also cross resistant to sulfonylurea, aryloxyphenoxypropanoate, triazine, triazinone, imidazolinone, chloroacetanilide, cyclohexanedione, dinitroaniline, carbamate and thiocarbamate herbicides. Enhanced oxidative N-demethylation and ring-methyl hydroxylation of chlortoluron together with the inhibition of chlortoluron metabolism by aminobenzotriazole suggested that chlortoluron resistance mechanisms in the "Peldon" biotype mimic those found in wheat. Microsome preparations from "Peldon" biotype

cell suspension cultures oxidized chlortoluron in the presence of NADPH and contained higher levels of cytochrome P450 than microsomes from cell cultures of a chlortoluron susceptible "Rothamsted" biotype. In addition, Type I substrate binding spectra with chlortoluron were observed with microsomes from "Peldon" biotype cell cultures, but not with microsomes from "Rothamsted" biotype cultures /73/.

The evolution of grassy weed populations with polygenic cytochrome P450-dependent metabolism-based cross resistance to different classes of herbicides used for weed control in wheat poses a serious threat to world grain production /20,75,76/. Increased research on the biochemistry, molecular genetics and differential expression of microsomal cytochrome P450 monooxygenases associated with herbicide selectivity and multiple herbicide resistance in wheat is needed to meet the challenge of similar metabolism based cross resistance that has evolved in weed populations exposed to repeated herbicide selection pressure.

4. CONCLUSIONS

Recent studies with induced microsomal cytochrome P450-dependent monooxygenases from wheat have provided direct evidence for the participation of these important Phase I enzymes in the oxidative N-demethylation, O-demethylation, aryl hydroxylation, alkyl hydroxylation and N-dealkoxylation of a broad spectrum of herbicides used for selective weed control in wheat and other cereals. Eleven different herbicides with differing mechanisms of action from six of the major classes of wheat selective herbicides have now been shown to be oxidized by induced cytochrome P450-dependent monooxygenases associated with isolated microsome fractions from etiolated wheat seedlings or wheat cell suspension cultures.

Differential responses of microsomal cytochrome P450 monooxygenases to selected substrates, inhibitors and inducers of cytochrome P450 support the concept that wheat microsomes, like those from other plants, contain a number of inducible cytochrome P450 isoforms with narrow specificities towards different herbicide and endogenous substrates /77/. However, an inducible microsomal cytochrome P450-dependent hydroxylase that oxidizes diclofop also

appears to be responsible for the ω-1-hydroxylation of lauric acid /12,14/.

Substantial improvements in the procedures developed for the induction, isolation and purification of microsomes from etiolated wheat seedling shoots have selectively increased constitutive herbicide cytochrome P450 monooxygenase activities over 20-fold and also increased cytochrome P450 concentrations to levels that approximate those present in hepatic microsomes. With these higher levels of herbicide monooxygenase activity and increased concentrations of cytochrome P450, induced wheat seedling microsomes serve as an excellent system for the solubilization, isolation, reconstitution and genetic engineering of specific cytochrome P450 isoforms responsible for herbicide oxidative metabolism and detoxification in wheat and possibly similar isoforms that may be responsible for increased herbicide oxidation in evolved cross resistant ecotypes of grassy weeds.

REFERENCES

1. Frear DS, Hodgson RH, Shimabukuro RH, Still GG. Behavior of herbicides in plants. Advan Agron 1972; 24: 327-378.
2. Dohn DR, Krieger RI. Oxidative metabolism of foreign compounds by higher plants. Drug Metab Rev 1981; 12: 119-157.
3. Cole D. Oxidation of xenobiotics in plants. Prog Pest Biochem Toxicol 1983; 3: 199-254.
4. Owen WJ. Metabolism of herbicides—Detoxification as a basis of selectivity. In: Dodge AD, ed. Herbicides and Plant Metabolism. London: Cambridge Univ Press, 1989; 171-198.
5. Durst F. Metabolisation des herbicides. In: Scalla R, ed. Les Herbicides. Paris: INRA, 1991; 193-236.
6. Coupland D. The role of compartmentation of herbicides and their metabolites in resistance mechanisms. In: Caseley JC, Cussans GW, Atkin RK, eds. Herbicide Resistance in Weeds and Crops. Oxford: Butterworth-Heinemann Ltd., 1991; 263-278.
7. McFadden JJ, Frear DS, Mansager ER. Aryl hydroxylation of diclofop by a cytochrome P450 dependent monooxygenase from wheat. Pestic Biochem Physiol 1989; 34: 92-100.
8. Zimmerlin A, Durst F. Xenobiotic metabolism in plants: Aryl hydroxylation of diclofop by a cytochrome P450 enzyme from wheat. Phytochemistry 1990; 29: 1729-1732.
9. Mougin C, Cabanne F, Canivenc M-C, Scalla R. Hydroxylation and N-demethylation of chlorotoluron by wheat microsomal enzymes. Plant Sci 1990; 66: 195-203.

10. Mougin C, Cabanne F, Scalla R. Additional observations on the chlorotoluron hydroxylase and N-demethylase activities in wheat microsomes. Plant Physiol Biochem 1992; 30: 769-778.
11. Mougin C, Polge N, Scalla R, Cabanne F. Interactions of various agrochemicals with cytochrome P450-dependent monooxygenases of wheat cells. Pestic Biochem Physiol 1991; 40: 1-11.
12. Zimmerlin A, Durst F. Aryl hydroxylation of the herbicide diclofop by a wheat cytochrome P450 monooxygenase. Plant Physiol 1992; 100: 874-881.
13. Frear DS, Swanson HR, Thalacker FW. Induced microsomal oxidation of diclofop, triasulfuron, chlorsulfuron and linuron in wheat. Pestic Biochem Physiol 1991; 41: 274-287.
14. Zimmerlin A, Salaün J-P, Durst F, Mioskowski C. Cytochrome P450-dependent hydroxylation of lauric acid at the subterminal position and oxidation of unsaturated analogs in wheat microsomes. Plant Physiol 1992; 100: 868-873.
15. Frear DS, Swanson HR. Unpublished results. 1994.
16. Moreland DE, Corbin FT, McFarland JE, Fleischmann TJ. Comparative enhancement of cytochrome P450 monooxygenase activities by naphthalic anhydride and ethanol in sorghum, corn, wheat and mung bean. Eighth IUPAC International Congress of Pesticide Chemistry, Washington, DC 1994; Abstr No 843: p 944.
17. Thalacker FW, Swanson HR, Frear DS. Characterization, purification and reconstitution of an inducible cytochrome P450-dependent triasulfuron hydroxylase from wheat. Pestic Biochem Physiol 1994; 49; 209-223.
18. Frear DS, Swanson HR. Oxidation of the sulfonylurea herbicide CGA 152005 by wheat seedling microsomes. 207th ACS National Meeting, San Diego, CA 1994; AGRO Abstract No 70.
19. Frear DS, Swanson HR, Tanaka FS. Metabolism of flumetsulam (DE-498) in wheat, corn and barley. Pestic Biochem Physiol 1993; 45: 178-192.
20. Gressel J. Multiple resistances to wheat selective herbicides: New challenges to molecular biology. In: Miflin BJ, ed. News and Views: Oxford Surveys of Plant Molecular Biology. Oxford: Oxford Univ Press, 1988; 195-203.
21. Hall LM, Holtum JAM, Powles SB. Mechanisms responsible for cross resistance and multiple resistance. In: Powles SB, Holtum JAM eds. Herbicide Resistance in Plants: Biology and Biochemistry. Boca Raton, FL: CRC Press, 1994; 243-261.
22. Frear DS, Swanson HR, Tanaka FS. Herbicide metabolism in plants. In: Runeckles VC, Tso TC, eds. Structural and Functional Aspects of Phytochemistry (Recent Advances in Phytochemistry). New York: Academic Press, 1972; 5: 225-246.
23. Geissbuhler H, Martin H, Voss G. The substituted ureas. In: Kearney PC, Kaufman DD, eds. Herbicides: Chemistry, Degradation and Mode of Action (Second edition). New York: Marcel Dekker, 1975; 1: 209-291.
24. Gronwald JW. Resistance to photosystem II inhibiting herbicides. In: Powles SB, Holtum JAM, eds. Herbicide Resistance in Plants: Biology and Biochemistry. Boca Raton, FL: CRC Press, 1994; 27-61.

25. Gross D, Laanio T. Duphuis G, Esser HO. The metabolic behavior of chlortoluron in wheat and soil. Pestic Biochem Physiol 1979; 10: 49-59.
26. Ryan PJ, Gross D, Owen WJ, Laanio TL. The metabolism of chlortoluron, diuron and CGA 43057 intolerant and susceptible plants. Pestic Biochem Physiol 1981; 16: 213-221.
27. Ryan PJ, Owen WJ. The mechanism of selectivity of chlortoluron between cereals and grass weeds. Proc Br Crop Prot Conf - Weeds, 1982; 1: 317-324.
28. Ryan PJ, Owen WJ. Metabolism of chlortoluron in tolerant and sensitive cereal varieties. Aspects Appl Biol: Crop Sensitivity to Herbicides, 1983; 3: 63-72.
29. Cabanne F, Gaillardon P, Scalla R. Aminobenzotriazole as a synergist of urea herbicides. Proc Br Crop Prot Conf - Weeds 1985; 3: 1163-1170.
30. Gaillardon P, Cabanne F, Scalla R, Durst F. Effect of mixed function oxidase inhibitors on the toxicity of chlortoluron and isoproturon to wheat. Weed Res 1985; 25: 397-402.
31. Gonneau M, Pasquette B, Cabanne F, Scalla R, Longhman BC. Transformation of phenoxyacetic acid and chlortoluron in wheat, barren brome, cleavers and speedwell: effects of an inactivator of monooxygenases. Proc Br Crop Prot Conf - Weeds, 1987; 1: 329-336.
32. Cabanne F, Huby D, Gaillardon P, Scalla R, Durst F. Effect of the cytochrome P450 inactivatior 1-aminobenzotriazole on the metabolism of chlortoluron and isoproturon in wheat. Pestic Biochem Physiol 1987; 28: 371-380.
33. Gonneau M, Pasquette B, Cabanne F, Scalla R. Metabolism of chlortoluron in tolerant species: possible role of cytochrome P450 monooxygenases. Weed Res 1988; 28: 19-25.
34. Canivenc M-C, Cagnac B, Cabanne F, Scalla R. Induced changes in chlortoluron metabolism in wheat cell suspension cultures. Plant Physiol Biochem 1989; 193-201.
35. Shimabukuro RH. Selectivity and mode of action of the postemergence herbicide diclofop methyl. Plant Growth Regul Soc Am Quart 1990; 18: 37-53.
36. Devine MD, Shimabukuro RH. Resistance to acetyl coenzyme A carboxylase inhibiting herbicides. In: Powles SB, Holtum JAM, eds. Herbicide Resistance in Plants: Biology and Biochemistry. Boca Raton, FL: CRC Press, 1994; 141-169.
37. Shimabukuro RH, Walsh WC, Hoerauf RH. Metabolism and selectivity of diclofop-methyl in wild oat and wheat. J Agric Food Chem 1979; 27: 615-623.
38. Dusky JA, Davis DG, Shimabukuro RH. Metabolism of diclofop-methyl (methyl-2-[4-(2′,4′-dichlorophenoxy)phenoxy]propanoate) in cell suspensions of diploid wheat (*Triticum monococcum*). Physiol Plant 1980; 49: 151-156.
39. Donald WW, Shimabukuro RH. Selectivity of diclofop-methyl between wheat and wild oat: growth and herbicide metabolism. Physiol Plant 1980; 49: 459-464.

40. Gorecka K, Shimabukuro RH, Walsh WC. Aryl hydroxylation: a selective mechanism for the herbicides diclofop-methyl and clofop-isobutyl, in gramineous species. Physiol Plant 1981; 53: 55-63.
41. Dusky JA, Davis DG, Shimabukuro RH. Metabolism of diclofop-methyl in cell cultures of *Avena sativa* and *Avena fatua*. Physiol Plant 1982; 54: 490-494.
42. Tanaka FS, Hoffer BL, Shimabukuro RH, Wien RG, Walsh WC. Identification of the isomeric hydroxylated metabolites of 2-[4-(2,4-dichlorophenoxy)phenoxy]-propanoate (diclofop methyl) in wheat. J Agric Food Chem 1990; 38: 559-565.
43. Romano ML, Stephenson GR, Tal A, Hall JC. The effect of monooxygenase and glutathione S-transferase inhibitors on the metabolism of diclofop-methyl and fenoxaprop-ethyl in barley and wheat. Pestic Biochem Physiol 1993; 46: 181-189.
44. Loos MA. Phenoxyalkanoic acids. In: Kearney PC, Kaufman DD, eds. Herbicides: Chemistry, Degradation and Mode of Action (Second Edition). New York: Marcel Dekker, 1975; 1: 1-128.
45. Coupland D. Resistance to the auxin analog herbicides. In: Powles SB, Holtum JAM, eds. Herbicide Resistance in Plants: Biology and Biochemistry. Boca Raton, FL: CRC Press, 1994; 171-214.
46. Bristol DW, Ghanuni AM, Oleson AE. Metabolism of 2,4-dichlorophenoxyacetic acid by wheat cell suspension cultures. J Agric Food Chem 1977; 25: 1308-1314.
47. Scheel D, Sandermann H. Metabolism of 2,4-dichlorophenoxy acetic acid in cell suspension cultures of soybean (*Glycine max* L.) and wheat (*Triticum aestivum* L.): I General results. Planta 1981; 152: 248-252.
48. Scheel D, Sandermann H. Metabolism of 2,4-dichlorophenoxyacetic acid in cell suspension cultures of soybean (*Glycine max* L.) and wheat (*Triticum aestivum* L.): II Evidence for incorporation into lignin. Planta 1981; 152: 253-258.
49. Duke SO, Becerril JM, Sherman TD, Lydon J, Matsumoto H. The role of protoporphyrin IX in the mechanism of action of diphenyl ether herbicides. Pestic Sci 1990; 30: 367-378.
50. Matringe M, Camadro J-M, Block MA, Joyard J, Scalla R, Labbe P, Douce R. Localization within chloroplasts of protoporphyrinogen oxidase, the target enzyme for diphenylether-like herbicides. J Biol Chem 1992; 267: 4646-4651.
51. Sweetser PB, Schow GS, Hutchison JM. Metabolism of chlorsulfuron by plants: Biological basis for selectivity of a new herbicide for cereals. Pestic Biochem Physiol 1982; 17: 18-23.
52. Blair AM, Martin TD. A review of the activity, fate and mode of action of sulfonylurea herbicides. Pestic Sci 1988; 22: 195-219.
53. Beyer EM, Duffy MJ, Hay JV, Schlueter DD. Sulfonylureas. In: Kearney PC, Kaufman DD, eds. Herbicides: Chemistry, Degradation and Mode of Action. New York: Marcel Dekker, 1988; 3: 117-189.

54. Ray TB. Site of action of chlorsulfuron. Inhibition of valine and isoleucine biosynthesis in plants. Plant Physiol 1984; 75: 827-831.
55. Chaleff RS, Mauvais CJ. Acetolactate synthase is the site of action of two sulfonylurea herbicides in higher plants. Science 1984; 224: 1443-1445.
56. Saari LL, Cotterman JC, Thill DC. Resistance to acetolactate synthase inhibiting herbicides. In: Powles SB, Holtum JAM, eds. Herbicide Resistance in Plants: Biology and Biochemistry. Boca Raton, FL: CRC Press, 1994; 83-139.
57. Sweetser PB. Safening of sulfonylurea herbicides to cereal crops: mode of herbicide antidote action. Proc Br Crop Prot Conf-Weeds 1985; 1: 1147-1154.
58. Owen WJ, deBoer G. Plant metabolism and the design of new selective herbicides. In. Ragsdale NN, Kearney PC, Plimmer JR, eds. Conf Proc Eighth IUPAC International Congress of Pesticide Chemistry. Washington, DC: Amer Chem Soc, 1995; 257-268.
59. Kleschick WA, Gerwick BC, Carson CM, Monte WT, Snider SW. DE-498, a new acetolactate synthase inhibiting herbicide with multicrop selectivity. J Agric Food Chem 1992; 40: 1083-1085.
60. Gerwick BC, Subramanian MV, Loney-Gallant VI. Mechanism of action of the 1,2,4-triazolo[1,5a]pyrimidines. Pestic Sci 1990; 29: 357-364.
61. Kleschick WA, Gerwick BC. The chemistry and biochemistry of triazolopyrimidine sulfonanilide herbicides, a new class of acetolactate synthase inhibitors. In: Copping L, Dodge AD, Dalziel J, eds. Prospects for Amino Acid Biosynthesis Inhibitors in Crop Protection and Pharmaceutical Chemistry (British Crop Protection Monograph 42). Lavenham, UK: Lavenham Press, Ltd., 1989; 133-145.
62. Lebaron HM, McFarland JE, Simoneaux BJ, Ebert E. Metolachlor. In: Kearney PC, Kaufman DD, eds. Herbicides: Chemistry, Degradation and Mode of Action. New York: Marcel Dekker, 1988; 3: 335-382.
63. Moreland DE, Corbin FT, Novitsky WP. Metabolism of metolachlor by a microsomal fraction isolated from grain sorghum (*Sorghum bicolor*) shoots. Z Naturforsch 1990; 45c: 558-564.
64. Moreland DE, Corbin FT, McFarland JE. Oxidation of multiple substrates by corn shoot microsomes. Pestic Biochem Physiol 1993; 47: 206-214.
65. Christopher JT, Powles SB, Liljegren DR, Holtum JAM. Cross-resistance to herbicides in annual ryegrass (*Lolium rigidum*). II Chlorsulfuron resistance involves a wheat-like detoxification system. Plant Physiol 1991; 95: 1036-1043.
66. Christopher JT, Preston C, Powles SB. Malathion antagonizes metabolism-based chlorsulfuron resistance in *Lolium rigidum*. Pestic Biochem Physiol 1994; 49: 172-182.
67. Heap IM. Resistance to herbicides in annual ryegrass (*Lolium rigidum*) in Australia. In: Caseley JC, Cussans GW, Atkin RK, eds. Herbicide Resistance in Weeds and Crops. Oxford: Butterworth-Heinemann Ltd., 1991; 57-66.
68. Kreuz K, Fonn'-Pfister R. Herbicide-insecticide interactions in maize: Malathion inhibits cytochrome P450-dependent primisulfuron metabolism. Pestic Biochem Physiol 1992; 43: 232-240.

69. Burnet MWM, Hildebrand OB, Holtum JAM, Powles SB. Amitrole, triazine, substituted urea, and metribuzin resistance in a biotype of rigid ryegrass (*Lolium rigidum*). Weed Sci 1991; 39: 317-323.
70. Burnet MWM, Loveys BR, Holtum JAM, Powles SB. Increased detoxification is a mechanism of simazine resistance in *Lolium rigidum*. Pestic Biochem Physiol 1993; 46: 207-218.
71. Burnet MWM, Loveys BR, Holtum JAM, Powles SB. A mechanism of chlortoluron resistance in *Lolium rigidum*. Planta 1993; 190: 182-189.
72. Moss SR, Cussans GW. The development of herbicide-resistant populations of *Alopecurus myosuroides* (black-grass) in England. In: Caseley JC, Cussans GW, Atkin RK, eds. Herbicide Resistance in Weeds and Crops. Oxford: Butterworth-Heinemann Ltd., 1991; 45-46.
73. Jones OTG. Cytochrome P450 and herbicide resistance. In: Caseley JC, Cussans GW, Atkin RK, eds. Herbicide Resistance in Weeds and Crops. Oxford: Butterworth-Heinemann Ltd., 1991; 213-226.
74. Kemp MS, Moss SR, Thomas TH. Herbicide resistance in *Alopecurus myosuroides*. In: Green MB, LeBaron HM, Moberg WK, eds. Managing Resistance to Agrochemicals: From Fundamental Research to Practical Strategies. Washington, DC: Amer Chem Soc Symp Ser 421, 1990; 376-393.
75. Gressel J. Catch 22 - Mutually exclusive strategies for delaying/preventing polygenically vs monogenetically inherited resistances. Eighth IUPAC International Congress of Pesticide Chemistry, Washington, DC 1994; Abstr No 437: 493.
76. Rubin B. Herbicide resistance in weeds and crops, progress and prospects. In: Caseley JC, Cussans GW, Atkin RK, eds. Herbicide Resistance in Weeds and Crops. Oxford: Butterworth-Heinemann Ltd., 1991; 387-414.
77. Donaldson RP, Luster DG. Multiple forms of plant cytochromes P450. Plant Physiol 1991; 96: 669-674.