An Overview of the Mechanisms of Action of Herbicide Safeners

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Z. Naturforsch. **46c,** 819–827 (1991); received March 26, 1991

Herbicides, Safeners, Mechanisms of Action

Herbicide safeners are chemicals used for manipulating the tolerance of large-seeded grass crops to selected soil-applied herbicides. The physiological interactions of herbicides and their respective safeners are characterized by the following facts: a) safeners are most effective when applied prior to or simultaneously with the herbicides whose injury they prevent; b) safeners exhibit a high degree of botanical and chemical specificity protecting only certain grasses against injury caused from specific classes of herbicides; and c) protected grass crops are moderately tolerant to the antagonized herbicides. At the biochemical level, safeners may act either as "bioregulators" regulating the amount of a given herbicide that reaches its target site in an active form or as "antagonists" of herbicidal effects at a similar site of action. A safener-induced enhancement of herbicide detoxication in protected plants is currently viewed as the most apparent mechanism for the action of the currently available safeners. Safeners enhance the conjugation of carbamothioate and chloroacetanilide herbicides with glutathione either by elevating the levels of reduced glutathione (GSH) or by inducing the activity of specific glutathione S-transferases (GSTs). A safener-induced enhancement of the activity of other degradative enzymes such as the cytochrome P450-dependent mixed function oxidases or UDP-glucosyl transferases seems to be important for the protective action of safeners against injury from aryloxyphenoxypropionate, imidazolinone, and sulfonylurea herbicides. Metabolic processes related to acetyl-CoA metabolism have been implicated as likely target sites for a competitive antagonism between safeners and chloroacetanilide or carbamothioate herbicides. At the molecular level, the "gene activation" and "gene amplification" theories offer a likely explanation for the action of safeners.

Introduction

"Herbicide safeners" (also known as "herbicide antidotes") are chemical substances that selectively protect crop plants against herbicide injury [1]. The selectivity of herbicide safeners results either from a selective placement of the safener or is due to biochemical principles. Thus, some safeners are applied directly to crop seeds prior to planting, while others possess true selectivity and are applied to the crop and weeds as prepackaged mixtures with the herbicide [1].

Abbreviations: Alachlor, 2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide; Barban, 4-chloro-2-butynyl 3-chlorophenylcarbamate; BAS-145138, 1-dichloroacetyl-hexahydro-3,3-8α-trimethyl-pyrolo-[1,2-α]-pyrimidin-6-(2H)-one; BCS, 4-bromophenyl-chloromethyl sulfone; Benoxacor, 4-(dichloroacetyl)-3,4-dihydro-3-methyl-2H-1,4-benzoxazine; Bentazon, 3-(1-methylethyl)-(1H)-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide; CGA-185072, 5-chloro-8-quinolinoxy-acetic acid-1-methyl-hexylester; Chlorimuron, 2-[[[[(4-chloro-6-methyoxy-2-pyridinyl)amino]carbonyl]-amino]sulfonyl]benzoic acid; Chlorsulfuron, 2-chloro-N-[[(4-methoxy-6-methyl-1,3,5-traizin-2-yl)amino] carbonyl]benzenesulfonamide; Chlortoluron, N′, (3-chloro-4-methylphenyl)-N,N-dimethylurea; Cyometrinil, (Z)-α-[cyanomethoxy)benzeneacetonitrile; Dichlormid, 2,2-dichloro-N,N-di-2-propenylacetamide; Diclofop, (±)-2-[4-(2,4-dichlorophenoxy)phenoxy]propionic acid; Dimepiperate, S-(1-methyl-1-phenylethyl-piperidine-1-carbamothioate); EPTC, S-ethyl dipropylcarbamothioate; Fenchlorazole-ethyl, ethyl-1-(2,4-dichlorophenyl)-5-trichloromethyl-1H-1,2,4-triazole-3-carboxylate; Fenclorim, 4,6-dichloro-2-phenylpyrimidine; Flurazole, phenylmethyl 2-chloro-4-(trifluoromethyl)-5-thiazole-carboxylate; Fluxofenim, 2,2,2-trifluoro-4′-chloroacetophenone-0-(1,3-dioxolan-2-yl-methyl)oxime; Imazethapyr, (±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid; Metolachlor, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide; MG-191, 2-dichloromethyl-2-methyl-1,3-dioxolane; Naphthalic anhydride, naphthalene-1,8-dicarboxylic acid anhydride; Oxabetrinil, α-[(1,3-dioxolan-2-ylmethoxy)-imino]benzeneacetonitrile; Pretilachlor, 2-chloro-2′,6′-diethyl-N-(2-propoxyethyl)acetanilide; Primisulfuron, 2-[3-(4,6-bis(difluoromethoxy)-pyrimidin-2-yl)-ureido-sulfonyl]benzoic acid methylester; R-29148, 3-(dichloroacetyl)-2,2,5-trimethyl-1,3-oxazolidine.

* Based on a paper presented at the International Conference of Herbicide Safeners, August 12–15, 1990 in Budapest, Hungary

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0939–5075/91/0900–0819 $ 01.30/0

The successful herbicide-safener-crop combinations that have been exploited commercially are summarized in Table I. All of the currently marketed safeners are particularly effective in protecting large-seeded grass crops such as maize (*Zea mays* L.), grain sorghum [*Sorghum bicolor* (L.) Moench], rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) against injury caused by several, chemically-diverse, classes of herbicides such as the carbamothioates and chloroacetanilides, sulfonylureas, imidazolinones, aryloxyphenoxypropionates, cyclohexanediones, and isoxazolidinones [1, 2].

Commercialized safeners are members of diverse chemical groups including naphthopyranones (*e.g.* NA or naphthalic anhydride); dichloroacetamides (*e.g.* dichlormid, benoxacor, BAS-145138); oxime ether derivatives (*e.g.* oxabetrinil and fluxofenim); substituted thiazoles (*e.g.* flurazole); dichloromethyldioxolans (*e.g.* MG-191); phenylpyrimidines (*e.g.* fenclorim); triazole carboxylates (*e.g.* fenchlorazole-ethyl); and quinolinoxyacetates (*e.g.* CGA-185072).

The possible physiological or biochemical mechanisms of action of herbicide safeners have been investigated extensively in the last two decades. Most of this research has been reviewed previously [2–7]. The purpose of this brief review is to summarize recent developments in the physiology, biochemistry, and molecular biology of the action of herbicide safeners.

Physiological and Biochemical Aspects of Safener Action

A recent analysis of the literature related to the physiological aspects of the successful crop-herbicide-safener combinations [8] showed that the interactions of safeners and antagonized herbicides are characterized by three major facts: a) safeners are most effective when applied prior to or simultaneously with the herbicides whose injury they prevent; b) safeners exhibit a high degree of botanical and chemical specificity protecting only certain grass crops against injury from specific herbicides; and c) protected grass crops are moderately tolerant to the antagonized herbicides.

The capacity of plants to detoxify certain herbicides by specific biochemical reactions is not evenly distributed among various plant species and it has long been recognized as an important process contributing to the selectivity of herbicides [8]. Thus, it is reasonable to expect that the protective action of safeners may be related closely to the physiological or biochemical processes contributing to the moderate tolerance of the protected grass crops to the antagonized herbicides. However, the basis of the botanical specificity of commercialized safeners (only grass crops are protected) is still a puzzling enigma that remains to be solved.

The chemical specificity exhibited by the currently marketed herbicide safeners has been partially attributed to their structural similarity to some of the antagonized herbicides. A good example of such a case is the safener dichlormid which is structurally very similar to EPTC or other carbamothioate herbicides [9]. The chloroacetamide derivative allidochlor or CDAA, which is chemically almost identical to the safener dichlormid, acts both as a safener and as a herbicide depending on the concentration used [7]. In other cases, however, the structural similarity of herbicides and

Table I. Successful herbicide safener crop combinations.

Herbicide class	Safeners	Protected Crops
Carbamothioates	NA, dichlormid, BCS	maize, wheat, rice
Chroroacetanilides	NA, dichlormid, BAS-145138	maize
	oxime ethers, flurazole	grain sorghum
	fenclorim	rice
Sulfonylureas	Na, dichlormid	maize, grain sorghum
	dimepiperate	rice
Imidazolinones	NA	maize
Aryloxyphenoxy-	fenchlorazole-ethyl	wheat, oats
propionates	NA, CGA-185072	barley
Isoxazolidinones	NA, dichlormid	maize, wheat
Cyclohexanediones	NA, dichlormid	maize, grain sorghum

their respective safeners is not so obvious. Nevertheless, studies employing the use of computer-assisted molecular modelling (CAMM) showed that at the molecular level, herbicides and their respective safeners are quite similar [10]. Comparisons of the molecular parameters measured for the safener/herbicide pairs of dichlormid/EPTC, flurazole/alachlor, fluxofenim/metolachlor, fenclorim/pretilachlor, benoxacor/metolachlor showed that these molecules possess similar degrees of bonding and charge distribution as well as molecular volumes.

From the preceded discussion it is evident that herbicide safeners may act either as "bioregulators" influencing the amount of a herbicide that reaches its target site in an active form or as "antagonists" of herbicidal effects at a similar site of action [8]. Safeners may reduce the amount of a herbicide reaching its site of action by either reducing the rate of its uptake and/or translocation or by enhancing the rate of its metabolic detoxication.

Alteration of Herbicide Uptake and/or Translocation by Safeners

The emerging shoot (coleoptile) of grass crops has been long recognized as a preferential site for the uptake of carbamothioate and chloroacetanilide herbicides and their respective safeners [8, 11, 12]. Therefore, the possibility that safeners may protect grass crops by preventing herbicide uptake appears promising and has been tested. Selected examples of the results of studies on this topic are presented in Table II. These results have been variable and often contadictory and they have been reviewed previously [4, 8].

In general, the effects of safeners on herbicide uptake and/or translocation are dependent on the concentrations of herbicides and their respective safeners used as well as on the plant tissue examined. In addition, the safener-induced alterations in the rate of uptake and/or translocation of a herbicide could be either direct or indirect. Direct safener effects could result from an antagonistic interaction of the herbicide and its respective safener on membrane permeability or a competition for "active sites" of uptake [13]. Indirect safener effects may result from a safener-induced prevention of herbicidal effects on cuticular integrity which will decrease transpiration and reduce herbicide uptake in safener-treated plants [14]. Based on current evidence, it is safe to conclude that safener-induced effects on herbicide uptake/translocation cannot explain the protective action of herbicide safeners.

Enhancement of Herbicide Detoxication by Safeners

A safener-induced enhancement of herbicide detoxication in protected plants seems to be the major mechanism involved in the protective action of the currently developed safeners. Safeners enhance the glutathione conjugation of chloroacetanilide and sulfoxidized carbamothioate herbicides either by elevating the levels of reduced glutathione (GSH) or by inducing the activity of glutathione-dependent enzymes. A safener-induced enhancement of the activity of other degradative enzymes such as cytochrome P450-dependent mixed function oxidases and UDP-glucosyl transferases seems to be important for the protection of grass crops against injury from aryloxyphenoxypropionate, sulfonylurea and imidazolinone herbicides.

Table II. Alteration of herbicide uptake/distribution by safeners.

Herbicide	Safener	Plant species	Uptake	Reference
Barban	NA	oats	reduced	[15]
EPTC	dichlormid	maize	reduced	[13]
Metolachlor	NA, cyometrinil	grain sorghum	reduced	[16], [17]
Pretilachlor	fenclorim	rice	reduced	[18]
Alachlor	flurazole	grain sorghum	enhanced	[19]
EPTC	NA, dichlormid cyometrinil	maize	enhanced	[13], [20]
Metolachlor	oxabetrinil	grain sorghum	enhanced	[21], [22]

Effects of Safeners on Glutathione and Glutathione-Dependent Enzymes

Glutathione, found primarily in its reduced form (GSH), is the most important non-protein plant thiol needed for the normal function of key metabolic processes such as protein synthesis, protection of chloroplast membranes from peroxidative damage and detoxication of selected herbicides [23].

Safeners may elevate GSH levels in protected plants either directly or indirectly by: a) regulating the assimilatory sulfate reduction to cysteine; b) activating key enzymes involved in the biosynthesis of GSH; and c) inducing the activity of glutathione reductase. A summary of such effects is presented in Table III.

Adams *et al.* [24] showed that dichlormid increased GSH levels in maize and other plants by enhancing the activity of ATP-sulfurylase. This enzyme represents the first regulatory step in sulfate assimilation and catalyzes the reaction between ATP and sulfate to yield adenosine-5′-phosphosulfate. More recently, however, Farago and Brunold [25] showed that dichlormid and benoxacor increased cysteine and GSH levels in maize by elevating the activity of adenosine-5′-phosphosulfate sulfotransferase (APSSTase), the second key enzyme in the assimilatory sulfate reduction, rather than the activity of ATP-sulfotransferase. These results indicate that safeners may act by eliminating the feedback regulation of the activities of key enzymes involved in the assimilatory sulfate reduction caused by increased concentrations of the end products cysteine and GSH [25].

A direct activation of maize GSH synthetase II by the safener dichlormid has been reported by Carringer *et al.* [20], whereas Breaux *et al.* [29] have postulated the regulation of the enzyme glutamyl cysteine synthetase or GSH synthetase (EC 6.3.2.2) by the safener flurazole in maize and grain sorghum. Flurazole is known to conjugate with GSH in maize and grain sorghum [29] and it is likely that its GS-conjugate may bind to GSH synthetase I and override the feedback inhibition of GSH synthesis by the end product of the pathway, GSH. Such a mechanism has been well documented in regulatory studies of GSH synthesis in mamalian systems [30].

Safeners may elevate GSH levels in protected plants indirectly by inducing the activity of glutathione reductase (GR, EC 1.6.4.2). GR is a NADPH-dependent enzyme which catalyzes the reduction of oxidized glutathione (GSSG) to GSH. An induction of GR by safeners will maintain a high GSH/GSSG ratio in the cells of protected grasses compensating for GSH used as a reductant in the formation of the GS-conjugates of chloroacetanilide and sulfoxidized carbamothioates or in the ascorbate-dehydroascorbate redox system of the chloroplast. Examples of stimulatory effects of selected safeners on the GR activity of grass crops are shown in Table III.

Enhanced metabolism of chloroacetanilide and sulfoxidized carbamothioate herbicides by GSH conjugation could result also from a safener-induced increase of the activity of the respective glutathione-S-transferase enzymes (GSTs, EC 2.5.1.18) which catalyze this reaction in protected

Table III. Examples of safener-induced enhancement of the activity of selected enzymes of sulfate metabolism and glutathione synthesis in plants.

Enzyme	Plant	Safener	Reference
ATP-sulfurylase (EC 2.7.7.4)	maize	benoxacor	[25, 26]
	grain sorghum	dichlormid	[25]
	Amaranthus sp.	R-29148	[25]
Adenosine-5′-phosphosulfate sulfotransferase (APSSTase)	maize	benoxacor	[26]
		dichlormid	[26]
GSH synthetase II (EC 6.3.2.3)	maize	dichlormid	[20]
Glutathione reductase (EC 1.6.4.2)	maize	dichlormid, MG-191	[27]
	rice	fenclorim	[28]
	grain sorghum	fluxofenim	[29]

grass crops. Plants contain multiple forms of GST enzymes which exhibit a rather high degree of substrate specificity [31]. At present, however, only the GST enzymes from maize and grain sorghum have been studied in any detail [31, 32]. Three GST isozymes exhibiting a high specificity for chloroacetanilide herbicides and at least two isozymes with high specificity for *s*-triazine herbicides are known to exist in maize [31]. GST isozymes are usually dimeric proteins having an approximate molecular weight of 50,000 M_r.

A strong correlation between the efficacy of a safener in protecting grain sorghum from chloroacetanilide injury and its ability to increase GST activity has been demonstrated [33]. Flurazole was the most effective sorghum safener eliciting a 30-fold increase in GST activity. Oxabetrinil and NA were also effective safeners of sorghum against metolachlor injury causing a 20-fold and 17-fold increase of GST activity, whereas dichlormid was the least effective safener of sorghum causing only a 5-fold increase in GST activity. Mozer *et al.* [34] showed that flurazole not only enhanced the activity of maize GSTs that are constitutively present, but it also induced a novel GST isozyme with greater activity in conjugating chloroacetanilide herbicides with GSH. The exact mechanism of the safener-induced enhancement of GST activity is not known. It appears likely that safeners act by an enzyme induction process rather than an enzyme activation since in *in vitro* studies dichlormid and oxabetrinil did not alter the activity of GSTs. [32, 33].

Effects of Safeners on Other Metabolic Enzymes

Safeners may act also by inducing the activity or the *de novo* synthesis of cytochrome P450-dependent mixed function oxidases (MFOs, EC 1.14.14.1) involved in the metabolic detoxication of carbamothioate, aryloxyphenoxypropionate, sulfonylurea, and imidazolinone herbicides in protected grass crops [8, 35]. Such a hypothesis is supported by several indirect studies conducted with the use of selected antioxidants or insecticide synergists which act as inhibitors of MFO enzymes. More recently, several direct studies on the effects of herbicide safeners on the activity of specific cytochrome P-450 containing MFO enzymes have been reported. The results of such studies are summarized on Table IV. In all examples presented in Table IV, safener treatments caused an enhancement of the oxidative metabolism of selected herbicides. The only exception was the O-demethylation of metolachlor by grain sorghum microsomes which was depressed following pretreatments with the oxime ether safeners oxabetrinil and fluxofenim [47].

Since in most cases of oxidative herbicide metabolism by plants involving aryl or alkyl hydroxylation, the hydroxylated products are rapidly glucosylated it is reasonable to expect that herbicide safeners may also enhance the activity of UDP-glucosyl transferases (EC 2.4.1.71) which catalyze such glucosylation reactions. Indeed, a recent report by Lamoureux and Rusness [36] showed that the safener BAS 145138 partially protects maize from chlorimuron-ethyl injury by increasing the rate of herbicide metabolism by hydroxylation, glucosylation, and glutathione conjugation.

Table IV. Safener-induced alteration of oxidative metabolism of herbicides.

Herbicide	Safener	Plant	Oxidative reaction	Reference
Bentazon	NA	maize	aryl hydroxylation	[41]
	NA, oxabetrinil	grain sorghum	aryl hydroxylation	[47]
Chlorimuron	BAS-145138	maize	aryl hydroxylation	[37]
Chlorsulfuron	NA, cyometrinil	maize	aryl hydroxylation	[42]
Chlortoluron	benoxacor	maize	alkyl hydroxylation	[43]
Diclofopmethyl	NA	oats	aryl hydroxylation	[44]
EPTC	dichlormid	maize	sulfoxidation	[45]
Imazethapyr	NA	maize	aryl hydroxylation	[46]
Metolachlor	oxime ethers	grain sorghum	O-demethylation	[47]
Primisulfuron	benoxacor	maize	aryl hydroxylation	[48]

Other oxidative enzymes that have been studied in relation to the mechanism of action of herbicide safeners include peroxidase (EC 1.11.1.7) and polyphenol oxidase (EC 1.10.3.2). Peroxidases are hemoproteins which catalyze the oxidation of their substrates utilizing hydrogen peroxide. Apart from their association with lignification, peroxidases catalyze the oxidation of indoleacetic acid (IAA), the hydroxylation of proline, and they may participate in the metabolic detoxication of xenobiotics in plants [35, 37]. Harvey *et al.* [38] reported that the safener dichlormid reduced peroxidase activity in maize seedlings and counteracted the stimulatory effects of the herbicide EPTC on the activity of this enzyme. Polyphenol oxidase is a chloroplast oxidase with no established function [39]. Wilkinson [40] reported that the safener NA stimulated the activity of this enzyme in maize.

Interactions of Herbicides and Safeners at Target Sites

The chemically diverse groups of herbicides that are antidoted by safeners on grass crops exert their action by a variety of biochemical mechanisms which are well-defined for sulfonylureas, imidazolinones, isoxazolidinones, aryloxyphenoxypropionates, and cyclohexanediones, but still speculative for carbamothioates and chloroacetanilides [49].

Sulfonylurea and imidazolinone herbicides are potent inhibitors of the enzyme acetohydroxyacid synthase or acetolactate synthase (AHAS or ALS, EC 4.1.3.18) and of the biosynthesis of branched chain amino acids [50]. Rubin and Casida [51] reported that pretreatment of maize with dichlormid elevated AHAS activity contributing partially to the protection of maize against injury from the herbicide chlorsulfuron. Polge *et al.* [52], however, reported that while NA and dichlormid enhanced the activity of AHAS in treated maize seedlings, AHAS extracted from safened plants was more sensitive to chlorsulfuron inhibition. In other studies, Barrett [46] failed to detect any measurable effects of the safeners NA, oxabetrinil, dichlormid and flurazole on extractable AHAS activity in shoots or roots of maize seedlings.

Aryloxyphenoxypropionate and cyclohexanedione herbicides have been recently identified as potent inhibitors of the enzyme acetyl-CoA carboxylase (ACCase, EC 6.4.1.2) [53]. Oxime ether safeners such as oxabetrinil and fluxofenim did not affect the activity of ACCase from grain sorghum [54]. Dichlormid antagonized partially the effects of sethoxydim on acetate incorporation into total lipids of isolated grain sorghum protoplasts, but it did not reverse the effects of sethoxydim on the target enzyme ACCase [55].

The symptomatology of carbamothioate and chloroacetanilide herbicides is not indicative of an acute blockage of a central metabolic reaction in susceptible plants, but it is rather consistent with a chronic loss of vital metabolic components [40]. Shoot deformations and growth inhibitions caused by these herbicides could result from their reported effects on cell division and cell elongation in tissues of treated grass plants [11, 56]. Some of the symptoms (*e.g.* stunting) caused by carbamothioate and chloroacetanilide herbicides on maize and grain sorghum seedlings are similar to those caused by classical growth retardants and could be prevented by exogenous applications of gibberellin (GA) [40]. At the cellular level, metabolic processes that are affected by chloroacetanilide and/or carbamothioate herbicides include synthesis of lipids, terpenoids, lignins, proteins, and nucleic acids, membrane function and ion transport [11, 12, 56]. Because many of the plant metabolic products affected by chloroacetanilide and carbamothioate herbicides are synthesized *via* acetyl-CoA intermediates, it has been proposed that the action of these herbicides may be related to some aspect of acetyl-CoA metabolism [56]. Antagonistic interactions between these herbicides and safeners at the aforementioned sites of action are possible and they have been reviewed [8].

Molecular Aspects of Safener Action

Alteration of structural genes, gene regulation, and gene amplification are currently recognized as significant genetic factors conferring herbicide tolerance or resistance on agronomic crops [50]. A number of selected plant enzymes catalyzing the metabolic detoxication of specific herbicides in higher plants have been isolated and partially characterized [57]. In most cases, however, the genes coding for these enzymes as well as their regulation by herbicide safeners have not been studied in detail.

As mentioned earlier, three GST isozymes, designated as GST I, GST II, and GST III, have been purified and characterized from maize [31]. All three isozymes catalyze the conjugation of chloroacetanilide herbicides with GSH, but while GST I and GST III are constitutively present in maize, GST II is seen only in safener-treated maize [34]. DNA sequences for the GST I and GST III isozymes from maize have been reported and they show some sequence similarity to each other and to other known GST sequences [31]. Wiegand *et al.* [58] showed that the safener flurazole may act at the transcriptional level inducing a 3- to 4-fold increase in the steady state level of mRNA encoding for the GST I gene in maize tissues grown from flurazole-treated seeds. Therefore, it appears that the "gene activation" theory which has been implicated in the action of natural or synthetic plant hormones [59] is also promising for explaining the protective action of herbicide safeners.

If safeners indeed act at a transcriptional level by regulating the expression of selected plant genes then their molecular mechanism of action should include an induction of mRNA, which is rapid, specific and unaffected by protein synthesis inhibitors. Wiegand *et al.* [58] showed that the induction of mRNA by flurazole is very specific and rapid, but studies on the effect of protein synthesis inhibitors on this effect of safeners are not available. The potential involvement of positive or negative control models in the regulation of safener-inducible genes for GSTs or other enzymes in protected plants has been postulated [8]. Undoubtedly, further research is needed to elucidate the molecular mechanisms of action of herbicide safeners.

Conclusion

The "enhanced herbicide detoxication" theory and the "competitive antagonism" theory represent the two most widely accepted approaches to explain the protective action of herbicide safeners. The currently available evidence providing support for and against these two theories of safener action is summarized in Table V. A safener-induced enhancement of herbicide detoxication in safened plants seems to be the major mechanism involved in the protective action of the currently developed safeners. Most safeners resemble structurally their respective herbicides and they induce the enzymes and cofactors needed for their own metabolism as well as that of the antidoted herbicides in safened plants. Although safeners can compete with herbicides at common target sites, such a mechanism seems unlikely. As shown in Table V, the ratio of safener-to-herbicide doses in prepackaged formulated mixtures of herbicides and safeners ranges from 1:6 to 1:30. Such ratios do not favor the "antagonist" theory of safener action since very little safener will be available at the site of action to compete effectively with its respective herbicide which would be present at considerably higher concentrations. A better understanding of the mechanisms of action of current safeners

Table V. Comparison of the evidence in support for or against the two major theories of safener action.

Enhanced Herbicide Detoxication
Support for:
– Diversity of chemistries and modes of action of antidoted herbicides
– Similarity of metabolic pathways detoxifying the antidoted herbicides in protected grass plants
– Preconditioning of susceptible crops following treatments with subtoxic doses of marginally selective herbicides
Support against:
– Weak correlation between the protective action of some safeners and their effects on GSH biosynthesis and GST activity
– Other chemicals known to increase GSH levels and GST activity in grass crops do not act as herbicide safeners
Competitive Antagonism Theory
Support for:
– Structural similarity of herbicides and their respective safeners
– Antagonism of carbamothioate and chloroacetanilide herbicidal effects on vital physiological processes by safeners
Support against:
– Botanical specificity of safener action (only grass crops are protected)
– The ratios of safener:herbicide doses in successful herbicide/safener combinations are very low (1:6 to 1:30)
– Safeners do not counteract the effects of herbicides with known mechanisms of action at the level of target enzymes (ACCase and ALS)
– Failure of safeners to protect grass crops against the herbicide dalapon whose injury symptoms resemble those caused by carbamothioate and chloroacetanilide herbicides

and herbicides will allow more positive attempts towards increasing the number of situations in which crop safeners for herbicides could be used. Continuing advancements in molecular biology techniques will undoubtedly contribute towards the achievement of these goals in the near future.

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