2 Biochemical Mechanisms, Inheritance, and Molecular Genetics of Herbicide Resistance in Weeds

Christopher Preston and Carol A. Mallory-Smith

CONTENTS

2.1 INTRODUCTION

As discussed in Chapter 1, herbicide resistance has been reported to most herbicide chemical classes and has been documented in 147 different weed species.[1] A complete and updated list can be found at http://www.weedscience.com. Resistance occurs as a result of heritable changes to biochemical processes that enable plant survival when treated with a herbicide. This chapter deals with the specific changes to plant biochemistry that endow resistance to herbicides. Any biochemical change that allows a plant to survive herbicide application can be selected. This means that even for the same herbicide, resistance can be endowed by a number of different biochemical mechanisms. Resistance can result from changes to the herbicide target site such that binding of the herbicide is reduced, or over-expression of the target site may occur. Alternatively, there may be a reduction in the amount of herbicide that reaches the target enzyme through detoxification, sequestration, or reduced absorption of herbicide. Finally, the plant may survive through the ability to protect plant metabolism from toxic compounds produced as a consequence of herbicide action. This chapter considers major mechanisms of resistance to herbicides in weed species where resistance has evolved in the field.

2.2 TARGET-SITE-BASED RESISTANCE

All herbicides act by binding or otherwise interacting with one or more proteins with consequent negative effects on plant metabolism or growth. Plants can become resistant to the effects of herbicides through modifications to these proteins that reduce or eliminate the ability of the herbicide to bind or interact. In such cases, resistance is described as being target-site-based. An alternative type of target-site-based resistance is over-production of herbicide-binding proteins. Target-site-based [2–4]

Reviews of target-site resistance to the PS II-,[5] ALS-,[6,7] and ACCase-[8,9] inhibiting herbicides have been extensive and, therefore, need not be repeated here. This section will only briefly cover areas reviewed previously and will focus on consideration of new information.

2.2.1 Resistance to Photosystem II-Inhibiting Herbicides

Photosynthetic electron transport is inhibited by a number of different herbicide chemical classes, including the triazines, ureas, and nitriles that block electron transport on the reducing side of photosystem II (PS II).[5] This blockage of electron flow leads to production of excess singlet oxygen, which results in destruction of lipids and chlorophyll.

The first example of resistance to PS II-inhibiting herbicides was reported in 1970,[10] and many new examples of resistance occurred shortly thereafter. By 1990, more than 50 weed species had populations with resistance to PS II-inhibiting herbicides.[11] Triazine resistance has now been documented in 61 different weed species around the world.[1] With few exceptions, resistance to PS II-inhibiting herbicides is target-site-based and due to changes in the herbicide-binding domain on

the D1 protein of PS II.[5] Cross-resistance patterns vary among the various chemical classes of PS II-inhibiting herbicides. Usually plants that are resistant to triazines are not cross-resistant to the substituted ureas or nitriles.[12] The molecular basis of most field-selected triazine-resistant weeds has been identified as a single amino acid substitution of Ser 264 to Gly in the D1 protein encoded by the chloroplastic *psbA* gene.[13] This amino acid substitution has been identified in resistant populations of numerous weed species and removes a hydrogen bond that is important for binding herbicides (Figure 2.1).

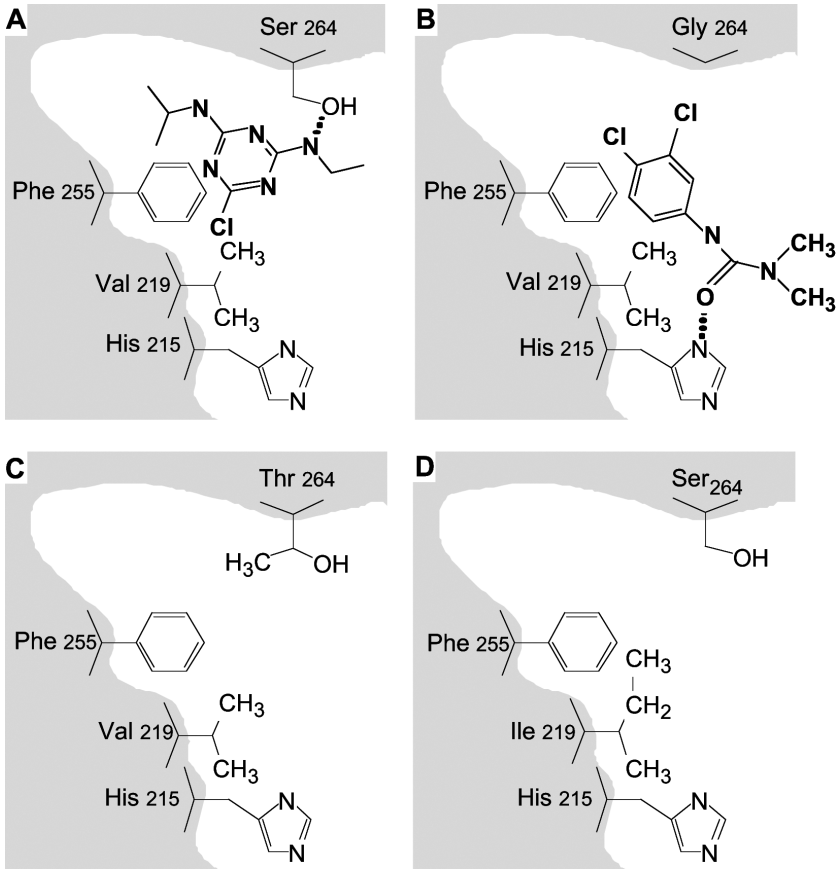

FIGURE 2.1 The effect on binding of herbicides of mutations within the D1 protein of PS II Wild type D1 protein (A), Gly 264 substitution (B), Thr 264 substitution (C), and Ile 219 substitution (D). Binding of atrazine (A) and diuron (B) are shown. Ser 264 provides a hydrogen bond to atrazine and the phenyl ring of Phe 255 is involved in stacking interactions with the triazine ring of atrazine. His 215 provides a hydrogen bond to diuron and the phenyl ring of Phe 255 is involved in stacking interactions with the phenyl ring of diuron. Val 219 is the amino acid sitting directly above His 215 in α-helix IV. The positions of amino acids and herbicides are based on the structures shown for the reaction center of *Rhodopseudomonus viridis* (as in [14]).

Apart from the Ser 264 to Gly change, there are only two other mutations in the D1 protein reported in weed populations resistant to PS II-inhibiting herbicides. A population of *Portulaca oleracea* resistant to linuron and atrazine has a single amino acid substitution at the Ser 264 resulting in a Thr substitution.[15,16] A Val 219 to Ile substitution confers diuron and metribuzin resistance in two different populations of *Poa annua* from fields in Oregon.[17] In the latter case, there was no change at Ser 264 or in any other positions in the herbicide-binding region. Previously, the Val 219 to Ile substitution had been reported in green algae but not in a higher plant species under field selection.[13]

The PS II reaction center proteins of higher plants are homologous to the reaction centers of photosynthetic bacteria such as *Rhodopseudomonas viridis*.[5,13] The reaction center of *R. viridis* has been crystallized, providing an insight into the likely structure of the plant PS II reaction center.[18] Crystals of the *R. viridis* reaction center containing bound herbicides[19–21] provide evidence for the role of important amino acids in herbicide binding (Figure 2.1). Ser 264 provides a hydrogen bond to triazine herbicides; however, substituted urea herbicides bind deeper into the Q_B binding site, hydrogen bonding with His 215. Phe 255 provides a hydrophobic interaction with both groups of herbicides. Thus the Ser 264 to Gly 264 change removes a hydrogen bond necessary for binding of triazine herbicides, but not substituted urea herbicides. Therefore, these mutants are highly resistant to triazine herbicides only.[13] The Ser 264 to Thr 264 change does not remove the hydrogen bond, but may distort its position. This change most likely causes interference with entry of herbicides to the Q_B site, or sterically interferes with herbicide interaction with Phe 255. In this case, resistance to both groups of herbicides is found.[15] The Val 219 to Ile 219 change is a conservative change. However, Val 219 is positioned immediately above His 215 in α-helix IV.[14] As Ile has a larger side chain than Val, this change may interfere with binding of herbicides to His 215. Therefore, the Val 219 to Ile 219 change would be predicted to result in substituted urea resistance. Triazine resistance also might be expected due to interference in the hydrophobic interactions between Phe 255 and the herbicide or changing the position of a tightly bound water molecule that provides an additional hydrogen bond to triazines.[21]

Because the mutations that confer resistance to PS II-inhibiting herbicides are encoded on plastid DNA, resistance is maternally inherited.[22] However, very occasional transmission of PS II resistance through pollen is reported, particularly where large numbers of crosses are made.[23] In addition, recent reports have indicated heterogeneity of chloroplasts within plants.[24] This raises the possibility that some plants resistant to PS II inhibitors may revert to the susceptible type with time through decreases in the proportion of plastids containing the mutation.

2.2.2 Resistance to Microtubule Assembly-Inhibiting Herbicides

The dinitroaniline chemical family, which contains several herbicides including ethalfluralin, pendimethalin, prodiamide, oryzalin, and trifluralin, inhibits the assembly of microtubules. The microtubules are formed when heterodimer subunits

of α- and β-tubulin polymerize. The dinitroaniline herbicides bind to tubulin, prevent polymerization, and so prevent cell division and cell elongation.[25]

Resistance to the dinitroanilines has been reported in seven species,[1] of which dinitroaniline-resistant *Eleusine indica* has been the most widely studied. *E. indica* populations with either high (R) or intermediate (I) dinitroaniline resistance have been identified. When dinitroaniline resistance was last reviewed,[26] the mechanism of resistance in *E. indica* was suggested to be a difference in tubulin structure based on differences in electrophoresis of tubulin subunits. Since then, the mechanism of resistance and the mutations endowing resistance have been identified in *E. indica*. Resistance has been established to result from single amino acid substitutions in α-tubulin.[27–29] When sequences of the cDNA of α-tubulin were compared, base changes were identified in the R and I types compared to α-tubulin cDNA from susceptible (S) populations. The R population contained two point mutations that resulted in amino acid changes from Thr 239 to Ile and Ala 340 to Thr in the TUA1 and TUA2 α-tubulin genes, respectively.[29] The authors speculated the change in hydrophobicity with these amino acid substitutions could alter the binding of herbicides to the protein. The I population had a different substitution, from Met 268 to Thr in TUA1, which also would decrease hydrophobicity.[29] The difference in amino acid substitutions between the R and I populations demonstrates that different mutations within α-tubulins differentially affect binding of dinitroaniline herbicides.

Four β-tubulin genes from the R and S populations were sequenced and no mutations that would result in amino acid replacements were found.[30] This provides further evidence that β-tubulin genes are not involved in dinitroaniline resistance in *E. indica*. Phylogenetic analysis of the β-tubulin genes from resistant and susceptible populations indicated that resistance in *E. indica* originated independently at multiple locations rather than spreading from one site.[30]

Definitive evidence that resistance to dinitroaniline herbicides is due to amino acid modifications in the α-tubulin gene was obtained when the gene containing the Thr 239 to Ile substitution was used to transform maize and tobacco. The transforming α-tubulin gene was shown to confer dinitroaniline resistance in maize cells and regenerated tobacco plants.[27,31] The substitution either changes the configuration to the herbicide-binding site or increases the stability of the interaction between α- and β-tubulin.[28]

With the dinitroaniline-resistant *E. indica*, reciprocal crosses between the R and S or I and S populations and subsequent analysis of the F_2 and F_3 generations established that dinitroaniline resistance is controlled by a single, recessive nuclear gene.[32] No plants with intermediate resistance were found when crosses between the R and S populations were made, which indicated that the I population is not a hybrid between the R and S populations. This was confirmed when the α-tubulin gene of the I population was sequenced showing an amino acid substitution different from the R population.[29] Analysis of crosses between the I and S populations showed that intermediate resistance also is controlled by a single, recessive nuclear gene.[33] Similarly, Jasieniuk et al.[34] reported that a single, recessive nuclear gene also controls trifluralin resistance in *Setaria viridis*. However, when Wang et al.[35] crossed the trifluralin-resistant *S. viridis* with *S. italica* (foxtail millet), trifluralin resistance in the F_2 populations derived from selfed F_1 plants was recessive, but apparently not

monogenic. This indicates that minor genes play a part in trifluralin resistance in the interspecific hybrid. The authors suggested several possibilities for the difference in reports of the inheritance of trifluralin resistance. Segregation may have been affected by interspecific hybridization or the resistance gene is tightly linked to a detrimental factor that is conserved over backcross generations. They further suggested that there might be two linked loci, one more important for resistance than the other, with the additive action of both loci required for resistance to high doses of trifluralin. This study points out that the inheritance of resistance may be more complex than is sometimes reported.

2.2.3 Resistance to Acetolactate Synthase-Inhibiting Herbicides

At least five different chemical families of herbicides inhibit acetolactate synthase (ALS), also called acetohydroxyacid synthase. These are the sulfonylurea, imidazolinone, triazolopyrimidine, pyridinyloxybenzoate, and sulfonylaminocarbonyltriazolinone chemistries.[36] ALS is the first common enzyme in the biosynthetic pathway for the production of the branched-chain amino acids. The exact binding site for herbicides on the enzyme has not been determined but has been suggested to be a residual quinone-binding site based on homology with pyruvate oxidase.[37]

The ALS-inhibiting herbicides were introduced into world agriculture in the 1980s and have been used extensively worldwide[38] with the result that resistance has evolved in a large number of weed species.[1] The number of weed species with populations resistant to ALS-inhibiting herbicides has increased rapidly over the past decade and now numbers 58, second only to triazine resistance.[1] In all but a few instances, resistance to the ALS-inhibiting herbicides is due to a less sensitive ALS. In contrast to triazine resistance, target-site-based resistance to the ALS-inhibiting herbicides can be conferred by a number of different point mutations. Amino acid substitutions that have been reported to provide resistance to ALS-inhibiting herbicides in weeds are listed in Table 2.1. Differences occur in target-site cross-resistance among the different chemical classes of herbicides that inhibit ALS. The differences are related to particular amino acid substitutions that occur within the binding region. Saari et al.[6] suggested that target-site cross-resistance between the sulfonylureas and the triazolopyrimidines is common and related to mutations at Pro 197, while target-site cross-resistance between the sulfonylureas and imidazolinones is less predictable.[50]

Multiple point mutations at Pro 173 (equivalent to Pro 197 in *Arabidopsis thaliana*) in ALS in *Kochia scoparia* have been reported.[42,44] Indeed, six different substitutions of Ala, Arg, Glu, Leu, Ser, or Thr for Pro 173 have been observed in different *K. scoparia* populations. In addition to having different amino acid substitutions, the populations were from different geographic regions, indicating that there were multiple independent selections of ALS-resistant populations.

Analysis of three *Sisymbrium orientale* populations resistant to ALS inhibitors revealed differences in amino acid substitutions in ALS among populations.[41] Two of the populations, from different geographic regions, had the same Trp to Leu change, while the third population had a Pro to Ile substitution, the latter resulting

TABLE 2.1
Amino Acid Substitutions Endowing Resistance to ALS Inhibitors in Resistant Weed Populations

Species	Population	Amino Acid Substitution[a]	Ref.
Amaranthus sp.	Iowa	Trp 591 · Leu	39
A. rudis	Illinois	Trp 591 · Leu	40
Brassica tournefortii	WBT1	Pro 197 · Ala	41
Kochia scoparia	KS-R	Pro 197 · Thr	42
	ND-R	Pro 197 · Arg	42
	MAN-R	Pro 197 · Leu	42
	MT-R	Pro 197 · Gln	42
	ID#5-R, TX-R	Pro 197 · Ala	42
	SLV-R	Pro 197 · Ser	42
	Illinois	Trp 591 · Leu	43
Lactuca serriola	Idaho	Pro 197 · Thr	44
	SLS1	Pro 197 · His	45
Salsola iberica	Washington	Pro 197 · Leu	Cited in 3
Sisymbrium orientale	SS01	Pro 197 · Ile	41
	NS01, SS03	Trp 591 · Leu	41
Xanthium sp.	MO-XANST	Trp 591 · Leu	46
	MI-XANST	Ala 122 · Thr	47
		Ala 205 · Val	48

[a] To aid comparison, the position numbers of amino acids for the various species have been altered to those of *Arabidopsis thaliana*.[49]

from two nucleotide substitutions.[41] Whether the two mutations occurred at the same time or whether they were due to separate events cannot be determined. However, differences in the point mutations among the populations provide strong evidence that there are independent selections for ALS resistance. No differences in the substrate binding of the enzyme were measured, but two of the resistant populations had a higher maximum rate of reaction, which could indicate over-expression of ALS in these populations.

The different patterns of target-site cross-resistance among ALS-inhibiting herbicides suggest that the sulfonylurea and imidazolinone herbicides bind to different areas of the herbicide-binding pocket on ALS (Figure 2.2). Trp 591 most likely interacts via hydrophobic interactions with aromatic ring systems of the herbicides. Substitution of Leu for Trp removes this hydrophobic interaction. This mutation results in cross-resistance to sulfonylurea and imidazolinone herbicides (Table 2.1). Substitutions at Ala 122 result in resistance to imidazolinone herbicides only.[47] Clearly, this amino acid must be positioned in proximity to where imidazolinone herbicides bind. Substitutions at Pro 197 commonly result in strong resistance to sulfonylurea herbicides with little or no resistance to imidazolinone herbicides.[6] However, some mutations result in resistance to imidazolinone herbicides as well.[41]

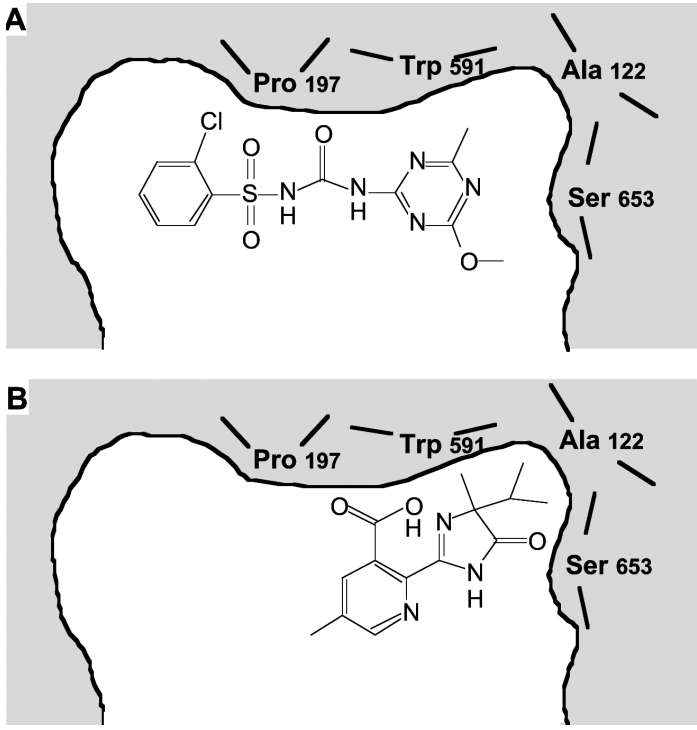

FIGURE 2.2 Proposed interaction of sulfonylurea (A) and imidazolinone (B) herbicides with amino acids of acetolactate synthase. This interpretation is based on cross-resistance patterns of ALS mutants.[3] The sulfonylurea herbicides interact with Trp 591, probably through hydrophobic interactions, and amino acids around Pro 197. The imidazolinone herbicides interact with Trp 591 and Ser 653; the latter amino acid may provide a hydrogen bond to the herbicide.

Substitutions at Pro 197 would change the tertiary structure of ALS and may displace an amino acid essential for binding of sulfonylurea herbicides. Depending on the amino acid substitution at Pro 197, interference with binding of imidazolinone herbicides might also occur.

Isolines of *Lactuca sativa* differing in resistance to ALS-inhibiting herbicides were produced by hybridizing *L. serriola* resistant to ALS inhibitors with herbicide-susceptible *L. sativa*, and then back-crossing to *L. sativa* for five generations.[51] The resistant isoline contained a Pro 197 to His substitution, previously identified in the resistant *L. serriola* parent, and a Ser 431 to Asn substitution. The amino acid substitutions in the resistant isoline had an adverse effect on the enzyme and the specific activity of ALS from the resistant isoline was reduced compared to the specific activity of the susceptible isoline. Feedback inhibition by valine, leucine, and isoleucine also was reduced in the resistant isoline, while the concentration of all three amino acids was higher in seed, and valine and leucine were higher in the leaves of the resistant isoline when compared to the susceptible isoline. Despite reduced specific activity of ALS, biomass production of the resistant isoline was

faster than biomass production for the susceptible isoline, and seed size also was larger. These studies revealed differences in the two isolines that have not been shown to occur at the whole plant level in fitness and competition studies with ALS-resistant and -susceptible weeds.[52]

Resistance to the ALS-inhibiting herbicides in all species investigated is controlled by a single, nuclear-encoded gene that is either dominant or has incomplete dominance (also referred to as semidominant).[41,53–55] Heterozygous plants often show symptoms when treated with ALS-inhibiting herbicides but do not die.[53] The response of F_1 hybrids, produced by crossing ALS-inhibiting herbicide-resistant and -susceptible *S. orientale* populations, to chlorsulfuron was intermediate to that of the parents, indicating that the trait is nuclear encoded and not cytoplasmic.[41] The F_1 hybrid was more sensitive to chlorsulfuron than the resistant parent, which suggests the gene has incomplete rather than complete dominance. F_2 generations of several different species show segregation ratios of either 3:1 or 1:2:1 for the resistance trait, which indicates it is controlled by a single gene.[41,53–55] This pattern of inheritance of resistance to ALS-inhibiting herbicides appears independent of the specific amino acid modifications that endow resistance. This suggests that each of the known amino acid substitutions within ALS will result in dominant-resistance inheritance.

2.2.4 Resistance to Acetyl-Coenzyme A Carboxylase-Inhibiting Herbicides

Acetyl-coenzyme A carboxylase (ACCase) is the target site of the aryloxyphenoxypropanoate (APP) and cyclohexanedione (CHD) herbicides.[56,57] ACCase is the first dedicated enzyme in the biosynthetic pathway for lipid synthesis.[8,9] Herbicides that inhibit ACCase are often termed graminicides as they only affect grass species with virtually no effect on a wide range of dicotyledonous species.[8,58] For this reason, ACCase-inhibiting herbicides are used for grass weed control in dicotyledenous crop species. Grasses contain two different ACCase enzymes with about 80% of the activity being associated with the plastid form of the enzyme. The plastidic enzyme of nongrasses is structurally different from that of grasses, being composed of different subunits as compared to a single protein of about 220 to 230 kDa in grasses.[59] All plant species have a separate cytoplasmic multidomain ACCase. Only the plastidic enzyme of grasses is sensitive to the APP and CHD herbicides.[60]

Resistance in grass weeds following widespread usage of ACCase-inhibiting herbicides has been widely reported,[1,8,58] with 19 different grass weeds with populations resistant to ACCase inhibitors.[1] With few exceptions, resistance to the ACCase-inhibiting herbicides has been attributed to an insensitive ACCase; however, the difficulty in extracting and purifying ACCase has raised questions about the conclusions reached in some studies.[9,61] The mutation(s) responsible for resistance to the ACCase-inhibiting herbicides have not been identified, unlike PS II and ALS resistances. Therefore, studies on the molecular genetics of target-site-based resistance to ACCase-inhibiting herbicides have not been conducted. However, it is clear from differences in target-site cross-resistance patterns of different resistant populations that multiple mutations within ACCase conferring resistance are possible.[3,62]

The plastidic ACCase from grass species is a large enzyme containing each of the four domains that in nongrasses are encoded on separate proteins.[59] These are the biotin carboxylase, biotin carboxy carrier protein (BCCP), and carboxyltransferase α and β (Figure 2.3). Therefore, the plastidic ACCase of grasses is similar in structure to the cytoplasmic ACCase of all species, including grasses. The only difference is that the grass plastidic ACCase is susceptible to herbicides whereas the cytoplasmic ACCase is tolerant. Elegant experiments by Nikolskaya et al.[63] constructed chimeric ACCase from wheat by combining parts of the herbicide-sensitive plastidic and herbicide-tolerant cytoplasmic proteins. These experiments localized herbicide susceptibility of the plastidic ACCase to a 411-amino acid region containing the carboxyltransferase β domain (Figure 2.3). Therefore, it seems likely that when amino acid modifications responsible for resistance to ACCase-inhibiting herbicides are discovered they will occur around the carboxyltransferase β domain.

A single dominant or partially dominant gene confers target-site resistance to ACCase-inhibiting herbicides in resistant populations of *Avena* spp.[66–68] and *Lolium* spp.[69,70] A single gene conferred resistance to both APP and CHD herbicides in an *A. fatua* population.[67] In a subsequent study, resistance in two different populations was controlled by different alleles of the same gene.[71]

2.2.5 Resistance to Glyphosate

Glyphosate, the world's most widely used herbicide, inhibits 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, an enzyme in the biosynthetic pathway for the production of aromatic amino acids.[38,72] Glyphosate is a nonselective herbicide effective in controlling many plants. Given the importance of glyphosate, it is understandable that considerable effort was expended attempting to create herbicide-resistant forms of EPSP synthase, including site-directed mutation of the active site of the enzyme.[73] However, these experiments demonstrated the difficulty in achieving a glyphosate-resistant, yet catalytically active, EPSP synthase. The conclusion drawn from these studies was that target-site-based resistance to glyphosate would be very unlikely to evolve in the field.[72]

Resistance to glyphosate appeared in the field in populations of *Lolium rigidum* in Australia in 1995 following more than 15 years of use of the herbicide.[74,75] Since

FIGURE 2.3 Linear structure of plastidic acetyl-coenzyme A carboxylase from wheat showing biotin carboxylase, biotin carboxyl carrier protein, and carboxyltransferase β and α domains. The solid line underneath indicates the position of the 411 amino acid region that endows herbicide sensitivity in the plastidic ACCase.[63] (From a comparison of the plastidic sequence[64] with the cytoplasmic sequence.[65])

then glyphosate resistance has occurred in populations of *Eleusine indica* in Malaysia.[76] Importantly, one glyphosate-resistant population of *E. indica* from Malaysia has been found to contain an EPSP synthase with reduced sensitivity to glyphosate.[77] The enzyme of the resistant plants is fivefold less sensitive to glyphosate while at the whole plant level there is threefold resistance.[77]

Two amino acid modifications were identified when the gene for EPSP synthase of the resistant population of *E. indica* was sequenced.[78] One of the amino acid substitutions, Ser substitution for Pro 106, occurred within the putative glyphosate-binding site.[73] This amino acid substitution in EPSP synthase is known to confer about tenfold resistance to glyphosate.[73]

2.2.6 Conclusions — Target-Site-Based Resistance

The early identification of target-site-based resistance to the PS II-inhibiting herbicides and the identification of the single amino acid modification involved had tended to influence thinking about herbicide resistance. To some extent, a dogma of only one amino acid substitution endowing resistance to herbicides prevailed. Therefore, assumptions were made that the same mutation had occurred. For example, until the identification of the Val 219 to Ile substitution in *Poa annua*, only changes at Ser 264 had been identified in field-selected PS II-resistant weed species.[13,17] It may be that few mutations will confer resistance to the PS II inhibitors or it may be that researchers were too quick to accept that resistance would be conferred by the same mutation in all species. The actual mutation in many species has not been elucidated; therefore, there may be other mutations present in weed species that have resistance to the PS II inhibitors. In contrast, many different point mutations have been identified in weed populations with resistance to the ALS-inhibiting herbicides. These point mutations are related to patterns of target-site cross-resistance. Likewise, the different target-site cross-resistance patterns that have been identified for ACCase resistance suggest that different point mutations in the ACCase gene will account for the different patterns of cross-resistance to these herbicides. Thus far, only two mutations have been shown to be responsible for dinitroaniline resistance providing different levels of resistance and a single mutation identified in EPSP synthase. Based on experience with PS II, researchers should not reject the idea that multiple mutations that confer resistance to herbicides may be possible in other target enzymes.

2.3 HERBICIDE DETOXIFICATION-BASED RESISTANCE MECHANISMS

The vast majority of herbicides can be detoxified to some extent by plants. The exceptions are herbicides such as paraquat and glyphosate, which are poorly metabolized by plants[72,79] and are thus used as nonselective herbicides. Indeed, the very concept of selective herbicides, lethal to weed species but not to the crop, usually depends on more rapid metabolism of the herbicide by the crop species.[80] For example, chlorsulfuron and other wheat-selective herbicides do not kill wheat plants because wheat rapidly detoxifies them. Often the targeted weed species have some ability to metabolize the herbicide, but this is insufficient to stop the weed from

being killed.[81] Generally, similar enzymatic systems are responsible for metabolism in crops and weeds, the difference being the rate and extent of metabolism are much greater in the crop.

Populations of 18 weed species have evolved resistance to herbicides through increased rates of herbicide detoxification (Table 2.2). Studies of herbicide resistance in weeds due to increased herbicide detoxification are extensive at the biochemical level; however, less information is available concerning the genetic control of resistance and none concerning the specific mutations endowing resistance. Three enzyme systems are thus far implicated in metabolism-based resistance to herbicides: the glutathione transferases, the aryl acylamidases, and the cytochrome P450 monooxygenases. The majority of examples of resistance due to increased herbicide metabolism are catalyzed by cytochrome P450 monooxygenases. These are also the most complex examples of resistance, frequently resulting in nontarget-site cross-resistance to unrelated herbicides.

TABLE 2.2
Weed Species with Populations Resistant to Herbicides as a Result of Enhanced Herbicide Detoxification

Species	Proposed Enzymatic System	Herbicides	Ref.
Alopecurus myosuroides	Cytochrome P450	Chlorotoluron Diclofop-methyl Propaquizafop Chlorsulfuron	82–89
	GST	Fenoxaprop-*p*-ethyl	90, 91
Abutilon theophrasti	GST	Atrazine	92, 93
Avena fatua	Cytochrome P450? (loss of activation)	Triallate	94
A. sterilis	Cytochrome P450	Diclofop-methyl	95
Digitaria sanguinalis	Unknown, possibly cytochrome P450	Fluazifop-*p*-butyl	96
Echinochloa colona	Aryl acylamidase	Propanil	97
E. crus-galli	Aryl acylamidase	Propanil	98
Hordeum leporinum	Unknown	Fluazifop-*p*-butyl	99
Lolium rigidum	Cytochrome P450	Diclofop-methyl Chlorsulfuron Chlorotoluron Metribuzin Simazine Chlorotoluron	100–110
	Unknown, possibly cytochrome P450	Tralkoxydim Fluazifop-*p*-butyl	108, 111
Phalaris minor	Cytochrome P450	Isoproturon	113
Sinapis arvensis	Cytochrome P450	Ethametsulfuron-methyl	114
Stellaria media	Cytochrome P450	Mecoprop	115

2.3.1 Glutathione Transferases

Glutathione transferases (historically abbreviated as GSTs) belong to a family of enzymes that attach the tripeptide glutathione through the cysteine residue to electrophilic, hydrophobic compounds.[116] Although the endogenous roles of GSTs are yet to be fully elucidated, they are involved in stress responses, often being induced following the onset of stress or pathogen attack.

Enzymes from this family have been well characterized because of their role in tolerance of maize and sorghum crops to triazine and chloroacetanilide herbicides.[117] GSTs function as dimers of subunits ranging from about 25 to 29 kDa. Different combinations of subunits, either as homodimers or heterodimers, may have different specificities.[116,118–120] The reaction mechanism involves a nucleophilic displacement of an electrophilic group, with glutathione binding to the substrate through the cysteine sulfur (Figure 2.4). With some herbicides a nucleophilic displacement ether cleavage occurs. Once glutathione becomes bound to the substrate, the glutathione conjugate is normally exported to the vacuole for further processing.[122]

Several populations of *Abutilon theophrasti* from the United States are resistant to the PS II-inhibiting triazine herbicides as a result of increased herbicide metabolism catalyzed by GSTs.[92,93] Further investigations of one resistant population have established that activity of two GST isoenzymes with atrazine as the substrate are highly elevated in the resistant individuals. These isoenzymes have specific activity for triazine herbicides and consequently the resistant populations are only resistant to triazine herbicides.[92] More recent studies with purified GST from the resistant

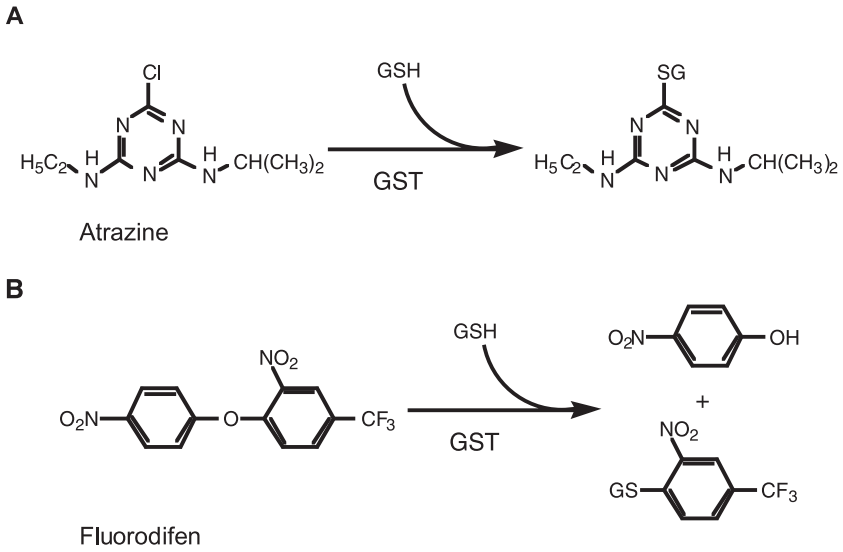

FIGURE 2.4 Glutathione transferase-mediated detoxification of herbicides by direct conjugation, atrazine (A), and by ether cleavage followed by conjugation, fluorodifen (B). (Reaction schemes after Reference 121.)

A. theophrasti indicate resistance is the result of increased catalytic activity rather than over-expression of GST.[123]

Cummins et al.[90] suggested that GSTs play a role in resistance to fenoxaprop-*p*-ethyl and other herbicides in some resistant populations of *Alopecurus myosuroides*. Indeed, these resistant populations have higher GST content and higher expression of certain isoenzymes; however, fenoxaprop-*p*-ethyl metabolism by GST *in vitro* is low.[90,91] One suggestion is that glutathione peroxidase activity associated with the GST is responsible for protection of the resistant plant from the effects of the herbicide.[124] However, this hypothesis is unable to account for the more rapid metabolism of fenoxaprop and diclofop by resistant *A. myosuroides.*[86] Clearly, further investigation is required to determine the role of glutathione peroxidase activity in herbicide resistance in *A. myosuroides*.

Genetic studies conducted with one atrazine-resistant population of *Abutilon theophrasti* indicate that GST-endowed resistance is inherited as a single nuclear-encoded gene with partial dominance.[125] Further studies demonstrated the level of atrazine metabolism in all F_1 progeny, regardless of maternal parent, was intermediate between that of the parental populations.[92]

2.3.2 Aryl Acylamidases

Aryl acylamidase is an enzyme that catalyzes the hydrolysis of certain acylamides.[126] The endogenous role of aryl acylamidase is not known, but it may be involved in nitrogen metabolism in plants.[127] This enzyme is also responsible for the metabolism of propanil in rice, thereby providing selectivity to this herbicide.[128]

Several populations of *Echinochloa colona* and *E. crus-galli* have evolved resistance to propanil in rice culture at various locations around the world (see Chapter 6 and [129,130]). In both *E. colona* and *E. crus-galli*, propanil resistance is the result of increased rates of propanil detoxification.[97,98] In one population of *E. colona*, elevated aryl acylamidase activity against propanil was demonstrated.[97] In crude enzyme extracts, the resistant population had a threefold enhancement of propanil metabolism by aryl acylamidase. The K_m for propanil of the resistant enzyme is unchanged, indicating resistance is probably the result of a threefold over-expression of the enzyme rather than a more efficient enzyme.[97] The resistant population also had increased aryl acylamidase activity against a range of other chemically related compounds.

Aryl acylamidase activity in propanil-resistant *E. colona* can be inhibited by a number of carbamate and organophosphate pesticides.[97] As described in Chapter 6, this has led to management of propanil-resistant *E. colona* populations in the field by a mixture of propanil and piperophos herbicides.[130,131]

2.3.3 Cytochrome P450 Monooxygenases

The cytochrome P450 monooxygenases are a large family of enzymes responsible for the addition of a single oxygen atom to hydrophobic substrates.[132] In plants, these enzymes are involved in a myriad of biosynthetic pathways including the synthesis of heme, allelochemicals, phytoalexins, and suberins.[133,134] These are membrane-bound heme-containing proteins and require the addition of two electrons from NADPH

reductase, but occasionally from other electron donors.[134] The reaction sequence catalyzed by cytochrome P450 monooxygenases involves the concerted splitting of molecular oxygen and addition of a single oxygen atom to the substrate (Figure 2.5). This reaction occurs in four steps. First, the substrate is bound to the enzyme, displacing water as a ligand to the heme iron. Next, an electron is added from NADPH, then O_2 is bound. Addition of another electron causes splitting of the oxygen molecule with a hydroxyl group inserted into the substrate and the other oxygen atom removed as water. The hydroxylated substrate then leaves the enzyme. During this process, the oxidation state of the heme iron cycles between Fe^{2+} and Fe^{3+}.[136]

Cytochrome P450 monooxygenases are important for selectivity of a large number of herbicides within certain crops that are able to rapidly metabolize herbicides to inactive compounds.[80] The reactions catalyzed by cytochrome P450 monooxygenases are diverse; however, herbicides are typically hydroxylated or dealkylated by this enzyme system. Hydroxylation is generally followed by conjugation to sugars, often glucose.[80] These conjugates are subsequently exported to the vacuole and/or incorporated in the cell wall.[137]

Cytochrome P450 monooxygenases are the major enzymatic system implicated in detoxification-based herbicide resistance in grasses. Table 2.2 details examples where cytochrome P450-dependent monooxygenases are deduced to endow herbicide resistance. Cytochrome P450-dependent herbicide metabolism is generally difficult to study because of problems in purifying active enzyme and the low rate of metabolism of herbicides *in vitro*.[138] Therefore, these studies have typically used inhibitors of cytochrome P450 monooxygenases, such as 1-amino benzotriazole (ABT) or piperonyl butoxide (PBO), or identification of signature hydroxylated or dealkylated products to demonstrate the involvement of cytochrome P450 monooxygenases. To date, microsomes isolated from these resistant weed populations have not convincingly demonstrated herbicide metabolism *in vitro*.

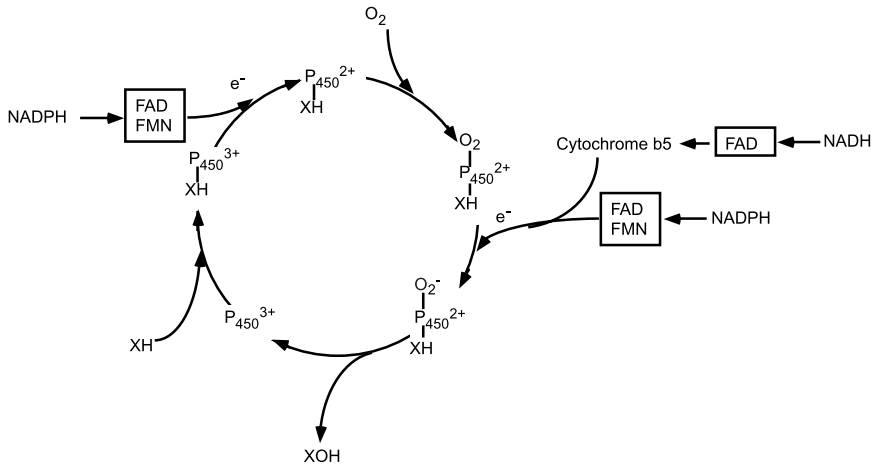

FIGURE 2.5 Reaction mechanism for cytochrome P450 monooxygenases. (From Davies, J. and Caseley, M., Herbicide safenes: a review, *Pestic. Sci.*, 55, 1043, 1999. © Society of Chemical Industry. With permission.)

TABLE 2.3
Cytochrome P450 Monooxygenase Inhibitors Differentially Inhibit Herbicide Metabolism in Multiple-Resistant *Lolium rigidum*

	Herbicide Remaining Unmetabolized (%)				
Inhibitor	**Diclofop-methyl + diclofop acid**	**Tralkoxydim**	**Simazine**	**Chlorotoluron**	**Chlorsulfuron**
None	38	23	35	45	78
1-Amino-benzotriazole	52*	22^{ns}	61*	64*	84^{ns}
Malathion	38^{ns}	26^{ns}	48^{ns}	54^{ns}	91*
Piperonyl butoxide	44^{ns}	17^{ns}	63*	63*	78^{ns}
Tetcyclasis	45^{ns}	18^{ns}	89**	82**	83^{ns}

Note: Values are herbicide remaining 24 h (diclofop-methyl, simazine, and chlorotoluron) or 6 h (tralkoxydim and chlorsulfuron) after treatment.

ns = not significantly different to metabolism in the absence of inhibitor.
* = significant decrease in herbicide metabolism ($P < 0.05$).
** = very significant decrease in herbicide metabolism ($P < 0.01$).

Source: Collated from Reference 108.

Over 180 genes or gene sequences belonging to the cytochrome P450 family have been identified in *Arabidopsis thaliana*.[139] This huge diversity of genes raises the possibility that individual herbicides are likely to be metabolized by specific enzymes. This has been established for one population of *Lolium rigidum* from Australia that is resistant to a large number of herbicides.[108] In this population, metabolism of five herbicides from different chemistries was, based on inhibition profiles, due to at least four different cytochrome P450 enzymes (Table 2.3).

This diversity of genes encoding cytochrome P450 monooxygenases also means that in different weed species, different enzymes may be recruited to metabolize a single herbicide. For example, isoproturon is metabolized by ring-hydroxylation in resistant *Phalaris minor* from India;[112,113] however, this population is not resistant to chlorotoluron,[140] a very similar chemical (Figure 2.6). In contrast, a population of *L. rigidum* from Australia resistant to chlorotoluron, mainly due to enhanced ring-hydroxylation,[100,109] also is resistant to isoproturon,[109] as is a population of *A. myosuroides* from the U.K.[82] Clearly, cytochrome P450 monooxygenases of different substrate specificity have been recruited to metabolize substituted urea herbicides in these weed species. One consequence of cytochrome P450 monooxygenase diversity in plants is that there is probably an enzyme available in all plants capable of metabolizing any herbicide that cytochrome P450 monooxygenases can attack.

Some *A. fatua* populations from Canada resistant to triallate demonstrate a different example of metabolism-based herbicide resistance. Rather than enhanced

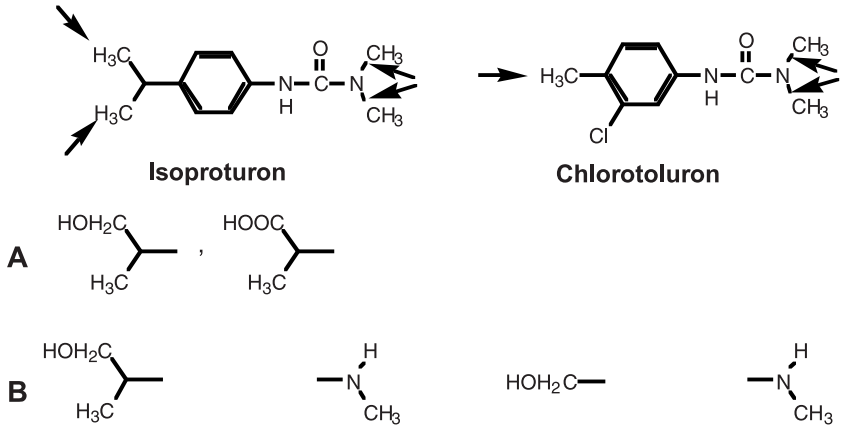

FIGURE 2.6 Cytochrome P450 monooxygenase-dependent metabolism of isoproturon and chlorotoluron. Arrows indicate points of attack. Cytochrome P450 monooxygenase reactions that are elevated in resistant populations compared to susceptible populations of *Alopecurus myosuroides* (A) or *Phalaris minor* (B) are indicated. (Collated from References 82 and 113.)

rates of metabolism, these populations show reduced metabolism of triallate compared to susceptible types.[94] Triallate requires activation to the sulfone for activity and this activation step is probably catalyzed by cytochrome P450 monooxygenases.[141] In the case of triallate-resistant *A. fatua*, reduced activation of triallate is suggested to be the resistance mechanism.[94]

The genetic inheritance of enhanced metabolism resistance mechanisms is poorly understood. Studies by Chauvez (cited in [142]) on *A. myosuroides* resistant to chlorotoluron suggested that resistance alleles at two or more genes contributed to resistance. Studies with *L. rigidum* from Australia resistant to a wide range of herbicides show that resistance to both simazine and chlorotoluron is nuclearly inherited with partial dominance (Figure 2.7). The F_1 plants show intermediate resistance compared to the susceptible and resistant parents. Further studies with one population are consistent with a major gene involvement in resistance for each herbicide, but do not rule out some contribution from minor genes.[143]

2.4 MECHANISMS OF HERBICIDE RESISTANCE OTHER THAN TARGET-SITE MODIFICATIONS OR HERBICIDE METABOLISM

As stated in the introduction to this chapter, herbicide resistance may result from one or more of a variety of biochemical mechanisms. Resistance to several groups of herbicides has been identified with resistance mechanisms that do not involve reduced target-site sensitivity or increased herbicide detoxification. In many instances, the actual mechanism is still unknown largely due to the difficulties in identifying alternative mechanisms of herbicide resistance.

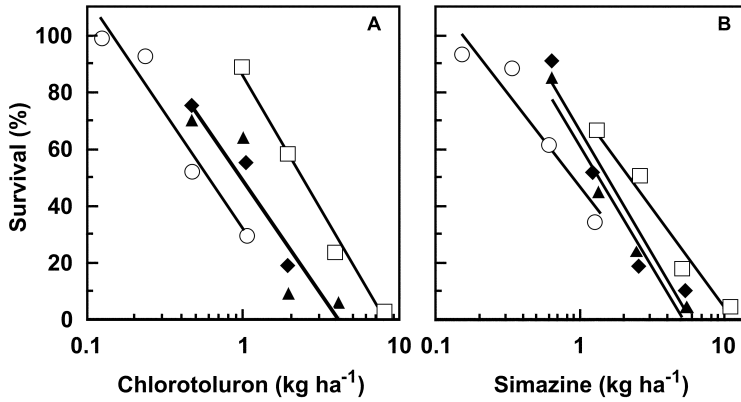

FIGURE 2.7 Dose response to chlorotoluron (A) and simazine (B) of susceptible VLR 1 (○), resistant VLR 69 (□), and reciprocal crosses (▲, ◆) between susceptible and resistant populations of *Lolium rigidum*.[143]

2.4.1 Resistance to Bipyridyl Herbicides

The bipyridyl herbicides, paraquat and diquat, are nonselective and active on a wide range of annual plants. These herbicides interact with the photosynthetic electron transport chain accepting electrons from the reducing side of PS I and are thereby reduced. The resulting anion radical reacts with oxygen and forms the highly reactive superoxide anion and hydroxyl radical. These radicals in turn cause lipid peroxidation and the destruction of cell membranes.[144] The bipyridyl herbicides act rapidly in strong light, producing symptoms within hours of application.

Paraquat resistance has evolved in populations of 26 weed species,[1] mainly in perennial cropping systems, including tree crops, vine crops, and alfalfa,[144] but also in broad area annual cropping.[145] The mechanism of resistance to paraquat has been studied extensively in a few resistant populations. Despite this effort, no conclusive mechanisms have been determined for resistance to the bipyridyl herbicides.

Decreased translocation and decreased penetration to the active site were proposed as the mechanism of resistance to the bipyridyl herbicides in *Hordeum glaucum* and *H. leporinum*.[146,147] In other studies with the same population of *H. glaucum*, increased sequestration in the vacuole of the resistant plants was suggested as the mechanism of resistance.[148] This hypothesis is not in conflict with the conclusion that resistance is due to decreased penetration to the active site. Paraquat-resistant populations of *H. glaucum* and *H. leporinum* displayed unusual temperature sensitivity, being highly resistant in winter but weakly resistant in summer (Figure 2.8). It was established that at 30°C the resistance mechanism breaks down such that the resistant population is not able to survive high rates of paraquat.[149] Under high temperatures, there was increased translocation of paraquat and more of the herbicide reached the active site in the resistant populations of the *Hordeum* spp.[149] This

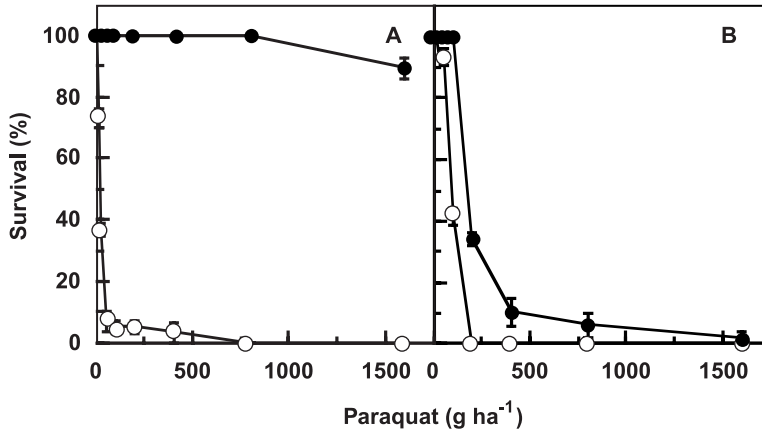

FIGURE 2.8 Dose response of susceptible (○) and resistant (●) populations of *Hordeum glaucum* to paraquat under cool (A) or warm (B) conditions showing the dramatic reduction in resistance under warm conditions. (From Purba, E., Preston, C., and Powles, S. B., The mechanism of resistance to paraquat is strongly temperature dependent in resistant *Hordeum leporinum* Link and *H. glaucum* Steud., *Planta*, 196, 1995. © Springer-Verlag. With permission.)

response supports the argument that resistance is related to decreased amounts of the herbicide reaching the active site.

In *Arctotheca calendula*, resistance did not appear to be related to decreased translocation of diquat but reduced herbicide penetration to the active site was proposed as the mechanism.[150] Further experiments with *A. calendula* have demonstrated that long-distance translocation of paraquat is dependent on the amount of leaf damage occurring. The resistant population has less leaf damage following paraquat application; therefore, paraquat translocation does not occur as rapidly.[151] These experiments support the hypothesis that paraquat is kept from the active site in the resistant population.

Paraquat sequestration has also been proposed as a resistance mechanism in *Conyza bonariensis*.[152] In these studies, there was much reduced lateral movement of paraquat in the resistant population compared to the susceptible population. Results of previous studies showed that paraquat was not metabolized in either the resistant or susceptible population.[153] Paraquat-resistant *Erigeron philadelphicus* and *E. canadensis* also showed restricted movement of the paraquat in leaves compared to susceptible individuals of these species.[154]

In *H. glaucum*, *H. leporinum*, and *A. calendula*, a single, incompletely dominant gene confers paraquat resistance.[155,156] However, the degree of dominance varies. Dominance is low for the grass species, heterozygotes are 2- to 8-fold resistant compared to 250-fold resistant for homozygotes, but higher for *A. calendula*.[156] In contrast to the *Hordeum* spp., paraquat resistance in *E. canadensis* is inherited as a single gene with a high level of dominance.[157] These differences in dominance of the resistant allele suggest that the biochemical basis of paraquat resistance might be different in *A. calendula* and *E. canadensis* compared to *H. glaucum* and

H. leporinum. With the exception of *E. canadensis*,[157] the populations segregated in a 1:2:1 ratio in the F_2 generation, with the intermediate plants presumed to be heterozygous. In *H. glaucum*, the F_3 generation progeny from the intermediate F_2 plants segregated again in a 1:2:1 ratio, while plants produced from the resistant plants were all resistant.[155] In *A. calendula*, back-crosses from the F_1 generation segregated in a 1:1 ratio, confirming the single gene hypothesis.[156] In *E. canadensis*, the F_2 generation segregated in a 1:3 ratio, indicating a single dominant gene was responsible for resistance.[157]

An alternative mechanism proposed to explain paraquat resistance is the elevation of oxygen radical detoxifying enzymes in the chloroplasts of resistant plants. Elevated activities of such enzymes have been measured in resistant populations of *Conyza bonariensis*[158,159] and *E. philadelphicus*,[160] and inferred for *C. canadensis*.[161] In *C. bonariensis*, elevated activities of a whole host of enzymes including superoxide dismutase, ascorbate peroxidase, glutathione reductase, and glutathione peroxidase were recorded.[158,159,162–164] In addition, the resistant population has elevated polyamine contents and increased activity of ornithine decarboxylase, an enzyme important to polyamine synthesis.[165] Activity of oxygen radical detoxifying enzymes was induced dramatically by paraquat application to resistant plants and to a lesser extent in susceptible plants.[166] Paraquat resistance in *C. bonariensis* is proposed due to a single, partially dominant gene co-segregating with increased activity of detoxification enzymes.[167] Other studies on resistant populations of *C. bonariensis* and *C. canadensis* have disputed a major role for oxygen radical detoxifying enzymes in resistance.[153,168,169] This dispute has been considered in detail elsewhere,[144,170] with the conclusion that without some way of removing or immobilizing paraquat the oxygen radical detoxification mechanism could only provide a modest degree of resistance.

2.4.2 Resistance to Glyphosate

As described in Section 2.2.5, glyphosate resistance has evolved in several populations of both *Lolium rigidum* and *Eleusine indica*. While the mechanism of glyphosate resistance in one population of *E. indica* has been established as a target-site modification (see Section 2.2.5), glyphosate resistance in *L. rigidum* is not due to reduced sensitivity of EPSP synthase to glyphosate.[171,172] The resistance mechanism remains to be determined. Studies with two different glyphosate-resistant *L. rigidum* populations have found no differences in glyphosate absorption, translocation, or metabolism compared to susceptible populations.[171,173] One resistant *L. rigidum* population (Echuca) had a higher level of EPSP synthase activity than a susceptible population;[172] however, the other glyphosate-resistant population (Orange) did not have increased EPSP activity.[171] Reduced movement of glyphosate to its site of action in the plastid was proposed as a possible mechanism of resistance in the Orange population.[171]

A single nuclear gene with incomplete dominance controls resistance in the Orange population.[174] In contrast, Pratley et al.[75] reported increased levels of resistance in subsequent generations of the Echuca population that had been selected

with glyphosate and suggested the involvement of more than one gene. However, the number of genes involved in the resistance has not been determined.[75]

Several populations of *Convolvulus arvensis* with differences in sensitivity to glyphosate were identified in 1984.[175] The authors did not refer to these populations as resistant; however, the differences in sensitivity to glyphosate are of the same magnitude reported for resistant *L. rigidum* and *E. indica* populations and for the rest of this discussion will be referred to as resistant. In this case, resistance has not evolved in the populations in response to herbicide selection pressure, but elucidation of the biochemical mechanisms of resistance may prove illuminating. Increased activity of the shikimate pathway has been suggested as the mechanism that provides glyphosate resistance in one *C. arvensis* population.[176] The resistant population contains a higher level of 3-deoxy-D-*arabino*-heptulosonate-7-phosphate synthase (DAHP synthase), the first enzyme in the shikimate pathway, than the susceptible population. The authors suggest there is higher activity of the shikimate pathway and that greater carbon flow through the pathway along with a greater phenylalanine pool in the resistant population allows this population to be less affected by glyphosate. The authors also hypothesize that multiple mechanisms were responsible for the resistance. Five populations with differing sensitivities to glyphosate were used as parents in a diallel cross experiment. Genetic analysis indicated that multiple genes were involved in the resistance and that maternal influences were important.[177]

2.4.3 Resistance to Auxin Mimics

The auxin mimic herbicides, which include the phenoxy acetic acid, benzoic, and picolinic herbicide families, have been widely used in agriculture since the 1940s.[178] They are termed auxin mimics because they mimic the action of the natural plant hormone indol acetic acid (IAA) to excess. Despite the extensive use of these herbicides, resistance to the auxin mimic herbicides has been identified in 19 weed species only.[1] Even though these herbicides have been used since 1945, the exact details on how the herbicides act are still unknown. Dicotyledonous plants treated with auxin mimic herbicides display multiple symptoms including epinasty, abnormal cell elongation, and adventitious root initiation. Resistance to auxin mimic herbicides was extensively reviewed by Coupland,[178] who identified that in many cases the basis of the resistance had not been fully elucidated. With one exception,[115] differences in absorption, translocation, or metabolism of herbicides were not responsible for resistance.[178] Since that time, resistance in two species, *Centaurea solstitialis* and *Sinapsis arvensis* (sometimes identified as *Brassica kaber*), has been extensively researched.

The resistant population of *C. solstitialis* had similar amounts of foliar absorption, translocation, metabolism, and cellular absorption of picloram compared to a susceptible population.[179] The picloram-resistant *C. solstitialis* population is cross-resistant to clopyralid. Foliar absorption, translocation, metabolism, and cellular absorption of clopyralid are also similar between resistant and susceptible populations.[180] In addition, there were no differences in ethylene production between resistant and susceptible plants.[180,181] Although studies were conducted to examine differences in

auxin/picloram binding sites between the populations, auxin/picloram binding could not be consistently detected and so it is still unknown whether such differences in picloram recognition might be involved with the resistance mechanism. Following picloram application, several genes are expressed differentially in resistant plants compared to susceptible plants, but these have not yet been characterized.[182]

Resistance in *S. arvensis*/*B. kaber* was not found to be due to differences in herbicide absorption, translocation, or metabolism.[183] After application of picloram, the susceptible plants did produce more ethylene.[184] This difference was attributed to differences in pathway regulation. Differences in ATP-dependent activity were measured and the authors attribute these differences to modulation of calcium ion channels. When auxin-binding proteins were compared, it was found that in the susceptible population the auxin-binding protein was more sensitive to picloram and dicamba than that in the resistant population.[185] This was confirmed in other experiments, which demonstrated differential effects of picloram on auxin-induced activities in resistant plants.[186] In addition, cytokinin levels were higher in the resistant population than in the susceptible population, which may mean that there are differences in hormonal regulation between the populations.[187]

There have been limited studies of inheritance of resistance to auxin mimic herbicides. However, it is known that a single, recessive nuclear gene controls picloram resistance in *C. solstitialis*,[188] whereas a single, dominant nuclear gene[189,190] controls dicamba resistance in *S. arvensis*/*B. kaber*. These differences in inheritance indicate there may be more than one mechanism responsible for auxin mimic herbicide resistance.

2.5 MULTIPLE-RESISTANCE MECHANISMS

Multiple-resistance is defined as resistance due to more than one resistance mechanism.[191] In the worst cases, this may result in weed populations with simultaneous resistance to many different herbicides. These populations can be particularly difficult to control as there may be few herbicide options remaining. Understanding the biochemistry and genetics of multiple-resistance is considerably more challenging as a result of having to separate the resistance mechanisms. Therefore, while a number of cases of multiple-resistance have been suspected, few have followed up with detailed biochemical studies to determine the mechanisms of resistance (Table 2.4), and even fewer genetic studies have occurred. The examples in Table 2.4 show that multiple-resistance can occur via combinations of target-site-based mechanisms, target-site- and metabolism-based mechanisms, and other combinations of mechanisms.

2.5.1 Evolution and Biochemistry of Multiple-Resistance

Multiple-resistance can arise within a weed population in several ways: through a change in selection history; through selection of multiple mechanisms by a single herbicide; or through crossing of individuals containing different resistance mechanisms. Examples of all three processes are known.

TABLE 2.4
Populations of Weed Species Showing Examples of Multiple Herbicide Resistance

Species and Population	Herbicides	Mechanisms	Ref.
Alopecurus myosuroides	ACCase inhibitors	Target site	86, 89, 192
Lincs E1	PS II inhibitors	Metabolism	
Amaranthus rudis Illinois	ALS inhibitors	Target site	40
	PS II inhibitors	Target site	
Avena fatua	Triallate	Reduced activation	94, 193
	Difenzoquat	Increased binding?	
A. sterilis NAS 4	ACCase inhibitors	Target site	95
		Metabolism	
Brachypodium distachyon	PS II inhibitors	Target site	194
		Metabolism	
Conyza canadensis	PS II inhibitors	Target site	195
	Paraquat	Unknown	
Echinochloa crus-galli	Atrazine	Target site	196
	Quinclorac	Unknown	
Galium spurium	ALS inhibitors	Target site	197
	Quinclorac	Unknown	
Hordeum leporinum	ACCase inhibitors	Target site	99
		Metabolism	
Kochia scoparia	ALS inhibitors	Target site	43
	PS II inhibitors	Target site	
Lolium rigidum SLR 31	ACCase inhibitors	Target site	102, 104, 105, 198
	ALS inhibitors	Metabolism	
	Tubulin elongation inhibitors	Others?	
L. rigidum VLR 69	ACCase inhibitors	Target site	100, 101, 108, 199
	ALS inhibitors	Metabolism	
	PS II inhibitors		
L. rigidum WLR 1	ALS inhibitors	Target site	200
		Metabolism	
L. rigidum WLR 2	PS II inhibitors	Metabolism	100, 101, 106
	Amitrole	Unknown	
Setaria faberi	PS II inhibitors	Target site	201
		Metabolism	
S. viridis	PS II inhibitors	Target site	201
		Metabolism	

2.5.1.1 Multiple-Resistance by Sequential Herbicide Selection

There are several examples of multiple-resistance evolving sequentially as a result of a change in selection history (Table 2.4). The example of triazine- and sulfonylurea-resistant *Kochia scoparia* will be used to illustrate this process.[43] Triazine herbicides were used extensively to control weeds, including the widespread weed

K. scoparia across the northern United States and Canada. Triazine resistance subsequently evolved in *K. scoparia* populations.[202] Following the introduction of the ALS-inhibiting sulfonylurea herbicides, these were used extensively to control *K. scoparia* and other weeds. Inevitably, ALS-inhibiting herbicides were used against populations that had previously evolved resistance to the triazine herbicides. At least one population of triazine-resistant *K. scoparia* subsequently evolved resistance to ALS-inhibiting herbicides.[43] As expected, these multiple-resistant plants contain two distinct resistance mechanisms. The resistant population has a D1 protein insensitive to triazine herbicides due to a substitution of Gly for Ser at position 264. In addition, a substitution of Leu for Trp at position 570 in ALS resulted in a modified ALS enzyme insensitive to sulfonylurea and imidazolinone herbicides.[43] A similar case has been reported for triazine and ALS-inhibiting herbicide-resistant *Amaranthus rudis*.[40] Given the past and continuing widespread use of the PS II- and ALS-inhibiting herbicides in world agriculture, it is likely that numerous more examples of multiple-resistance to these two modes of action will occur.

Selection for multiple-resistance does not have to be sequential. In principle, similar results could occur from rotating herbicide modes of action. While there is anecdotal evidence for resistance occurring in this fashion,[38,203] there are few documented examples.

2.5.1.2 Multiple-Resistance by Selection with a Single Herbicide

Selection of multiple mechanisms of resistance by a single herbicide also can occur. This type of multiple-resistance is less commonly reported, probably because many researchers examine for a single major resistance mechanism. Two such examples are a *Brachypodium distachion* population resistant to triazine herbicides[194] and a *Lolium rigidum* population resistant to sulfonylurea herbicides.[200] In both cases there is an increased rate of herbicide metabolism; however, a strongly insensitive target enzyme dominates resistance. A more clear-cut example of multiple-resistance to a single herbicide is diclofop-methyl resistance in an *Avena sterilis* population (NAS 4). This population is strongly resistant to diclofop-methyl; however, it has an ACCase enzyme with only tenfold resistance to diclofop acid.[95] This population also demonstrated more rapid metabolism of diclofop acid. In this population at least two genes must encode herbicide resistance, one endowing resistance due to a modified ACCase and at least one endowing resistance due to increased detoxification of diclofop acid. As *A. sterilis* is a self-pollinated species, these genes most likely have been selected together.

Cross-pollinating weed species can also accumulate resistance mechanisms through gene flow. This may occur through selection with a single herbicide where two individuals, each containing a different resistance mechanism, cross. Some of the resulting progeny will inherit both resistance mechanisms.[62,108,203] However, where there is also a complex herbicide selection history or gene flow from other fields with different herbicide use patterns, particularly complicated patterns of multiple resistance can occur. This happens with *L. rigidum* populations in Australia.[62,203] Such

populations may evolve resistance to a wide range of herbicides and be particularly difficult to control. *L. rigidum* population VLR 69 is a particularly good example of multiple-resistance.[204] This population contains an ACCase resistant to aryloxyphenoxypropanoate herbicides and enhanced metabolism of triazine, substituted urea, triazinone, aryloxyphenoxypropanoate, cyclohexanedione, and sulfonylurea herbicides.[108,203] In addition, about 5% of the population also contains a herbicide-resistant ALS.[199] As described above, at least four different cytochrome P450 monooxygenases contribute to herbicide resistance in this population.[108]

2.5.2 Genetics of Multiple-Resistance

There is a direct relationship between genes and resistance mechanisms such that a single gene will contribute to a single mechanism. Therefore, all cases of multiple-resistance, by definition, must be encoded by more than one gene. However, it is also theoretically possible for more than one gene to contribute to the same resistance mechanism. Therefore, sorting out the genetics of multiple-resistance can be a formidable task.

The example of a *K. scoparia* population resistant to both triazine and ALS inhibitors described above[43] is relatively simple, as only two genes, one encoding a modification to PS II and the second a modification to ALS, contribute to resistance. However, where enhanced metabolism of herbicides is one of the mechanisms of resistance, the genetics can be considerably more complicated. This is because a single gene may control increased expression of several genes through coordinate expression. Alternatively, some herbicides, such as atrazine, can be metabolized by more than one enzyme system.[80] In this way, more than one gene could contribute to metabolism-based resistance.

An illustration of the complexity of genetics of multiple-resistance comes from studies with *L. rigidum* population VLR 69. This population would be expected to have different genes endowing target-site-based resistance at ACCase and ALS, as well as an additional gene(s) endowing metabolism-based resistance.[203] Studies were conducted to determine the linkage between the metabolism-based resistances in *L. rigidum* population VLR 69. To do this, segregating F_2 families were selected with high rates of several herbicides. The survivors were crossed within treatments, and the progeny analyzed for cross-resistance. This analysis demonstrated distinct linkages of resistance (Figure 2.9). For example, selection of the F_2 with chlorotoluron resulted in more progeny with resistance to simazine and chlorotoluron than with resistance to tralkoxydim or chlorsulfuron. Similar results were obtained with selection of the F_2 with simazine.[143] This demonstrates that resistance mechanisms for simazine and chlorotoluron are closely linked and probably controlled by a single gene. In all, at least five different genes appear to contribute to multiple-resistance in *L. rigidum* population VLR 69.[143]

2.6 CONCLUSIONS AND PROSPECTS

The intensive use of herbicides to control weeds inevitably has resulted in the evolution of herbicide-resistant weed populations. On surveying the large body of

FIGURE 2.9 Cross-resistance and lack of cross-resistance to (left to right) chlorsulfuron, chlorotoluron, tralkoxydim, or simazine in progeny of simazine-selected F_2 families created from a cross between *Lolium rigidum* populations VLR 69 and VLR 1.[143]

work on resistance mechanisms, it is clear that the majority of studied examples relate to changes in the target enzyme. This may reflect biological reality but a note of caution, in that it may be biased by the relative ease with which target-site changes can be detected. Despite this, it is clear that single amino acid changes to target enzymes can be easily selected by herbicides in the field. However, it must be remembered that target-site changes are not the only mechanism that can endow resistance to herbicides. Any biochemical change that allows a plant to survive application of herbicide can be selected. Other resistance mechanisms detected include increased herbicide detoxification and reduced penetration of herbicide to its active site. It is also possible for a single herbicide to select different resistance mechanisms in different populations, or indeed the same population, of a species.

No herbicide should be considered resistance proof. The ability of weeds to evolve resistance to paraquat, where functional target-site modifications are very unlikely and herbicide metabolism is nonexistent,[144] demonstrates that resistance is possible through other mechanisms. However, there are herbicides that are much more prone to resistance evolution than others. The rarity of resistance to the widely used auxin mimics is testament to the difficulty of evolving herbicide resistance to some modes of action.[178]

Overwhelmingly, the genetic basis of resistance can be attributed to a single gene, usually a single base pair change in DNA resulting in a single amino acid change in a protein. In cases of multiple-resistance it appears that several genes, each endowing resistance to non-overlapping groups of herbicides, are involved. Even in these cases, the direct linkage of one gene to one resistance mechanism appears to hold. As herbicides provide an enormous selection pressure, it is to be expected that single gene mechanisms endowing a large change in phenotype will be selected.[205,206] The few examples where more than one gene contributes to a single resistance mechanism are worthy of note. As yet, such examples are poorly understood; however, full elucidation of the genetic and biochemical behavior of these populations will be illuminating.

The evolution of herbicide resistance in weed populations has not led to less herbicide application or a dramatic increase in nonchemical control methods. Instead, alternative herbicides have been used to manage resistance weeds. This has resulted

in, and will continue to result in, ever more complicated patterns of multiple herbicide resistance. The use of other herbicide strategies such as mixtures and variable dose rates to delay the onset of resistance has been promoted[207,208] but not widely adopted. An understanding of the mechanisms and genetics of resistance has helped elucidate why these strategies will not be widely effective. As resistance is for the most part endowed by single, mostly dominant genes of large effect, such strategies are unlikely to greatly delay the inevitable.[206] The only certain way to delay the evolution of herbicide resistance is to use each herbicide less often and to introduce significant nonchemical control methods.

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