1
HRAC Classification of Herbicides and Resistance Development

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1.1
Introduction

The use of herbicides has caused significant changes in the production systems of all major crops globally. The highly effective chemical control of weeds has replaced manual, animal, and mechanical weed control, has increased the productivity, and has also enabled the development of larger farm sizes and an improved subsistence for farmers. However, herbicides have not resulted in the extinction of weeds; rather, they cause – together with other influencing factors – a continuous selection of plants to occur which are able both to survive and to reproduce. As a consequence, these plants with such survival properties are able to become dominant and to become distributed over increasingly large areas.

The first cases of herbicide resistance were reported around 1970, since then the resistance of both mono- and dicotyledonous weeds to herbicides has become an increasing problem worldwide. At the end of 2010, the International Survey of Herbicide-Resistant Weeds recorded 348 herbicide-resistant biotypes with 194 weed species – 114 dicotyledonous and 80 monocotyledonous (I. Heap, 2010, personal communication, *http://www.weedscience.com*). The relatively steady increase in the number of new cases of resistance since 1980 accounts for the increasing importance of herbicide resistance in weeds in the major agricultural regions (Figure 1.1).

During the period between 1970 and 1990, most documented cases of resistance concerned the triazines. The introduction of new herbicides with different sites of action (SoA) resulted in a shift, so that more recently both acetolactate synthase (ALS) and acetyl-CoA carboxylase (ACCase)-resistant weeds have been reported (Figure 1.2). Additionally, the rapid adoption of glyphosate-resistant crops in North and South America, and the use of glyphosate as a pre-sowing treatment in different cropping systems, have resulted in increasing cases of glyphosate resistance (I. Heap, 2010, personal communication, *http://www.weedscience.com*). The probability of resistance developing towards glyphosate had been expressed as being likely but underestimated, though less frequently in comparison with most other SoA classes [1].

Modern Crop Protection Compounds, Second Edition. Edited by Wolfgang Krämer, Ulrich Schirmer, Peter Jeschke, and Matthias Witschel.

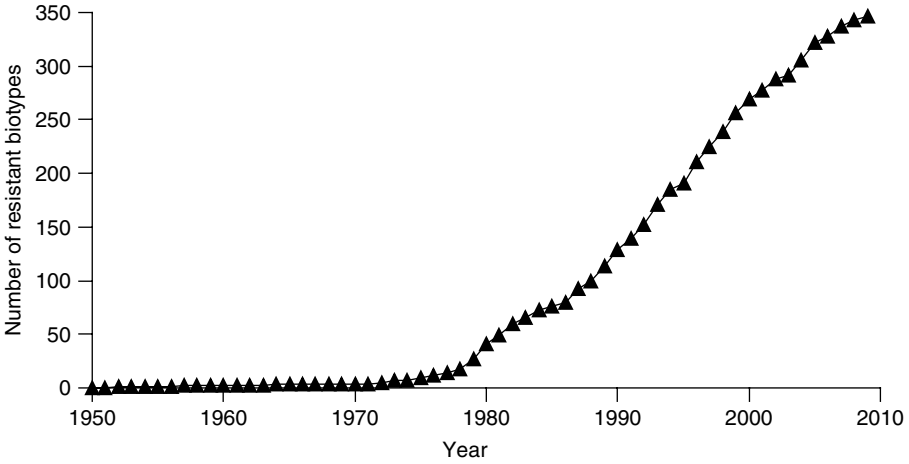

Figure 1.1 The recent chronological increase in the number of herbicide-resistant weeds worldwide. I. Heap (2010), personal communication; *http://www.weedscience.com*.

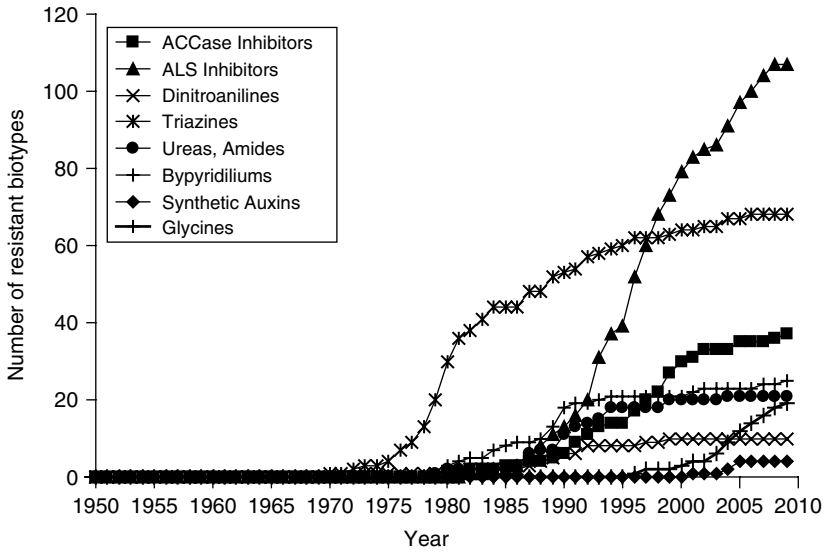

Figure 1.2 The recent chronological increases in the numbers of herbicide-resistant weeds for the different herbicide classes. I. Heap (2010), personal communication; *http://www.weedscience.com*.

1.2
HRAC Classification System of Herbicides

The Herbicide Resistance Action Committee (HRAC) is an international body founded by the agrochemical industry as part of the Global Crop Protection Federation (GCPF) organization. The aims of the HRAC are to support cooperative approaches to the management of herbicide resistance by fostering understanding, cooperation, and communication between industry, government, and farmers.

The global HRAC group proposed a classification system for herbicides according to their target sites, their SoA, the similarity of induced symptoms, or their chemical classes (Table 1.1). This system proved to be the most comprehensive classification system of herbicides globally, although with the Weed Science Society of America (WSSA) Code System and Australian Code System two similar classification systems had been developed at an earlier stage for regional needs. The use of different numbers and letters in the different classification systems very often led to confusion and misunderstanding on the global level. Therefore, it was considered that one common global system would be highly desirable for all users, and would also provide a better understanding of the differences between molecular classes. In particular, all single systems should support and advise all users of herbicides; moreover, such advice should be descriptive and also state how the individual active compounds must be applied in order to achieve the best results in terms of weed control.

The classification system describes not only the chemical family belonging to a specific SoA but also all compounds (via their common names) belonging to each family. This is shown in Table 1.2 for the SoA such as ''Inhibition of dihydropteroate (DHP) synthase,'' ''Microtubule assembly inhibition,'' ''Inhibition of mitosis/microtubule organization,'' ''Inhibition of very long-chain fatty acid synthases (VLCFAs; Inhibition of cell division),'' and ''Inhibition of cell wall (cellulose) synthesis,'' as examples. (Note: these are not mentioned in other chapters of this book; for a more detailed table, see *www.hracglobal.com*). The scheme ''The World of Herbicides,'' which is available under this internet address, also lists all the chemical structures of the various herbicides belonging to the different chemical families.

1.3
Herbicide Resistance

Among the weed population, herbicide resistance is a natural phenomenon that occurs at a low frequency and which has evolved over millions of years. Herbicide applications select only for these weeds in a population, but do not cause resistance nor alter the plant genetics. Increasing problems with herbicide-resistant weed populations have occurred predominantly in countries with intensive agriculture cropping systems. The reliance on a few available weed management tools, coupled with a disregard of the principles of Integrated Weed Management (IWM), are closely related to changes in the weed population community. Changes in the

Table 1.1 HRAC classification system in comparison to Weed Science Society of America (WSSA) and Australian code system. Adapted from Refs [2–4].

Site of action	Chemical family	HRAC group	WSSA group[a]	Australian group[a]
Inhibition of acetyl CoA carboxylase (ACCase)	Aryloxyphenoxy-propionate, cyclohexanedione, phenylpyrazoline	A	1	A
Inhibition of acetolactate synthase (ALS) (acetohydroxyacid synthase; AHAS)	Sulfonylurea, imidazolinone, triazolopyrimidine, pyrimidinyl(thio)benzoate, sulfonylaminocarbonyl-triazolinone	B	2	B
Inhibition of photosynthesis at PS II	Triazine, triazinone, triazolinone, uracil, pyridazinone, phenyl-carbamate	C1	5	C
Inhibition of photosynthesis at PS II	Urea, amide	C2	7	C
Inhibition of photosynthesis at PS II	Nitrile, benzothiadiazinone, phenyl-pyridazine	C3	6	C
Photosystem I-electron diversion	Bipyridylium	D	22	L
Inhibition of protoporphyrinogen oxidase (PPO)	Diphenylether, phenylpyrazole, *N*-phenylphthalimide, thiadiazole, oxadiazole, triazolinone, oxazolidinedione, pyrimidindione, other[d]	E	14	G
Inhibition of the phytoene desaturase (PDS)	Pyridazinone, pyridinecarboxamide, other[d]	F1	12	F
Inhibition of 4-hydroxyphenyl-pyruvate-dioxygenase (4-HPPD)	Triketone, isoxazole, pyrazole, other[d]	F2	27	H
Inhibition of carotenoid biosynthesis (unknown target)	Triazole, diphenylether, urea (also C2)	F3	11	Q
Inhibition of 1-deoxy-D-xylose 5-phosphate synthase (DOXP synthase)	Isoxazolidinone	F4	13	Q
Inhibition of EPSP synthase	Glycine	G	9	M
Inhibition of glutamine synthetase	Phosphinic acid	H	10	N
Inhibition of dihydropteroate (DHP) synthase	Carbamate	I	18	R
Inhibition of microtubule assembly	Dinitroaniline, phosphoroamidate, pyridine, benzamide, benzoic acid	K1	3	D

Inhibition of mitosis/microtubule organization	Carbamate	K2	23	E
	Arylaminopropionic acid	K2	25	Z
Inhibition of VLCFAs (inhibition of cell division)	Chloroacetamide, acetamide, oxyacetamide, tetrazolinone, isoxazoline[b], other[d]	K3	15	K
Inhibition of cell wall (cellulose) synthesis	Nitrile	L	20	O
	Benzamide	L	21	O
	Triazolocarboxamide	L	28	
	Alkylazine	L	29[c]	
Uncoupling (membrane disruption)	Dinitrophenol	M	24	
Inhibition of lipid synthesis – not ACCase inhibition	Thiocarbamate, phosphorodithioate	N	8	J
	Benzofuran	N	16	J
	Chloro-carbonic-acid	N		J
Action like indole acetic acid (synthetic auxins)	Phenoxy-carboxylic-acid, benzoic acid, pyridine carboxylic acid, quinoline carboxylic acid, and other[d]	O	4	I
Inhibition of auxin transport	Phthalamate, semicarbazone	P	19	P
UnknownNote: While the mode of action of herbicides in Group Z is unknown, it is likely that they differ in mode of action between themselves and from other groups.	Pyrazolium	Z	26	
	Organoarsenical	Z	17	Z
	Other[d]	Z	26	

[a] Not all chemical classes are classified.
[b] Proposed.
[c] Proposed by WSSA.
[d] Includes additional molecules which belong to the same SoA (e.g., PPO) but have no special chemical family such as "thiadiazole or oxazolidinedione."
VLCFA, very long-chain fatty acid synthase.

Table 1.2 Selected groups of the HRAC classification system with examples of the active ingredients, which are not mentioned in following chapters. Adapted from Refs [2–4].

Site of action	Chemical family	Active ingredient	HRAC group	WSSA group[a]	Australian group[a]
Inhibition of dihydropteroate (DHP) synthase	Carbamate	Asulam	I	18	R
Microtubule assembly inhibition	Dinitroaniline	Benefin=benfluralin	K1	3	D
		Butralin			
		Dinitramine			
		Ethalfluralin			
		Oryzalin			
		Pendimethalin			
		Trifluralin			
	Phosphoroamidate	Amiprophosmethyl			
		Butamiphos			
	Pyridine	Dithiopyr			
		Thiazopyr			
	Benzamide	Propyzamide= pronamide tebutam			
	Benzoic acid	DCPA=chlorthal-dimethyl			
Inhibition of mitosis / microtubule organisation	Carbamate	chlorpropham	K2	23	E
		propham			
		carbetamide			
	Arylaminopropionic acid	Flamprop-m	K2	25	Z
Inhibition of VLCFAs (inhibition of cell division)	Chloroacetamide	Acetochlor	K3	15	K
		Alachlor			
		Butachlor			
		Dimethachlor			
		Dimethanamid			
		Metazachlor			
		Metolachlor			
		Pethoxamid			
		Pretilachlor			
		Propachlor			
		Propisochlor			
		Thenylchlor			
	Acetamide	Diphenamid			
		Napropamide			
		Naproanilide			
	Oxyacetamide	Flufenacet			
		Mefenacet			
	Tetrazolinone	Fentrazamide			
		Ipfencarbazone			

Table 1.2 (*continued*)

Site of action	Chemical family	Active ingredient	HRAC group	WSSA group[a]	Australian group[a]
	Isoxazoline[b]	Pyroxasulfone			
	Other	Anilofos			
		Cafenstrole			
		Piperophos			
Inhibition of cell wall (cellulose) synthesis	Nitrile	Dichlobenil	L	20	O
		Chlorthiamid			
	Benzamide	Isoxaben		21	
	Triazolocarboxamide	Flupoxam		28	
	Alkylazine	Indaziflam		29[c]	
		Triaziflam			

[a] Not all chemical classes are classified.
[b] Proposed.
[c] Proposed by Weed Science Society of America (WSSA).
VLCFA, very long-chain fatty acid synthase.

farming environment – and, specifically, the economic pressure on farming – are key factors that force farmers to change their practices to those that encourage resistance development.

The limitation in cropping systems, a lack of rotation of herbicide chemistry, or SoA, a limitation in weed control techniques, and the reduction of dose rates represent some of the major drivers for the selection of herbicide resistances. Regular country-based surveys often make it clear that farmers are aware of the problems and their causes. In fact, a survey conducted in Germany in 2004 showed that 94% of farmers were aware that the repeated use of the same herbicide, and 89% that a reduction in dose rates, would cause the development of herbicide resistance. However, 86% of the farmers were forced to reduce their costs and thus did not have available a wide range of weed management techniques (BCS, 2004, Internal communication).

As mentioned above, the planting of herbicide-resistant crops worldwide, which increased from 1.7 mio ha^{-1} in 1996 to about 134 mio ha^{-1} in 2009, has changed the farmers' weed control tactics completely [5]. These systems have provided farmers with favorable economic advantages, simplicity of a better weed management system, as well as more cropping flexibility. In Canada, the adoption of herbicide-resistant cropping systems reached 93% of the canola production in 2009. Moreover, with a total of 21.4 mio ha (an increase of 5.6 mio ha, or 35%), Brazil has in recent years become the second largest grower of biotech crops worldwide [5, 6].

The reliance on one herbicide has reduced the number of applications, and also the number of SoA used. In 2006, glyphosate was applied to 96% (87% in 2004, 62% in 2000, 25% in 1996) of the entire herbicide applied acreage of soybeans

(98% of the total area) in the US. No other herbicide was applied to more than 7% (2004) of the applied acreage (four herbicides with >10% in 2000) [7].

The continued use of herbicide-resistant cropping systems, with an over-reliance on single weed management techniques, selects for weeds that have already evolved resistance to the applied herbicide. Additionally, among the plant population specific weed species that previously were less frequent but naturally resistant can become dominant and, therefore, more difficult to control. It was suspected that a weed population shift would have a greater impact on the cropping system than would the selection of resistant weeds [8, 9]. The results of recent studies have suggested that resistance in weeds and shifts in weed populations occur more quickly than might have been expected [10]. Indeed, statistical observations have shown that the use, dose rates, and application frequency have already changed. For example, in the USA in 1996, glyphosate was applied on average to soybeans 1.2 times at 841 g a.i. ha^{-1}; this was subsequently increased to 1.4 applications (at 1065 g a.i. ha^{-1}) in 2000, and to 1.7 applications (at 1569 g a.i. ha^{-1}) in 2006 [7]. Similar trends can be observed for corn and cotton and also for soybeans in other countries such as Argentina and Brazil.

In the past, intensive soil cultivation techniques and stubble burning were commonly used control techniques in agricultural areas. However, the increasing limitation or banning of stubble burning caused an increasing weed coverage, an increasing soil seed bank, and the development of herbicide resistance in many agricultural regions. Several different investigations have highlighted the fact that the burning of straw drastically decreased weed densities, an example being the number of water plants (*Echinochloa* spp.) in comparison to the incorporation of rice straw into the soil [11]. Australian farmers, in particular, have sought alternative weed control techniques during their harvest operations, because of the limited choice of chemical solutions available during the growing season. The method used to bale of straw, such as a trailing baler attached to the harvester, or the physical destruction of weed seeds during the harvesting operation (''Rotomill''), represent additional possibilities [12].

During recent years, the economic pressure placed on farmers to produce at the lowest costs, coupled with changing environmental influences such as soil erosion or water availability, have led to the adoption of no-till practices. Typically, soybean farmers in the USA increased their planted areas with no-till from 45.3% in 2006 to almost 50% in 2009 [13], and the use of no-till is expected to further increase globally. In most cases, the shift to a no-till system causes an over-reliance on herbicides, and the price erosion of herbicides during the past few years has played a significant role in the adoption of no-till practices. Recent surveys have shown that farmers are aware that no-till practices increase herbicide costs and also herbicide resistance – in particular, to glyphosate. Nevertheless, the acreage for no-till is expected to continue increasing, especially in areas where the level of no-till approach is currently low [14]. However, growers with increasing herbicide-resistance problems are planning to reduce the use of no-till where no other herbicide option is no longer available.

The results of simulation studies have shown that the risk of adopting no-till, as well as the development of herbicide resistances, can be reduced by alternating between minimum and no-tillage systems, or by alternating between nonselective herbicides for pre-sowing weed control [15]. The most efficient weed control strategy for conserving susceptibility in no-tillage systems was the ''double knockdown'' pre-sowing application scheme of glyphosate and paraquat in sequence.

One of the most effective tools in the management of herbicide resistance and weed density is to include a diverse crop rotation practice. Weed species are typically associated with crops, and crop rotations determine their specific weed population over time [16]. A high diversity provides the farmer with more opportunities and more flexibility with respect to the growing conditions, tillage practices and planting time, the selection of crop cultivars, the rotation of herbicides with different SoA, the variation of application timings of herbicides across the years to a specific weed emergence period, and/or the inclusion of nonchemical management techniques [17]. Consequently, these practices provide farmers with opportunities either to prevent or to slow down the selection and development of herbicide resistance. Selected resistance can persist among field populations for many years, with the populations remaining stable until resistant weed seeds disappear from the soil seed banks, which occurs very seldom. Investigations with triazine-resistant weed populations have shown that resistant weed seeds remained in the soil, despite changes in crop rotation and an absence of triazine herbicides (J. Gasquez, 2003, personal communication). Similar results were obtained from studies in which the effect of management practices on ACCase-resistant *Alopecurus myosuroides* was evaluated in the field [18]. In this case, the percentage of resistant plants remained unchanged during a three-year period, even without the application of ACCase inhibitors. The density of blackgrass plants decreased, especially when spring crops were part of the crop rotation.

Neither cropping systems nor the single weed management tactic can be applied to solve specific weed problems on a long-term basis. The use of all possible practices to prevent and to manage herbicide resistances in an integrated fashion should be the long-term goal for agricultural production.

The continuous application of herbicides leads to the selection of rare genotypes of weeds that are resistant to the herbicide and, eventually, at the same time are already cross-resistant to other herbicides which have not been used previously (as noted above). These genotypes may already exist in a weed population in very low frequency, before the introduction the selecting herbicide.

More comprehensive overviews of herbicide resistance in general, including additional examples that are not described in this book, are available elsewhere (see Refs [19–22]).

1.3.1 Biochemistry of Herbicide Resistance

Resistance to herbicides can be based on one of the following biochemical mechanisms [20, 22]:

- **Target-site resistance:** This is due to a reduced (or even lost) ability of the herbicide to bind to its target protein. The effect usually relates to an enzyme with a crucial function in a metabolic pathway, or to a component of an electron-transport system. Target-site resistance may also be caused by an overexpression of the target enzyme (via gene amplification or changes in a gene promoter).
- **Non-target-site resistance:** This is caused by mechanisms that reduce the amount of herbicidal active compound reaching the target site. One important mechanism is an enhanced metabolic detoxification of the herbicide in the weed, which leads to insufficient amounts of the active substance reaching the target site. A reduced uptake and translocation, or sequestration of the herbicide, may also result in an insufficient herbicide transport to the target site.
- **Cross-resistance:** In this case, a single resistance mechanism causes resistance to several herbicides. The term *target-site cross-resistance* is used when the herbicides bind to the same target site, whereas *non-target-site cross-resistance* is due to a single non-target-site mechanism (e.g., enhanced metabolic detoxification) that entails resistance across herbicides with different SoA.
- **Multiple resistance:** In this situation, two or more resistance mechanisms are present within individual plants, or within a plant population.

1.3.1.1 Target-Site Resistance

Cases analyzed to date have shown that herbicide resistance is very frequently based on a target-site mutation. Within the past 40 years, weed species have developed target-site resistance to most known herbicide chemistries; those of major importance are discussed in the following subsections.

1.3.1.1.1 Inhibitors of Photosystem II (PS II) Early reports on the resistance of weeds to photosystem (PS) II inhibitors of the triazine group first appeared around 1970. Since then, triazine resistance has been reported for numerous – mainly dicotyledonous – weed species.

Investigations into the mechanism of resistance to triazines have revealed that, in most cases, such resistance is due to a mutation that results in a modification of the target site; this is known to be the Qb site of the D1 protein in the PS II reaction center. The triazine herbicides bind to this site, thereby inhibiting the photosynthetic electron flow. In resistant mutants, the triazine binding is markedly reduced; for example, the concentration of atrazine required to achieve a 50% inhibition of photosynthetic electron flow in isolated chloroplasts of *Chenopodium album* was at least 430-fold higher for chloroplasts from an atrazine-resistant mutant than for those from wild-type plants [23].

In many cases, the mutants of weed species with target-site resistance to triazines showed a lower growth rate and ecological fitness than the susceptible wild-type, when analyzed in the absence of a triazine herbicide as selection agent. The quantum yield of CO_2 reduction in resistant biotypes was decreased; furthermore, electron transfer between the primary and secondary quinones in the PS II reaction center was slowed. The latter effect may have been the cause of an increased susceptibility to photoinhibition in the resistant biotypes [24, 25].

The D1 protein is encoded by the chloroplast *psb*A gene, which is highly conserved among higher plants, algae, and cyanobacteria [26]. In almost all investigated cases of the resistance of field-grown weed species to triazines, resistance was attributed to a mutation in the *psb*A gene with a resultant Ser264 → Gly change in the herbicide-binding niche of the D1 protein. Consequently, this resistance is usually maternally inherited. Although herbicides of the phenylurea group are also inhibitors of the PS II system, a cross-resistance of atrazine-resistant mutants with a Ser264 → Gly change has not been observed with phenylureas. It has been proposed that the binding sites of triazines and phenylureas are not identical, but rather overlap [27–29], with Ser264 providing a hydrogen bond to atrazine or other herbicides of the triazine group. The substitution of Ser264 by glycine removes this bond, which is important for binding the triazines. According to the concept of overlapping binding sites, hydrogen bonding to Ser264 is not important for phenylureas, due to a different binding geometry; consequently, the binding of phenylurea will not be affected by the Ser264 → Gly mutation.

In 1999, Masabni and Zandstra described a mutant of *Portulaca oleracea* with a resistance pattern to PS II inhibitors, but which was different from most triazine-resistant weeds [30]. In fact, the mutant was not only resistant to the phenylureas linuron and diuron, but was also cross-resistant to atrazine and other triazines. Sequencing of the D1 protein revealed that, in the resistant biotype, Ser264 had been replaced by Thr, and not by Gly, this being the first report of a Ser264 → Thr mutation on a whole-plant level. It was suggested that the Ser → Thr mutation had modified the conformation of the herbicide-binding niche at the D1 protein, in such a way that the binding of phenylureas and triazines was also reduced.

An additional novel mutant was identified when field accessions of *P. annua* with a known resistance to PS II inhibitors, collected in Western Oregon, were analyzed after amplification of the herbicide-binding region (933 bp fragment) of the chloroplast *psb*A gene, using the polymerase chain reaction (PCR). A sequence analysis of the fragment from a mutant which showed resistance to diuron and metribuzin (resistance factors of between 10 and 20) revealed a substitution from Val219 to Ile in the D1 protein encoded by the *psb*A gene. This amino acid substitution had previously been identified following the mutagenesis of laboratory cultures of algae and cell cultures of *Chenopodium rubrum*. The finding of a Val219 → Ile substitution in *P. annua*, however, was the first reported case of a weed species with resistance to PS II inhibitors in the field, that was due to a *psb*A mutation other than at position 264. As noted above, the electron-transfer processes in the PS II reaction center of weeds with a mutation at position 264 were slowed, and the ecological fitness of the mutants reduced. In contrast, no effect on electron transfer in the PS II reaction center was found for the *P. annua* mutant with the Val219 → Ile change, and it was supposed that this mutant (in ecological terms) may be as fit as its wild-type counterpart [31]. The same mutation was also found among *Kochia scoparia* and *Amaranthus powelli* populations [32, 33]. Further mutations from a selection of non-triazine PS II inhibitors have evolved in *Senecio vulgaris* from Asn266 to threonine [34], in *C. album* from Ala251 to Val [35], and

Capsella bursa-pastoris from Phe255 to Ile [36]. All of these populations remained sensitive to triazine herbicides, including atrazine and simazine.

1.3.1.1.2 Inhibitors of Acetyl-CoA Carboxylase (ACCase) The enzyme ACC catalyzes the carboxylation of acetyl-CoA, which results in the formation of malonyl-CoA. In plastids, this reaction is the initial step of *de novo* fatty acid biosynthesis and is, therefore, of crucial importance in plant metabolism. Species of the Poaceae family (grasses) have in their plastids a homomeric, multifunctional form of ACCase with the following domains: biotin carboxy carrier protein (BCCP); biotin carboxylase (BC); and carboxyltransferase (CT). Other monocotyledonous species examined to date, as well as most dicotyledonous species, have in their plastids a heteromeric, multisubunit type of ACCase with the BCCP, BC, and CT domains encoded on separate subunits. In addition, all dicotyledons and monocotyledons (including the Poaceae) have a cytosolic ACCase, which belongs to the homomeric type. The ACCase-inhibiting herbicides inhibit only the plastidic homomeric ACCase in grasses (Poaceae), but have no inhibitory effect on the plastidic heteromeric form of other monocotyledonous and dicotyledonous species, nor the homomeric ACCase in the cytosol. Therefore, while these herbicides will have a selectively lethal effect only on grass species, they are tolerated by other monocotyledonous and by dicotyledonous species. To date, three different chemical groups of ACCase inhibitor have been identified: (1) the aryloxyphenoxypropionates (APPs); (2) the cyclohexanediones (CHDs), which have been developed during the past 15–20 years to provide a very important herbicide family with a selective action on a broad spectrum of grass weed species; and (3) the phenylpyrazoline (PPZ) group.

Until now, a target-site resistance of biotypes to ACCase inhibitors has been confirmed for several grass weed species of economic importance. The earliest cases of target-site based resistance were reported for biotypes of *Lolium multiflorum* from Oregon, USA [37], and for *Lolium rigidum* from Australia [38].

ACCase prepared from the resistant *L. multiflorum* biotype, which had been selected by the field use of diclofop, was inhibited by the APPs diclofop, haloxyfop, and quizalofop with IC_{50}-values (the herbicide concentration required for 50% enzyme inhibition) that were 28-, 9-, and 10-fold higher than for ACCase from a susceptible biotype. There was no cross-resistance to the CHD herbicides sethoxydim or clethodim [39]. ACCase resistance was subsequently also confirmed for *L. multiflorum* biotypes from other countries. In a resistant biotype selected by diclofop in Normandy, the resistance factor (ratio of the IC_{50} for ACCase from the resistant to the IC_{50} for ACCase from the susceptible biotype) was 19 for diclofop, and 5 for haloxyfop, but only 2 for the CHDs clethodim and sethoxydim [40]. Interestingly, a different ACCase resistance pattern was found for the resistant *L. multiflorum* biotype Yorks A2, although field selection was apparently also mainly by diclofop. The resistance factors were 3 and 9, respectively, for the APPs diclofop and fluazifop, but 20 for the CHD herbicide cycloxydim [41].

The first biotypes of *L. rigidum* with target-site resistance to ACCase inhibitors were identified during the early 1990s in Australia. Selection either with an APP

or with a CHD herbicide resulted in target-site cross-resistance to both herbicide groups. However, regardless of whether the selection was by an APP or a CHD compound, the level of resistance in these biotypes was higher towards APP than towards CHD herbicides. The ACCase resistance factors were 30 to 85 for diclofop, >10 to 216 for haloxyfop, and 1 to 8 for sethoxydim [38, 42, 43].

Biotypes with target-site-based resistance to ACCase inhibitors were also selected in wild oat species (*Avena fatua*, *Avena sterilis*). The resistance patterns were found to be variable; for example, the resistance factors for ACCase from the Canadian *A. fatua* biotype UM1 were 105 for sethoxydim, 10 for tralkoxydim, and 10 for diclofop and fenoxaprop, whereas for the *A. fatua* biotype UM33 from Canada the ratios were 10.5 for fenoxaprop, 1.2 for diclofop, 5 for sethoxydim, and 1.7 for tralkoxydim. It was proposed that this effect was due to different point mutations, each being associated with a characteristic resistance pattern [44]. However, another reason might be the frequency of homozygote and heterozygote resistant and susceptible plants within a tested population.

During studies of resistance conducted with *Alopecurus myosuroides* populations from the UK, two biotypes – Oxford A1 and Notts A1 – were identified which were highly resistant to fenoxaprop, diclofop, fluazifop, and sethoxydim due to an insensitive ACCase. Genetic studies revealed that the target-site resistance in the two *A. myosuroides* biotypes was monogenic and nuclear inherited, with the resistant allele showing complete dominance [45].

Target-site based resistance to ACCase has also been reported for several other grass weeds, including two biotypes of *Setaria viridis* from Manitoba, Canada. One of these (UM8) confers high levels of ACCase insensitivity to fenoxaprop and sethoxydim, while the ACCase of biotype UM 131 was highly insensitive to sethoxydim, but only moderately to fenoxaprop (for a review, see Ref. [43]). Biotypes of *Setaria faberi* and *Digitaria sanguinalis*, obtained in a vegetable cropping system in Wisconsin, USA, each had an ACCase which was highly insensitive to sethoxydim but only moderately insensitive to clethodim and fluazifop [46]. Based on the patterns of target-site-based cross-resistance of weeds to APP and CHD herbicides, it was postulated that the two classes of ACCase inhibitor do not bind in an identical manner to the target site (''overlapping binding sites''), and that different point mutations at the target enzyme accounted for the variable resistance patterns. Molecular investigations with chloroplastic ACCase from wheat indicated, first, that a 400-amino-acid region in the CT domain was involved in insensitivity to both APP and CHD herbicides [47]. Subsequent follow-up studies with a chloroplastic ACCase of *L. rigidum* showed that the resistance to ACCase inhibitors was due to a point mutation, which resulted in an isoleucine to leucine change in the CT domain of the enzyme [48]. Previously, Tal and Rubin investigated the molecular basis of ACCase resistance in a *L. rigidum* biotype from Israel, with resistance to CHD and APP herbicides [49]. Following the amplification (by PCR) of a 276 bp DNA encoding the CT domain of ACCase, the substitution of a single isoleucine by leucine was also found in this resistant biotype. The results of inheritance studies conducted by the same authors suggested that the alteration of ACCase in *L. rigidum* was governed by a single nuclear codominant gene.

It was shown that a point mutation resulting in an isoleucine to leucine substitution within the chloroplastic ACCase CT domain is also responsible for the target-site resistance of *A. fatua* [50], *A. myosuroides* [51], and *S. viridis* [52]. The mutant leucine ACCase allele in the *Setaria* species was characterized to be dominant, and no negative effect was detected on ACCase function of the mutant. It was suggested that the change in ACCase conformation caused by the isoleucine to leucine mutation was only minor, yet sufficient to prevent (or at least strongly reduce) the herbicide binding to the enzyme. Brown *et al.* [51] also pointed to the very interesting fact that the leucine found in the plastidic homomeric ACCase of mutated resistant grass weeds is also found in the heteromeric plastidic enzyme of non-grass species, and also in the cytosolic homomeric enzymes that are ''naturally'' resistant to these herbicides. Hence, the selective action of ACCase-inhibiting herbicides appears to reside at this enzyme site [43].

Further studies conducted in France by Délye and coworkers, with *A. myosuroides* accessions from different sites, shed more light on the molecular basis of the different resistance patterns to ACCase inhibitors, thus providing further support to overlapping binding sites for APP and CHD herbicides at the ACCase enzyme [53, 54]. Meanwhile, different point mutations were identified in different grass weed species that gave rise to insensitive ACCase due to the exchange of one amino acid: in addition to the Ile → Leu (position 1781), the Trp → Cys (pos. 1999), Trp → Cys (pos. 2027), Ile → Asn (pos. 2041), Ile → Val (pos. 2041), Asp → Gly (pos. 2078), Cys → Arg (pos. 2088), and Gly → Ala (pos. 2096) could be identified ([55, 56]; for a review, see Ref. [22]). The Ile → Leu mutation (pos. 1781) is the most common, and results in a resistance to mainly all ACCase herbicides. However, the determination of cross-resistance patterns and resistance levels for the different mutations cannot be generalized, and these differ between grass weed species. Both, high-level and low-level resistance to the individual ACCase herbicides is given for the different mutations based on evaluation criteria, including the herbicide used and the dose rate, the homozygosity/heterozygosity of mutations, and the presence of non-target-site resistance mechanisms.

Recently, the PCR amplification and sequencing of plastidic ACCase domains involved in herbicide resistance have been employed to screen a spectrum of 29 grass species for target-site-based resistance to ACCase inhibitors by direct comparison of the sequences of plastidic ACCase around the critical codons [57]. In *P. annua* and *Festuca rubra*, it was found that a leucine residue occurred at position 1781, while the wild types of all other grass species had an isoleucine in this position. *P. annua* and *F. rubra* are already known (based on enzyme inhibition assays) to possess a plastidic ACCase that is markedly less susceptible to ACCase inhibitors than the ACCase of other grass species. Thus, the leucine in position 1781 can clearly be regarded as the basis, or a substantial part, of the natural inherent tolerance of both species to ACCase-inhibiting herbicides.

A different mechanism of target-site resistance to ACCase inhibitors that should be mentioned here was identified in a *Sorghum halepense* biotype from Virginia, USA, which had been selected in the field by quizalofop applications. The resistance level of this biotype *in vivo* was relatively low, with resistance factors [based on ED_{50}

(effective dose) values] ranging between 2.5 and 10 for quizalofop, sethoxydim, and fluazifop. No difference was found between the herbicide susceptibility of ACCase from the resistant biotype and a susceptible standard. However, the specific activity of ACCase in the resistant biotype was found to be two- to threefold greater than in susceptible plants. These results suggested that an overproduction of ACCase was the mechanism that conferred a moderate level of resistance to these herbicides. Owing to the enzyme overproduction the resistant biotype was able, presumably, to sustain a level of malonyl-CoA production necessary for survival of herbicide treatment. To date, however, this has been the only reported case for this mechanism in a naturally occurring biotype [58].

1.3.1.1.3 Inhibitors of Acetolactate Synthase (ALS/AHAS) The enzyme ALS plays an essential role in branched-chain amino acid biosynthesis in plants. In the pathway leading to valine and leucine, ALS catalyzes the formation of 2-acetolactate from two pyruvate molecules, and in the pathway to isoleucine the formation of 2-acetohydroxybutyrate from 2-ketobutyrate and pyruvate. Due to this double function, the enzyme is also referred to (with a more general term) as acetohydroxyacid synthase. ALS is inhibited by several groups of herbicides, mainly the sulfonylureas (SUs), imidazolinones (IMIs), triazolopyrimidines (TPs), pyrimidinylthiobenzoates (PTBs), and sulfonylaminocarbonyltriazolinones (SCTs) (see Chapter 2.1).

Resistant biotypes which were being reported during the early 1990s were selected by chlorsulfuron or metsulfuron-methyl in wheat-growing areas, or by sulfometuron-methyl in non-crop areas. While the resistance of *L. rigidum* to ALS-inhibitors was attributed to an enhanced herbicide metabolism [59], it was shown, for *Lolium perenne* and dicotyledonous species such as *Stellaria media*, *Kochia scoparia*, *Salsola iberica*, and *Lactuca serriola*, that resistant biotypes had a mutated ALS with a reduced susceptibility to ALS-inhibiting herbicides [60–62]. The IC_{50}-values for the SUs, which were determined *in vitro* with ALS isolated from *S. media*, *S. iberica*, and *L. perenne*, were increased by four- to 50-fold in the resistant biotypes. Smaller increases, from about two- to sevenfold, were determined in the same biotypes for the IMI herbicide, imazapyr [62].

Later, ALS-inhibitors were developed for selective use in rice, and this led to the selection of resistant rice weed biotypes. A biotype of *Monochoria vaginalis*, discovered in Korea, showed high levels of cross-resistance to bensulfuron-methyl, pyrazosulfuron-ethyl, and flumetsulam. The resistance factors determined for ALS *in vitro* were 158 to bensulfuron-methyl and 58 to flumetsulam, but only 1.6 to imazaquin [63]. In rice fields in Japan, a biotype of *Scirpus juncoides* was selected which exhibited a high degree of resistance to imazasulfuron (resistance factor of 271, calculated from ED_{50}-values for growth inhibition). Inhibition studies with isolated ALS revealed an IC_{50} of 15 nM for the enzyme from susceptible plants, but of more than 3000 nM for ALS isolated from the resistant biotype; this suggested that the resistance was due to an altered ALS enzyme [64].

It appears that a reduced sensitivity of the target enzyme is the predominant cause of resistance to ALS inhibitors, and that resistance is conferred by a single, dominant, or at least partial dominant, nuclear-encoded gene. The results of

molecular studies revealed that resistance is caused by single substitution of one of seven highly conserved amino acids in the ALS enzyme. These are the following, with 22 known resistance substitutions (amino acid number standardized to the *Arabidopsis thaliana* sequence): Pro197, Ala122, and Ala205, located at the amino-terminal end, Asp376, and Trp574, Ser653, and Gly654, located near the carboxy-terminal end [65, 66]. For more details, see also Chapter 2.1.

When, in the ALS of a *Lactuca serriola* biotype, which was highly resistant to SUs and moderately resistant to IMIs, Pro197 was substituted by His, the pyruvate-binding domain on the ALS enzyme was found not to be altered by the mutation [67]. From *K. scoparia* it was reported that several substitutions of Pro197 by another amino acid (Thr, Arg, Leu, Gln, Ser, Ala) would confer resistance to SUs [68]. In the same species, it was found later that the substitution of Trp574 by Leu would also cause resistance to SUs and, in addition, a cross-resistance to IMIs [69]. The latter substitution was also detected in resistant biotypes of several other dicotyledonous weed species.

In a biotype of *Amaranthus retroflexus* from Israel, resistance was caused by a change of Pro197 to Leu. This biotype exhibited cross-resistance to SUs, IMIs, TPs, and also to pyrithiobac-sodium *in vivo* and on the ALS enzyme level [70]. In mutations of *Amaranthus rudis*, Ser653 was found to be exchanged by Thr or Asn; such mutants were only resistant to IMIs [71].

It was concluded from the multiplicity of amino acid substitutions carried out, that the herbicide-binding site of the ALS can tolerate substitutions of each of the seven conserved amino acids, without causing any major consequences to normal catalytic functions. It was, therefore, speculated that the herbicide-binding site and the active site of ALS are different, despite probably their being in close proximity. In the absence of herbicide selection, the weed biotypes with mutated ALS showed, in most cases, no reduction (or only a negligible reduction) of fitness (for reviews, see Refs [65, 72]), whereas others [73] found for the Trp574 → Leu substitution in *Amaranthus powellii* a substantial fitness cost. A review of the fitness costs of herbicide resistance alleles was recently produced [74].

1.3.1.1.4 Glyphosate Today, glyphosate has become the most important herbicide worldwide, and is widely used as nonselective herbicide in different indications, and also as a selective herbicide in transgenic crops. The introduction of transgenic crops in 1996 changed the use pattern of the compound and the weed management system, as discussed above [6]. Glyphosate inhibits the chloroplast enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which catalyzes the reaction of shikimate-3-phosphate (S3P) and phosphoenolpyruvate (PEP) to form 5-enolpyruvylshikimate-3-phosphate (EPSP). The inhibition of EPSPS activity disrupts the shikimate pathway and aromatic amino acid production, which finally causes the plant to be destroyed.

Since the introduction of glyphosate in 1974, there have been no reports of evolved glyphosate-resistant weeds during a 20-year period of intensive use [75]. Even in 1997, following the introduction of transgenic crops, it was not believed that glyphosate resistance in weeds would ever become a major problem [76]. Due

to the loss of diversity in weed management systems, however, the simplicity and flexibility of this technology was changed, such that resistance to glyphosate has emerged and has been confirmed in at least 20 weed species in 16 countries (I. Heap, 2010, personal communication, *http://www.weedscience.com*). Both, target-site and non-target-site resistance mechanisms have evolved in different weed species. In resistant accessions of *Eleusine indica* from Malaysia, this was found to have resulted from point mutations of the target enzyme EPSPS. By using PCR amplification and the sequence analysis of an EPSPS fragment, an exchange of Pro106 by Ser was found in two resistant accessions, and an exchange of Pro106 by Thr in a third resistant accession [77, 78]. This mutation Pro106, with exchanges by Ser, Thr, and Ala, was also found in different *L. rigidum* and *L. multiflorum* biotypes from different locations in Australia, USA, Chile, and South Africa (for a review, see Ref. [79]). In contrast to other target-site mutations (see ACCase and ALS), the amino-acid substitution at position Pro106 resulted in a modest degree of glyphosate resistance of 2- to 15-fold in most cases [79]. Recent investigations have led to the identification of just over a 160-fold EPSPS gene amplification, resulting in an increased EPSPS overexpression in glyphosate-resistant *Amaranthus palmeri* biotypes. The gene amplification was not due to genome duplication, however. The results of these studies showed that the increasing number of copies conferred an increasing glyphosate resistance level in plants [80].

1.3.1.2 Non-Target-Site Resistance by Enhanced Metabolic Detoxification

Typically, crop and weed species dispose of the enzyme systems that catalyze the metabolic conversion of herbicides such that the metabolites, which usually are more polar than the parent compound, are either nonphytotoxic at all or have a reduced phytotoxic potential. Among the various enzyme systems involved in metabolic herbicide detoxification, two are of particular importance in weeds and crops:

- **The cytochrome-P450 monooxygenase system:** This system catalyzes oxidative transformations of the herbicide molecule (e.g., hydroxylations and oxidative dealkylations). In fact, the system is a member of a large enzyme family that consists of multiple cytochrome-P450 monooxygenases with diverse substrate specificities.
- **Glutathione-*S*-transferase (GST):** This family of enzymes catalyzes conjugation reactions that result in the nucleophilic displacement of aryloxy moieties, chlorine, or other substituents by the tripeptide glutathione (GSH). The GSTs also occur in various isoforms that differ in their catalytic properties.

The herbicide tolerance of crop species has been found to be based frequently on differential rates of metabolic herbicide detoxification in crop and weed species. Whilst the rates of herbicide detoxification among weed species are too low to prevent the binding of a lethal herbicide dosage at the target site, the tolerant crop is able metabolically to detoxify the herbicide at such a high rate that binding of the herbicide at the target site in sufficient amounts to cause irreversible herbicidal effects will be prevented. If weed biotypes with an improved ability for herbicide detoxification,

comparable to the tolerant crop species, occur in a population they will survive herbicide application and will thus be selected. These enzyme system-based resistance mechanisms can affect herbicides from different SoA, and (potentially) cause unexpected cross-resistances to herbicides that have not been used.

To date, several weed biotypes have been described for which herbicide resistance was related to an enhanced metabolic herbicide detoxification. Indeed, several cases have been reported for *L. rigidum*. An early report from Christopher *et al*. stated that the excised shoots of biotype SLR 31 from Australia, which was resistant to diclofop, exhibited a cross-resistance to the SUs chlorsulfuron, metsulfuron-methyl, and triasulfuron [81]. Although the metabolite pattern of chlorsulfuron was identical in the resistant biotype and a susceptible standard, the resistant biotype metabolized the herbicide more rapidly. The pathway of chlorsulfuron detoxification in *L. rigidum* was similar to that described for wheat, with ring hydroxylation being followed by glucose conjugation. The time course of chlorsulfuron metabolism in the *L. rigidum* biotype SR 4/84 (resistant to diclofop and cross-resistant to chlorsulfuron) was analyzed separately in shoots and roots. The half-life of chlorsulfuron in susceptible plants was longer in the roots (13 h) than in the shoots (4 h), and was reduced in the resistant biotype to 3 h and 1 h, respectively. Detoxification of the herbicide by ring hydroxylation, most likely catalyzed by a cytochrome-P450-dependent monooxygenase, with subsequent glucose conjugation, was enhanced in the resistant biotype [59].

Two other *L. rigidum* biotypes from Australia (WLR2 and VLR69) developed metabolism-based resistance to PS II inhibitors. In this case, WLR2 was obtained from a field with selection pressure by atrazine and amitrole, but never by phenylureas, while VLR69 was obtained from a field with selection pressure by diuron and atrazine. Both biotypes were resistant to triazines and, despite the field selection by atrazine, resistance was more pronounced to the structurally related simazine. Furthermore, both biotypes were resistant to chlorotoluron, though only VLR69 had previously been exposed to phenylureas. The results of analytical studies revealed that, in both resistant biotypes, the metabolism of chlorotoluron and simazine was enhanced, and that the main route of their metabolism was via N-dealkylation reactions. This type of reaction, coupled to the fact that herbicide metabolism was inhibited by 1-aminobenzotriazole (ABT; an inhibitor of cytochrome-P450 monooxygenases) suggested an increased activity of cytochrome-P450 monooxygenases in the resistant biotypes [82, 83]. The mechanism of phenylurea resistance of *L. rigidum* biotypes from Spain has been studied [84]. A biotype (R3) selected in the field by applications of diclofop plus isoproturon or plus chlorotoluron had *in vivo* resistance factors [ED_{50} R (resistant)/ED_{50} S (susceptible)] of about 9.3 and 5.5 to chlorotoluron and isoproturon, respectively, and was also resistant to a broad spectrum of other phenylureas. Metabolism studies with chlorotoluron, in the absence and presence of the cyochrome-P450 monooxygenase inhibitor 1-ABT, suggested that resistance was due to an enhanced ability to degrade the molecule to nontoxic ring-alkylhydroxylated intermediates suitable for follow-up conjugation reactions. Thus, several biotypes of *L. multiflorum* from the UK, with resistance to diclofop, have been analyzed [41]. While one biotype had an insensitive ACCase, the

resistance of three other biotypes could be attributed to an enhanced metabolism of this herbicide.

The resistance of the grass weed *Phalaris minor* to isoproturon, and of the dicotyledonous weed species *Abutilon theophrasti* to atrazine, has also been attributed to an enhanced metabolism. Here, GST was noted as the enzyme responsible for atrazine detoxification in *A. theophrasti* [85], whereas in *P. minor* the cytochrome P450 monooxygenase was most likely involved in the enhanced detoxification of isoproturon [86].

An increasing occurrence of the resistance of *A. myosuroides* to herbicides in several European countries has prompted investigations into resistance mechanisms in this species. Aside from target-site-based resistance, cases of resistance due to an enhanced herbicide metabolism have also been reported. Two biotypes – Peldon A1 and Lincs. E1 – with *in vivo* resistance factors to isoproturon of 28 and 2.6, respectively, were shown to metabolize this herbicide faster than a susceptible standard, with the rate of metabolism being higher in Peldon than in Lincs. The addition of the cytochrome-P450 monooxygenase inhibitor 1-ABT lowered the rate of chlorotoluron metabolism, and correspondingly increased phytotoxicity; this suggested an involvement of the cytochrome-P450 monooxygenase system in the detoxification of the herbicide. However, the major detoxification reaction in these biotypes appeared to be the formation of a hydroxymethylphenyl metabolite [87].

The same biotypes, Peldon A1 and Lincs. E1, are also resistant to the graminicide fenoxaprop, which is used for the selective control of *A. myosuroides* and other grassy weeds in cereals (mainly wheat). On a whole-plant level, Lincs. E1 was more resistant than Peldon A1. The selectivity of this herbicide has been attributed to a rapid detoxification via GST-catalyzed conjugation in the cereal species. In both resistant *A. myosuroides* biotypes, the GST activities toward fenoxaprop were shown to be increased to a similar degree, when compared with a susceptible biotype. This was due to an increased expression of a constitutive GST, and to the expression of two novel GST isoenzymes. Furthermore, GSH levels were increased in the resistant biotypes, in Peldon more than in Lincs. These data pointed to an involvement of GST activity and GSH levels in the resistance to fenoxaprop, although a lack of correlation to the whole-plant resistance of these biotypes did not permit definite conclusions to be drawn [88]. Recently, a range of European *A. myosuroides* biotypes with resistance to fenoxaprop has been investigated [89], and several of these biotypes – notably one from Belgium – were shown to detoxify the herbicide at an increased rate. The biotype from Belgium also had the highest GST activity towards the unspecific substrate chloro-dinitrobenzene (CDNB), although GST activity towards the herbicide was not tested.

Studies on the mode of inheritance of metabolic herbicide resistance in *A. myosuroides* and *L. rigidum* postulated that more than one gene is involved in cytochrome-P450 metabolism-based resistance in weed biotypes [90–92]. The occurrence of an enhanced metabolic detoxification can be associated with an ecological cost expressed in a reduction of the vegetative biomass and reproduction rate [74].

In contrast to the above-described cases, the herbicide propanil is detoxified in rice and weed species by the action of an aryl acylamidase

(aryl-acylamine amidohydrolase). A high activity of this enzyme in rice confers crop tolerance. In Colombia, a biotype of *Echinochloa colona* was found that is resistant to propanil; subsequent enzyme tests with extracts from this biotype revealed an almost threefold higher activity of aryl acylamidase in the resistant than in a susceptible biotype. Based on these findings, it was concluded that resistance of the *E. colona* biotype is related to an enhanced propanil detoxification [93].

1.3.1.3 **Non-Target-Site Resistance by Altered Herbicide Distribution**

Cases of non-target-site resistance by altered herbicide distribution have been reported for two important herbicides, paraquat and glyphosate.

The intensive use of paraquat has resulted in an evolution of resistance in various weed species. Subsequently, intensive investigations into the resistance mechanisms involved was mainly carried out using resistant biotypes from *Hordeum* spp. and *Conyza* spp., and an altered distribution of the herbicide in the resistant weeds was suggested as the cause – or at least the partial cause – of resistance. In resistant *Conyza canadensis*, it was supposed that a paraquat-inducible protein might function by carrying paraquat to a metabolically inactive compartment, either the cell wall or the vacuole. This sequestration process would prevent sufficient amounts of the herbicide from entering the chloroplasts, which is the cellular site of paraquat action. Inhibitors of membrane transport systems, such as *N,N*-dicyclohexylcarbodiimide (DCCD), caused a delay in the recovery of the photosynthetic functions of a paraquat-resistant biotype, when administered after the herbicide. The results of these transport inhibitor experiments supported the involvement of a membrane transporter in paraquat resistance [94].

Translocation studies with two paraquat-resistant biotypes of *Hordeum leporinum* revealed that the basipetal transport of paraquat in resistant *H. leporinum* was much reduced compared to susceptible plants. It was concluded, therefore, that a resistance to paraquat was the result of a reduced herbicide translocation out of the treated leaves [95]. It might be supposed that, also in this species, herbicide sequestration into the leaf vacuoles may have been the primary cause for the altered long-distance transport.

The high efficiency of glyphosate as a potent herbicide is based on its ability to translocate within the plant via xylem and phloem to the apical and root meristems, as well as to the reproductive organs of perennial plants. Independent populations of *L. rigidum* with resistance to glyphosate have been reported from different locations in Australia. One of these, with an approximately 10-fold *in vivo* resistance to glyphosate, was used to conduct intensive investigations into the mechanism of resistance. Neither a modification of the target enzyme EPSPS, nor of herbicide metabolism, contributed to the resistance in this case. However, translocation studies following foliar application revealed that, in the resistant biotype, glyphosate accumulated preferentially in the leaf tips, whereas in susceptible plants the accumulation was greater in the leaf bases and roots. These results suggested a shift of glyphosate transport in the resistant plants, from the phloem to the xylem system. Thus, it was speculated that the resistant biotype might have lost an efficiency to load glyphosate into the symplast, such that more

of the herbicide would remain in the apoplast and be translocated acropetally with the transpiration stream. Consequently, the concentration of glyphosate in the plastids of the sensitive meristematic tissues at the shoot base and in the roots would be reduced [96]. Meanwhile, a reduced glyphosate translocation within the plants and to the roots was confirmed for different *C. canadensis* and *L. rigidum* biotypes from different countries (for reviews, see Refs [79, 97]). It was speculated, that the membrane transporters were responsible for pumping the herbicide either into vacuoles or out of the chloroplast, such that the herbicide was unable to reach the target site [97].

1.3.1.4 **Multiple Resistance**

As defined above, multiple resistance means that more than one resistance mechanism occurs in a weed population or an individual plant. This can either mean that both target site-based and non-target-site-based mechanisms occur in the same biotype, or that a biotype is resistant to herbicides with different mechanisms of action. Multiple resistance can result in the resistance of a weed biotype to a very broad range of herbicide chemistries. Multiple resistance has been reported for several weed species, notably *Lolium rigidum*, *Alopecurus myosuroides*, *Kochia scoparia*, *Conyza canadensis*, and *Amaranthus rudis*. Such multiple resistance developed to a major extent especially in the Australian biotypes of *L. rigidum*, most likely as a result of agricultural conditions paired with biological characteristics of this weed (cross-pollinating species with a high genetic variability and seed production, and high plant numbers per area).

Multiple resistance can develop by selection with a single herbicide, or by selection with several herbicides that are used either sequentially or simultaneously. Moreover, cross-pollinating species may become multiple resistant when two individuals, each with a different resistance mechanism, undergo hybridization. An example of the selection of multiple resistance by a single herbicide (the ALS inhibitor chlorsulfuron) is the *L. rigidum* biotype WLR1. As the main mechanism of resistance, this biotype had an ALS with reduced sensitivity to chlorsulfuron, sulfometuron and imazamethabenz, and as additional mechanism an enhanced metabolism of chlorsulfuron [98]. Extreme cases of multiple resistance, due to an application history of many herbicides, were reported from Australia for several *L. rigidum* biotypes. For example, biotype VLR69 possessed the following mechanisms: An enhanced metabolism of ACCase-inhibiting herbicides; a resistant form of the ACCase enzyme; an enhanced metabolism of the ALS-inhibitor chlorsulfuron; and also a resistant form of the ALS enzyme in 5% of the population [43].

The selection of multiple resistance following the sequential use of different herbicides has been described for a biotype of *K. scoparia* from North America. In this case, many years of triazine usage resulted in the selection of a biotype with target-site resistance of the D1 protein in PS II. Following the subsequent use of ALS inhibitors, a point mutation in the gene encoding for ALS was selected in addition, which made this biotype target-site-resistant also to SUs and IMIs [69].

Some *Lolium* populations from Australia and South Africa have shown both target-site as well as a reduced translocation to glyphosate [79]. Further examples

of weed species and biotypes with multiple resistance mechanisms have been described in various reviews, and also in the database of the International Survey of Herbicide-Resistant Weeds (I. Heap, 2010, personal communication, *http://www.weedscience.com*) [19, 20, 22].

Clearly, multiple resistance leads to complex patterns of broad herbicide resistance, particularly in cross-pollinating weed species. This places a serious restriction on the remaining options for chemical weed control in agricultural practice.

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