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Herbicide Resistance Action Committee (HRAC): Herbicide Classification, Resistance Evolution, Survey, and Resistance Mitigation Activities

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1.1 Introduction

Crop losses and quality depreciation, due to harmful organisms, especially weeds, can be substantial and can be significantly reduced or even prevented by crop protection measures [1]. In combination with agronomic measures, herbicides are necessary tools of weed control in modern crop production systems; however due to natural selection process, herbicide-resistant weed populations can evolve rapidly [2].

The first cases of herbicide resistance (HR) were reported in 1957 on wild carrot (*Daucus carota*) resistant to 2,4-D [3]. Then during the second half of the 1970s, new cases were reported. Since then, resistance of mono- and dicotyledonous weeds to herbicides has become an increasing problem worldwide [4].

In September 2018, the International Survey of herbicide-resistant weeds (http://weedscience.org/; [4]) recorded 495 unique cases (species × site/mode of action (MoA)) of herbicide-resistant weeds globally, representing 255 species (148 dicotyledonous and 107 monocotyledonous) [4]. Weeds have evolved resistance to 23 of the 26 known herbicide sites/MoA and to 163 different herbicides. Herbicide-resistant weeds have been reported in 92 crops in 70 countries [4]. The relatively constant increase in the number of new cases of resistance since about 35 years accounts for the increasing importance of HR in weed control in the major agricultural regions (Figures 1.1 and 1.2).

During the period 1970–1990, a significant number of documented cases of resistance concerned the triazine resistance. The introduction of new herbicides with different MoAs that resulted in the evolution of new resistance cases related to acetolactate synthase (ALS) and acetyl-CoA carboxylase (ACCase)-resistant weeds, especially in grass weeds present in cereal-based cropping systems, has been reported (Figure 1.3).

Since 1996, the introduction of glyphosate-tolerant maize, soybean, and cotton in North and South America and the extensive and repeatedly use of glyphosate

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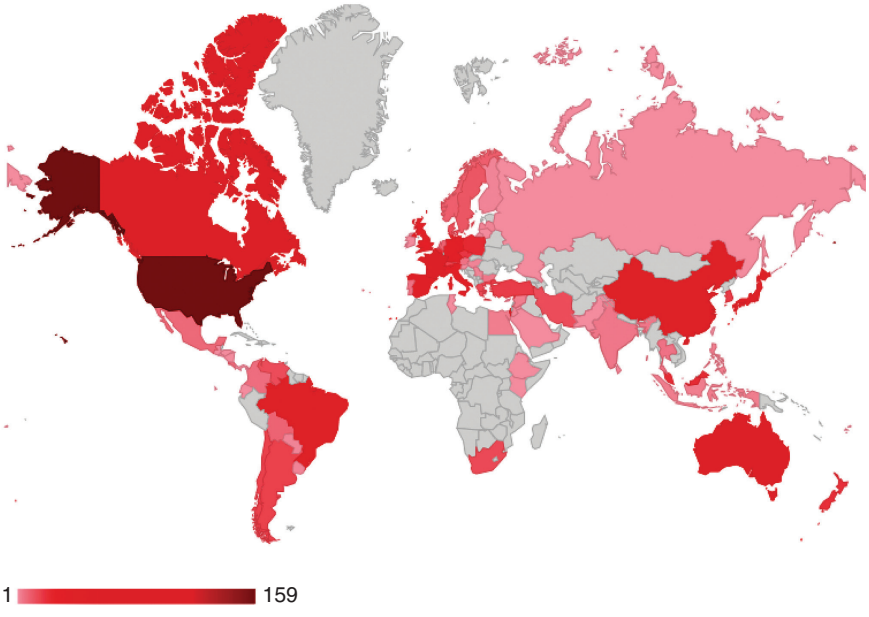

Figure 1.1 World map of the number of unique resistance cases reported per country. *Source:* Reproduced with permission of Heap 2017 [4].

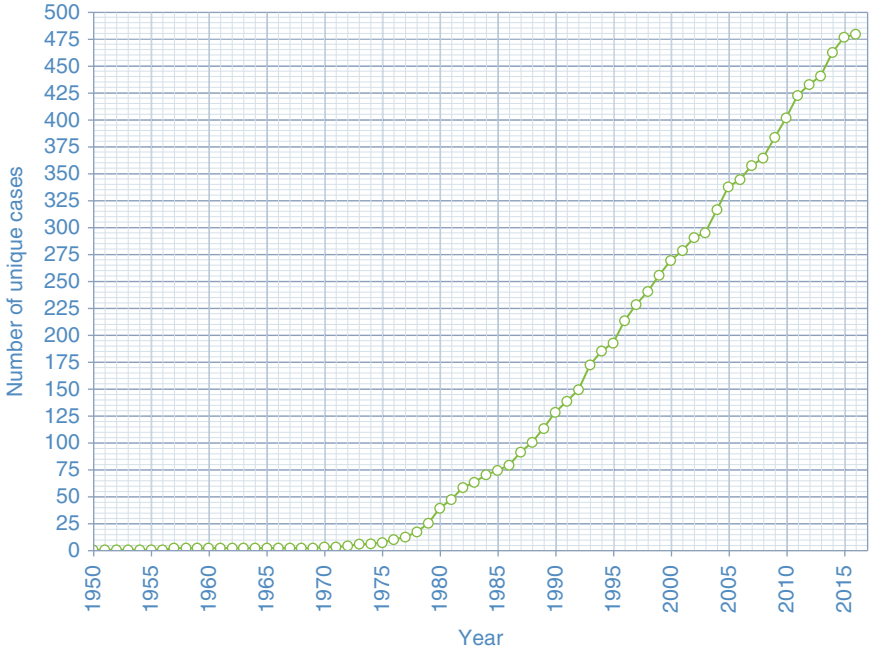

Figure 1.2 The recent chronological increase in the number of herbicide-resistant weeds worldwide. *Source:* Reproduced with permission of Heap 2017 [4].

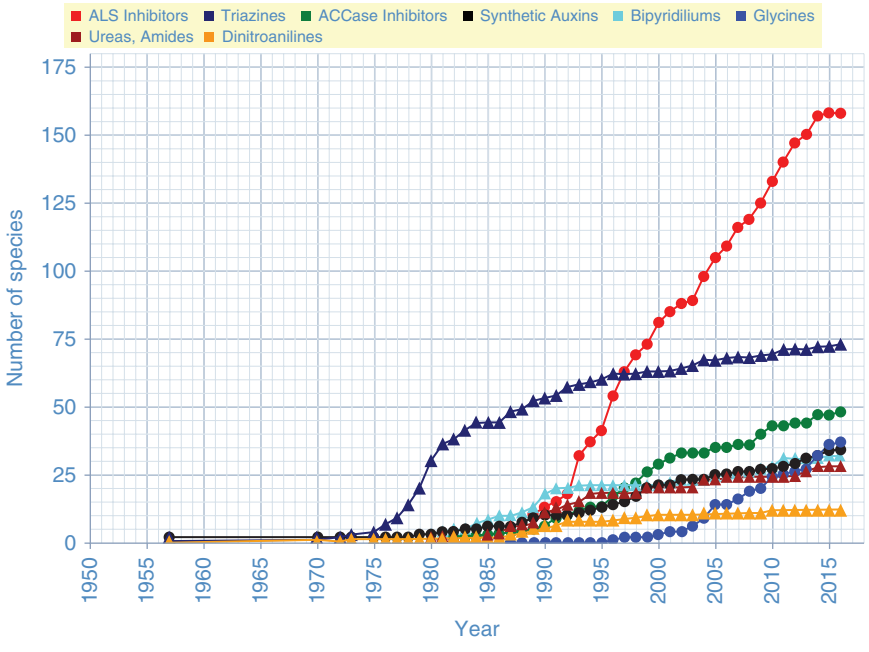

Figure 1.3 The recent chronological increase in the numbers of herbicide-resistant weeds for different herbicide classes reported by site/mode of action. *Source:* Reproduced with permission of Heap 2017 [4].

from presowing to posttreatments during the crop cycles have resulted in a strong increase of glyphosate resistance cases [4] as discussed by Heap and LeBaron [5].

1.2 HRAC Herbicide Classification System

The Global Herbicide Resistance Action Committee (HRAC) group has established a classification system for herbicides based on their *targeted inhibited protein* (*site of action*) or, when not defined, *their MoA*, i.e. *similarity of induced symptoms*, like inhibition of microtubule assembly. In addition for each site/MoA, herbicides were grouped in different chemical classes when appropriate (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) and the 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 some misunderstanding on the global level. Therefore, it was considered that one common global system would be highly desirable for all users in order to unequivocally define the differences between the different chemical classes of herbicides. This classification system is aiming to give support and advices to all users of herbicides in terms of chemical weed control management, in particular

Table 1.1 HRAC classification system in comparison to Weed Science Society of America (WSSA) and Australian code system.

Site/mode of action	Chemical family	HRAC Group	WSSA group[a)]	Australian group[a)]
Inhibition of acetyl-CoA carboxylase (ACCase)	Aryloxyphenoxypropionate, cyclohexanedione, phenylpyrazoline	A	1	A
Inhibition of acetolactate synthase (ALS) also named 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, phenylcarbamate	C1	5	C
Inhibition of photosynthesis at PS II	Urea, amide	C2	7	C
Inhibition of photosynthesis at PS II	Nitrile, benzothiadiazinone, phenylpyridazine	C3	6	C
Photosystem I-electron diversion	Bipyridylium	D	22	L
Inhibition of protoporphyrinogen (PPO)	Diphenyl ether, phenylpyrazole, *N*-phenylphthalimide, thiadiazole, oxadiazole, triazolinone, oxazolidinedione, pyrimidindione, other[d)]	E	14	G
Inhibition of 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, diphenyl ether, urea (also C2)	F3	11	Q
Inhibition of 1-deoxy-D-xylose-5-phosphate synthase (DOXP synthase)	Isoxazolidinone	F4	13	Q
Inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)	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, arylaminopropionic acid	K2	23	E
Inhibition of very-long-chain fatty acid synthesis (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	Thiocarbamate, phosphorodithionate	N	8	J
	Benzofuran	N	16	J
	Chlorocarbonic acid	N		J
Synthetic auxins (action like indole acetic acid)	Phenoxy-carboxylic acid, benzoic acid, pyridine carboxylic acid, quinolone carboxylic acid, and other[d]	O	4	I
Inhibition of auxin transport	Phtalamate, semicarbazone	P	19	P
Unknown: While the mode of action of herbicide in group Z is unknown, it is likely that they differ between themselves and from other groups	Pyrazolium	Z	26	
	Organoarsenical	Z	17	Z
	Other[d]	Z	26	Z

a) Not all chemical classes are classified.
b) Proposed.
c) Proposed by WSSA.
d) Includes additional molecules that belong to the same site of action (SoA) (e.g. PPO) but have no special chemical family such as "thiadiazole or oxazolidinedione."
Source: Adapted from Ref. [4].

in defining proper strategies of herbicide site/MoA rotation adapted to each cropping system, to achieve the best weed control and mitigate the evolution of HR.

The classification system describes not only the chemical family belonging to a specific site of action/MoA but also all compounds (via their common names) belonging to each family. This is shown in Table 1.2 for selected site/MoAs, e.g. "inhibition of dihydropteroate (DHP) synthase," "inhibition of microtubule assembly," "inhibition of mitosis/microtubule organization," "inhibition of very-long-chain fatty acid synthesis (VLCFAs; inhibition of cell division)," and "inhibition of cell wall (cellulose) synthesis." More details can be found in the intranet HRAC site (http://hracglobal.com/tools/classification-lookup). In addition, a synthetic map "The World of Herbicides" shows all chemical structures of the different herbicides grouped by chemical classes and their site/MoA (http://hracglobal.com/tools/world-of-herbicides-map).

1.3 Herbicide Resistance Survey

1.3.1 Herbicide Resistance Definition

HR is defined by WSSA as the acquired ability of weed populations to survive a herbicide application that previously was known to control them (http://wssa.net/).

1.3.2 Herbicide Resistance Population Evolution and Integrated Weed Management

At population level, HR is an evolutionary process based on the selection of few naturally resistant individuals present in a given population (1 by 1 million or 10 million) and selected by the application of a given herbicide. More treatments are repeated with a given herbicide (same site/MoA); higher will be the selection pressure and faster will the resistance to that herbicide evolve. This is even reinforced in simplified cropping systems based on simple crop rotation with any and/or no tillage [6]. HR evolution can be mitigated using recognized best management practices [7]. This involves chemical measures like the rotation of herbicides with different sites/MoA, in season (sequential application) or between seasons, and the use of herbicide mixtures with different sites/MoAs, effective on the targeted weeds. In addition the full recommended herbicide rate has to be used [2]. Furthermore, in combination with herbicides, the introduction of nonchemical measures like crop rotation, soil management and tillage, delayed sowing, and use of cover crops or harvest seed destructor are contributing to define the more appropriate integrated weed management (IWM) strategy to control weed development and contain locally the increase of the soil weed seed bank [2, 8, 9] at reasonable costs [8, 10].

The planting of herbicide-tolerant crops that has increased from 1.7 mio ha in 1996 to about 185.1 mio ha in 2016 has changed the farmers' weed control strategy [11]. These systems, in particular those based on glyphosate tolerance, have

Table 1.2 Selected group of the HRAC classification system with examples of the active ingredients, which are not mentioned in following chapters.

Site/mode of action	Chemical family	Active ingredient	HRAC group	WSSA group[a)]	Australian group[a)]
Inhibition of dihydropteroate (DHP) synthase	Carbamate	Asulam	I	18	R
Inhibition of microtubule assembly	Dinitroaniline	Benefin (benfluralin), butralin, dinitramine, ethalfluralin, oryzalin, pendimethalin, trifluralin	K1	3	D
	Phosphoroamidate	Aminoprophosmethyl, butamiphos			
	Pyridyne	Dithiopyr, thiazopyr			
	Benzamide	Propyzamide (pronamide tebutam)			
	Benzoic acid	DCPA (chlorthal dimethyl)			
Inhibition of mitosis/microtubule organization	Carbamate	Chlorpropham, propham, carbetamide	K2	23	E
	Arylaminopropionic acid	Flamprop-*m*	K2	25	Z
Inhibition of very-long-chain fatty acid synthesis (VLCFAs), inhibition of cell division	Chloroacetamide	Acetochlor, alachlor, butachlor, dimethachlor, dimethenamid, metazachlor, pethoxamid, pretilachlor, propachlor, propisochlor, thenychlor	K3	15	K
	Acetamide	Diphenamid, napropamide, naproanilide			
	Oxyacetamide	Flufenacet			
		Mefenacet			
	Tetrazolinone	Fentrazamide, ipfencarbazone			
	Isoxasoline[b)]	Pyroxasulfone			
	Other	Anilofos, cafenstrole, piperophos			

(Continued)

Table 1.2 (Continued)

Site/mode of action	Chemical family	Active ingredient	HRAC group	WSSA group[a)]	Australian group[a)]
Inhibition of cell wall (cellulose) synthesis	Nitrile	Dichlobenil, chlorthiamid	L	20	O
	Benzamide	Isoxaben		21	
	Triazolocarboxamide	Flupoxam		28	
	Alkylazine	Indaziflam, triaziflam		29[c)]	

a) Not all chemical classes are classified.
b) Proposed.
c) Proposed by Weed Science Society of America (WSSA).
Source: Adapted from Ref. [4].

provided the growers favorable economic advantages as well as more cropping flexibility. In addition, the decrease of tillage has contributed to soil preservation. In most cases, the reliance on one herbicide has reduced the number of applications and the number of sites/MoA used. In 2004, glyphosate was applied on 87% of the whole acreage of soybean in the United States, whereas it was 25% in 1996 [12]. No other herbicide was applied on more than 7% of the acreage. This trend showed the same evolution for soybean, maize, and cotton and reached a peak in 2015 (H. Strek and A.G. Bayer, personal communication). The unfortunate consequence of these simplified cropping systems is the evolution of HR, in particular to glyphosate, in the main driving weed species [2, 13].

It was suspected that weed population shift will have a bigger impact on the cropping system than the selection of resistance weeds [14, 15]. Nevertheless several studies showed that weed resistance evolved faster than expected [16, 17]. Intensive soil cultivation techniques and stubble burning were common weed control techniques in many agriculture areas by the past as reported, e.g. for *Echinochloa* spp. [18]. The limitation or ban of stubble burning caused increasing weed density and an increased soil seed bank and therefore favored the selection of HR by the necessity to have to control much bigger populations. The particular case of Australia, where limited solutions remain available to control ryegrass (*Lolium rigidum*) populations resistant to several herbicides representing different sites/MoA, has shown the development, the implementation, and the increasing use of new technologies like balling of straw by trailing baler attached to the harvester or destroying of weed seeds physically during the harvesting operation ("Rotomill") [9]. This last technology, harvest weed seed control, is now tested or in early implementation phase in the United States to control glyphosate-resistant palmer amaranthus (*Amaranthus palmeri* S. Wats) in soybean crop [19].

The increasing farm size to be managed, as well as economic pressure to farmers to maintain some profitability, and the changing environmental influence, like soil erosion or water availability, have led to the adoption of no-till practices. Modeling studies showed that the risk of adopting no-tillage and the evolution of HR can be reduced by alternating between minimum and no-tillage systems or by alternating herbicides with different sites/MoA [20]. A spatial and temporal study of resistance evolution of black grass (*Alopecurus myosuroides*) to ALS inhibitors in cereal systems in Germany during six years (2010–2016) clearly showed that the major factors mitigating the resistance evolution are agronomic factors, i.e. crop rotation (in particular the presence of spring crop in the rotation), tillage, or delayed sowing date [21].

Long-term studies in resistant ryegrass and blackgrass to ACCase and ALS inhibitors showed also that when resistant seeds are present in high frequency in the soil seed bank, they remained significant and did not disappear even after several years of proper resistance management [22–24]. Although the total seed density can be decreased, the frequency of resistant plants remained unchanged [23, 24].

Neither cropping systems nor single weed management tactic can solve specific weed problem on a long-term basis. In an IWM approach, the use of all possible practices, chemical and nonchemical techniques, in the right

combination(s) adapted to the local situation, should be the long-term goal for sustainable agricultural production and is promoted by the HRAC (http://hracglobal.com/).

1.3.3 Herbicide Resistance Mechanisms

One mission of the HRAC is to collect information helping to define HR management strategies. In that respect the knowledge of resistance mechanisms is one important aspect. Resistance mechanisms are multiple and can be grouped in the following categories [25]:

- *Target-site resistance*: This is due to reduced (or even lost) ability of the herbicide to bind to its target protein (site of action). The effect usually relates to an enzyme with a crucial function in the plant cell metabolism (e.g. ALS), or to a component of a photosystem (PS) including the electron transport, or to a component of the plant cell integrity (e.g. microtubules). Target-site resistance can be due to the presence of a mutation of the gene encoding the target protein. It can also occur when the target protein is overproduced due to increased transcription/translation or when its gene is present in multiple copies due to gene duplication, observed in the resistant plants as for the *5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)* gene encoding the target for glyphosate. The solution to overcome these resistance mechanisms consists in using a herbicide with another site/MoA for which the weed population has not developed a resistance.
- *Nontarget-site resistance*: This is caused by mechanisms that reduce the amount of active herbicide reaching the target site. An important mechanism is the ability of the resistant plant to detoxify the herbicide or enhanced metabolic resistance (EMR). Reduced uptake or translocation, or vacuole sequestration, may also lead to insufficient amount of active compound reaching the target site. Nontarget-site resistance is not related to the site/MoA of herbicides, but to their chemical structures. It can be broad spectrum, related to chemistries with different sites/MoA or novel chemistries not yet used. Overcoming it might be difficult, and all efforts to mitigate its evolution have to be done.

Two other definitions are important:

- *Cross-resistance*: In this case, a single resistance mechanism causes resistance to several herbicides. The term target-site cross-resistance is used when these herbicides bind to the same target site, whereas nontarget-site cross-resistance is due to a single nontarget-site mechanism (e.g. EMR) that entails resistance to several herbicides either with the same or with different sites/MoA.
- *Multiple resistance*: In this situation, two or more resistance mechanisms are present within individual plants or within a plant population.

1.3.3.1 Target-site Resistance

Cases analyzed to date have shown that HR is very frequently based on target-site mutations. Within the past 45 years, weed species have developed target-site resistance to most known herbicide chemistries. Those of major importance are discussed in the following subsections.

1.3.3.1.1 Inhibitors of Photosystem II (PS II) Early reports on resistance of weeds to 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 that is known to be the Qb site of the D1 protein in the PS II reaction center (EC 1.10.3.9). The triazine herbicides bind to this site, thereby inhibiting the photosynthetic electron flow. In the resistant mutants, the triazine binding is significantly 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 times higher for chloroplasts from an atrazine-resistant mutant than for those from wild-type plants [26].

In several cases, the mutants of weed species with target-site resistance to triazines showed 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 populations 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 populations [27, 28].

The D1 protein is encoded by the chloroplast *psbA* gene, which is highly conserved among higher plants, algae, and cyanobacteria [29]. In almost all investigated cases of the resistance of field-grown weed species to triazines, resistance was attributed to a point mutation in the *psbA* gene with a resultant Ser264 by a Gly change in the herbicide binding pocket 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, they are still active on the D1 protein bearing the S264G mutation, suggesting that the binding sites of triazines and phenylureas are not identical but overlapping [30, 31]. This can be explained by a hydrogen bond provided by the S264, necessary for the binding of the triazines, in particular atrazine, which is not necessary for the binding of the phenylureas.

In 1999 the replacement of S264 by a threonine was reported in *Portulaca oleracea* [32]. This mutant was resistant to both phenylureas (linuron and diuron) and triazines (in particular atrazine). This suggested that the S264T mutation modified the conformation of the herbicide binding pocket at the D1 protein conferring resistance to a broader chemical spectrum of PS II inhibitors. Another point mutation was described in the *psbA* gene of *Poa annua* resistant to both diuron and metribuzin, i.e. the replacement of valine 219 by an isoleucine in the D1 protein. That substitution conferring resistance to a broad spectrum PS II inhibitors (phenylureas and triazines) was found to not affect significantly the plant fitness in contrast to the amino acid substitutions found in position 264 [33].

1.3.3.1.2 Inhibitors of Acetyl-CoA Carboxylase (ACCase, EC 6.4.1.2) The enzyme ACCase 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

Poaceae family (grasses) have in their plastids a homomeric, multifunctional form of ACCase with the following domains: biotin carboxyl 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 by separated genes. In addition all di- and monocotyledonous (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*), which determine their selective lethal effects only on grass species. To date, there are three different chemical classes of ACCase inhibitors, the aryloxyphenoxypropionates (APPs or FOPs), the cyclohexanediones (CHDs or DIMs), and the phenylpyrazoline (PPZ or DEN).

Until now, target-site resistance in population not controlled by ACCase inhibitors has been reported for several grass weed species of economic importance. The earliest cases of target-site-based resistance were reported for populations of *Lolium multiflorum* from Oregon, USA [34], and for *L. rigidum* from Australia [35].

ACCase prepared from a resistant *L. multiflorum* population that had been selected by the field use of diclofop-methyl-methyl was inhibited by the APPs diclofop-methyl-methyl, 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 prepared from a susceptible population. There was no cross-resistance to the CHD herbicides sethoxydim or clethodim [36]. ACCase resistance was subsequently also confirmed for *L. multiflorum* populations from other countries. In a resistant population selected by diclofop-methyl-methyl in Normandy, the resistance factor (ratio of the IC_{50} for ACCase from the resistant to the IC_{50} for ACCase from the susceptible population) was 19 for diclofop-methyl-methyl and 5 for haloxyfop, but only 2 for the CHD clethodim and sethoxydim [37]. Interestingly, a different ACCase resistance pattern was found for the resistant *L. multiflorum* population Yorks A2, although field selection was apparently also mainly by diclofop-methyl-methyl. The resistance factors were 3 and 9, respectively, for the APPs diclofop-methyl-methyl and fluazifop, but 20 for the CHD herbicide cycloxydim [38].

The first populations 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 chemical classes. However, regardless of whether the selection was by an APP or a CHD compound, the level of resistance in these populations was higher toward APP than toward CHD herbicides. The ACCase resistance factors were 30–85 for diclofop-methyl-methyl, >10–216 for haloxyfop, and 1–8 for sethoxydim [35, 39, 40].

Populations with target-site-based resistance to ACCase inhibitors were also selected in wild oat species (*Avena fatua*, *Avena sterilis*). The resistance patterns related to the different ACCase-inhibitor herbicides were found to be variable between populations. It was proposed that this effect was due to different point mutations, each being associated with a characteristic resistance pattern [41]. However, another reason might be the frequency of homozygote- and heterozygote-resistant and susceptible plants within the tested populations or

the presence of additional uncharacterized resistance mechanisms in the populations.

Genetic studies of two *A. myosuroides* populations from the United Kingdom (Oxford Al and Notts Al) highly resistant to fenoxaprop, diclofop-methyl-methyl, fluazifop, and sethoxydim revealed that the target-site resistance in the two populations was monogenic and nuclear inherited, with the resistant allele showing complete dominance [42].

Target-site-based resistance to ACCase has also been reported for several other grass weeds, including *Setaria viridis*, *Setaria faberi*, and *Digitaria sanguinalis*, with different cross-resistance pattern related to the different APPs and CHD ACCase inhibitor herbicides [40, 43]. Based on that, it was postulated that the two chemical classes of ACCase inhibitors 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 [44]. 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 substitution in the CT domain of the enzyme [45, 46]. In addition, the results of inheritance studies suggested that the alteration of the ACCase in *L. rigidum* was determined by a single nuclear dominant gene. The same substitution was found in ACCase inhibitor-resistant *A. fatua* [47], *A. myosuroides* [48], and *S. viridis* [49]. The mutated leucine ACCase allele in the *Setaria* species was found to be dominant, and no alteration was observed on the ACCase function of the mutant plants. 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. [48] showed that the leucine found in the plastidic homomeric ACCase of mutated resistant grass weeds is also found in the heteromeric plastidic enzyme of nongrass species and in the cytosolic homomeric enzymes that are "naturally" insensitive (resistant) to these herbicides. Therefore the selective action of ACCase-inhibiting herbicides appears, at least in part, to be determined by the primary structure of the ACCase protein [40].

Further studies conducted in France by Délye and coworkers with *A. myosuroides* populations from different locations 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 herbicide chemical classes at the ACCase enzyme [50, 51]. Meanwhile, different point mutations were identified in different grass weed species that gave rise to insensitive ACCase due to the substitution of one amino acid: In addition to the Ile1781Leu, the Trp1999Cys, Trp2027Cys, Ile2041Asn, Ile2041Val, Asp2078Gly, Cys2088Arg, and Gly2096Ala could be identified ([52, 53]; for a review, see Ref. [25]). The Ile1781Leu is the most common substitution observed, and Ile1781Leu and Asp2078Gly confer resistance to mainly all ACCase-inhibitor 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 and populations. It can depend on the mutation present as

heterozygous or homozygous, especially for polyploidy species, and the presence in addition to target-site mutation(s) of other resistant mechanisms, in particular nontarget-site resistance mechanisms, e.g. inhibition of uptake or transport or the detoxification of the herbicide(s).

Comparison of the sequences of plastidic ACCase around the critical codons in 29 different species [54] showed that in *P. annua* and *Festuca rubra*, a leucine residue formed at position 1781, while the wild type of all other grass species had an isoleucine in that 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 presence of Ile at the position 1781 in the ACCase sequence can be considered crucial for the sensitivity of plants to ACCase-inhibitor herbicides.

A different mechanism of target-site resistance to ACCase inhibitors was identified in a *Sorghum halepense* population from Virginia, USA, which had been selected in the field by quizalofop applications. The specific activity of ACCase in the resistant population was found to be two- to threefold greater than in susceptible plants. These results and the absence of mutation suggested that an overproduction of ACCase was the mechanism that conferred a moderate level of resistance to these herbicides. To date, however, this has been the only reported case for this mechanism in a naturally occurring population [55].

1.3.3.1.3 Inhibitors of Acetolactate Synthase (ALS/AHAS, EC 2.2.1.6) 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 (AHAS). 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 populations that were being reported in the early 1990s were selected by chlorsulfuron or metsulfuron-methyl in wheat-growing areas, or by sulfometuron-methyl in noncrop areas. While the resistance of *L. rigidum* to ALS inhibitors was attributed to an enhanced herbicide metabolism (EMR) [56], it was shown, for *Lolium perenne* and dicotyledonous species such as *Stellaria media*, *Kochia scoparia*, *Salsola iberica*, and *Lactuca serriola*, that resistant populations had a mutated ALS with a reduced susceptibility to ALS-inhibiting herbicides [57–59]. 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 4- to 50-fold in the resistant populations. Smaller increases, from about two- to sevenfold, were determined in the same populations for the IMI herbicide, imazapyr [59].

Later, ALS inhibitors were developed for selective use in rice, and this led to the selection of resistant rice weed populations. A population 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 [60]. In rice fields in Japan, a population 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 population; this suggested that the resistance was due to an altered ALS enzyme [61].

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 substitutions 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) and Asp376, Arg377, Trp574, Ser653, and Gly654 (located near the carboxy-terminal end) [62, 63]. For more details see also Chapter 2.1 and the mutations reported in the International Database [4, 25].

When in the ALS of a *L. serriola* population, which was highly resistant to SUs and moderately resistant to IMIs, Pro197 was substituted by His, and the pyruvate-binding domain on the ALS enzyme was found not to be altered by the mutation [64]. 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 [65]. 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 [66]. The latter substitution was also detected in resistant populations of several other dicotyledonous weed species.

In a population of *Amaranthus retroflexus* from Israel, resistance was caused by a change of Pro197 to Leu. This population exhibited cross-resistance to SU, IMIs, and TPs and also to pyrithiobac-sodium *in vivo* and on the ALS enzyme level [67]. In *Amaranthus tuberculatus*, Ser653 was found to be exchanged by Thr or Asn; such mutants were only resistant to IMIs [68].

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 populations with mutated ALS showed, in most cases, no reduction (or only a negligible reduction) of fitness (for reviews see Refs. [62, 69]), whereas others [70] found for the Trp574Leu substitution in *Amaranthus powellii* a substantial fitness cost. Possible fitness costs of the resistance alleles were reviewed [71]. It has to be stressed that in many studies related to target-site analyses, nontarget-site mechanisms, in particular herbicide detoxification, were not studied. The presence of additional resistance mechanisms would change, at least partly, the conclusion(s) related to the analyses of multiresistance, cross-resistance, and fitness costs.

1.3.3.1.4 5-Enolpyruvylshikimate-3-phosphate Synthase (EPSPS, EC 2.5.1.19): Target of Glyphosate Glyphosate has become the most important herbicide worldwide and is widely used as nonselective herbicide in different indications as well 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 [2, 4, 5]. Glyphosate inhibits the chloroplast enzyme 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 [72]. Even in 1997, following the introduction of transgenic crops, it was not believed that glyphosate resistance in weeds would ever become a major problem [73]. 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 many species and countries [4]. Both target-site and nontarget-site resistance mechanisms have evolved in different weed species. In resistant accessions of *Eleusine indica* from Malaysia, a point mutation of the target enzyme EPSPS was observed. By using PCR amplification and the sequence analysis of an EPSPS fragment, an exchange of Prol06 by Ser was found in two resistant accessions and an exchange of Pro106 by Thr in a third resistant accession [74, 75]. This mutation Pro106, with exchanges by Ser, Thr, and Ala, was also found in different *L. rigidum* and *L. multiflorum* populations from different locations in Australia, the United States, Chile, and South Africa (for a review, see Ref. [76]). 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 [76]. Recently in *E. indica* a double mutation Thr102Ile and Pro106Ser was discovered conferring high resistance to glyphosate. The evolution of this two mutations in crop fields under glyphosate selection is likely a sequential event, with the P106S mutation being selected first and fixed, followed by the T102I mutation to create the highly resistant EPSPS [77]. More recently EPSPS gene amplification, up to more than 100 copies, in *Amaranthus* spp. was identified in highly glyphosate-resistant populations [78, 79]. The overexpression of the EPSPS was causing the resistance to glyphosate. Further studies have confirmed the presence of high EPSPS copy number in *L. perenne*, *K. scoparia,* and *Bromus diandrus*, glyphosate-resistant populations [80–82].

1.3.3.1.5 Protoporphyrinogen Oxidase (PPO, EC 1.3.3.4) Protoporphyrinogen oxidase (PPO) is an enzyme in the chloroplast cell that oxidizes protoporphyrinogen IX (PPGIX) to produce protoporphyrin IX (PPIX). PPIX is important because it is a precursor molecule for both chlorophyll (needed for photosynthesis) and heme (needed for electron transfer chains). Inhibitors of the oxidase enzyme, however, do more than merely block the production of chlorophyll and heme. The inhibition of PPO by inhibitors also results in forming highly reactive

molecules that attack and destroy lipids and protein membranes. When a lipid membrane is destroyed, cell becomes leaky and cell organelles dry and disintegrate rapidly [83].

PPO Inhibitors have limited translocation in plants and sometimes are referred to as contact herbicides. PPO Inhibitors injure mostly broadleaf plants; however, certain PPO Inhibitors have some activity on grasses. PPO Inhibitors usually burn plant tissues within hours or days of exposure. PPO Inhibitors used in the United States belong to eight different chemistries including diphenyl ethers, *N*-phenylphthalimides, oxadiazoles, oxazolidinediones, phenylpyrazoles, pyrimidinediones, thiadiazoles, and triazolinones. These herbicides are used to control weeds in field crops, vegetables, tree fruits and vines, small fruits, nurseries, lawns, and industry. Recent works have shown the evolution of different mutations, ΔG210, Arg98Gly, Arg98Met, and Arg98Leu in *A. tuberculatus*, *A. palmeri*, and *Ambrosia artemisiifolia* [84–86].

1.3.3.2 Nontarget-site Resistance by Enhanced Metabolic Detoxification

Plants dispose of enzyme systems that catalyze the metabolic conversion of xenobiotic, including herbicides. The metabolites that usually are more polar than the parent compound are either nonphytotoxic at all or have a reduced phytotoxicity. 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 (several protein families) 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 [87].
- *Glutathione S-transferase (GST, EC 2.5.1.18):* 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 [88].

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. While 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 to its target site in sufficient amounts to cause irreversible herbicidal effects will be prevented. If weed biotypes with an improved ability for herbicide detoxification, comparable with the tolerant crop species, occur in a population, they will survive herbicide application and will thus be selected. This enzyme system-based resistance mechanism is no more related to the target of the herbicide (i.e. its site/MoA) but rather to its chemical structure and therefore causes unexpected cross-resistance to herbicides from different chemical classes with different sites/MoA as well to herbicides that have not been so far used.

To date, many populations in several weed species have been described for which HR was related to an enhanced metabolic herbicide detoxification. An early report from Christopher et al. [89] stated that the excised shoots of *L. rigidum* SLR 31 population from Australia, which was resistant to diclofop-methyl-methyl, exhibited a cross-resistance to the SUs chlorsulfuron, metsulfuron-methyl, and triasulfuron. Although the metabolite pattern of chlorsulfuron was identical in the resistant population and a susceptible population, the resistant population metabolized faster the herbicide. The pathway of chlorsulfuron detoxification in *L. rigidum* was similar to that described for wheat with ring hydroxylation being followed by glycosyl conjugation. The time course of chlorsulfuron metabolism in the *L. rigidum* population SR 4/84 (resistant to diclofop-methyl-methyl 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 hours) than in the shoots (4 hours) and was reduced in the resistant population to 3 and 1 hours, 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 population [56]. Nevertheless, it is so far not shown at the gene level that the respective Cyt P450 and glycosyltransferase are encoded by homologous genes in both the crops and the weeds.

Two other *L. rigidum* populations 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 populations were resistant to triazines and, despite the field selection by atrazine, resistance was more pronounced to the structurally related simazine. Furthermore, both populations were resistant to chlorotoluron, though only VLR69 had previously been exposed to phenylureas. The results of analytical studies revealed that, in both resistant populations, 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 (1-ABT), an inhibitor of cytochrome P450 monooxygenases, suggested an increased activity of cytochrome P450 monooxygenases in the resistant populations [90, 91]. The mechanism of phenylurea resistance of *L. rigidum* populations from Spain has been studied [92]. A population (R3) selected in the field by applications of diclofop-methyl-methyl, and isoproturon or 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 cytochrome 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. In other studies, several populations of *L. multiflorum* from the United Kingdom with resistance to diclofop-methyl-methyl have been analyzed [38]. While one population had an insensitive ACCase, the resistance of three other populations could be attributed to an enhanced metabolism of this herbicide.

The resistances 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* [93], whereas in *P. minor* the cytochrome P450 monooxygenase was most likely involved in the enhanced detoxification of isoproturon [94].

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, resistance due to an enhanced herbicide metabolism has also been reported. Two populations – Peldon Al and Lincs El – with *in vivo* resistance factors to isoproturon of 28 and 2.6, respectively, were shown to metabolize this herbicide faster than a susceptible reference population 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 populations appeared to be the formation of a hydroxymethylphenyl metabolite [95].

The same populations, Peldon Al and Lincs El, 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 El was more resistant than Peldon Al. 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* populations, the GST activities toward fenoxaprop were shown to be increased, when compared with a susceptible population. 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 populations, 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 populations did not permit definite conclusions to be drawn [96]. Further work overexpressing in *Arabidopsis*, a GST overexpressed in herbicide-multiresistant *A. myosuroides*, Peldon population suggests its involvement in resistance to herbicides [97]. Recently, a range of European *A. myosuroides* populations with resistance to fenoxaprop has been investigated [98], and several of these populations – notably one from Belgium – were shown to detoxify the herbicide at an increased rate. The population from Belgium also had the highest GST activity toward the unspecific substrate chlorodinitrobenzene (CDNB) although GST activity toward the herbicide was not tested.

Studies on the mode of inheritance of metabolic HR in *A. myosuroides* and *L. rigidum* postulated that more than one gene is involved in cytochrome P450 metabolism-based resistance in weed populations [99–101]. Recent works using transcriptome analyses have allowed to make steps forward in the identification of several genes involved in herbicide detoxification [102–104]. 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

[71]. 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 population of *Echinochloa colona* resistant to propanil was found; subsequent enzyme tests with extracts from this population revealed an almost threefold higher activity of aryl acylamidase in the resistant than in a susceptible population. Based on these findings, it was concluded that resistance of the *E. colona* population is related to an enhanced propanil detoxification [105].

The HPPD inhibitors in particular the triketone chemistry (e.g. tembotrione and mesotrione) inhibit the oxidative decarboxylation and rearrangement of p-hydroxyphenylpyruvate (HPP) to homogentisate (HGA), which inhibits the catabolism of tyrosine and results in a deficiency of plastoquinone and α-tocopherols (vitamin E) [106]. Recent data suggested that detoxification involving Cyt P450 monooxygenase is involved in mesotrione resistance of *A. palmeri* [107].

Recent development in genomics has brought new insight in the characterization of the genes encoding for the enzymes involved in herbicide detoxification. This new knowledge could contribute in the next years to find novel solutions to mitigate nontarget-site HR by detoxification.

1.3.3.3 Nontarget-site Resistance by Altered Herbicide Distribution

Cases of nontarget-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 were mainly carried out using resistant populations 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 population when administered after the herbicide. The results of these transport inhibitor experiments supported the involvement of a membrane transporter in paraquat resistance [108].

Translocation studies with two paraquat-resistant populations of *Hordeum leporinum* revealed that the basipetal transport of paraquat was much reduced compared with susceptible plants. It was concluded therefore that a resistance to paraquat was the result of a reduced herbicide translocation out of the treated leaves [109]. 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 [110].

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 a herbicide metabolism contributed to the resistance in this case. However, translocation studies following foliar application revealed that in the resistant population, 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 population 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 [111]. Meanwhile, a reduced glyphosate translocation within the plants and to the roots was confirmed for different *C. canadensis* and *L. rigidum* populations from different countries (for reviews, see Refs. [76, 112]). 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 [112].

Plants can develop resistance to synthetic auxin herbicides like 2,4-D or dicamba via transport inhibitor mechanisms or metabolism or other mechanisms as reviewed [113]. Recent data suggest that transport inhibition plays an important role of resistance of *Papaver rhoeas* to 2,4-D [114].

1.3.3.4 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 nontarget-site-based mechanisms occur in the same population or that a population is resistant to herbicides with different mechanisms of action. Multiple resistance can result in the resistance of a weed population to a very broad range of herbicide chemistries. Multiple resistance has been reported for several weed species (Figure 1.4), notably *L. rigidum*, *A. myosuroides*, *K. scoparia*, *D. insularis*, *C. canadensis*, *A. palmeri*, and *A. tuberculatus* (http://weedscience.org/). Such multiple resistance developed to a major extent especially in the Australian populations 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). Similarly, *Amaranthus* spp. have evolved multiple resistance in the simplified agronomic practices used in the United States, and the same trend can be observed in South America (soybean and maize crops) and in grasses in Europe (cereal-based cropping systems).

Multiple resistance can develop by selection with a single herbicide or 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

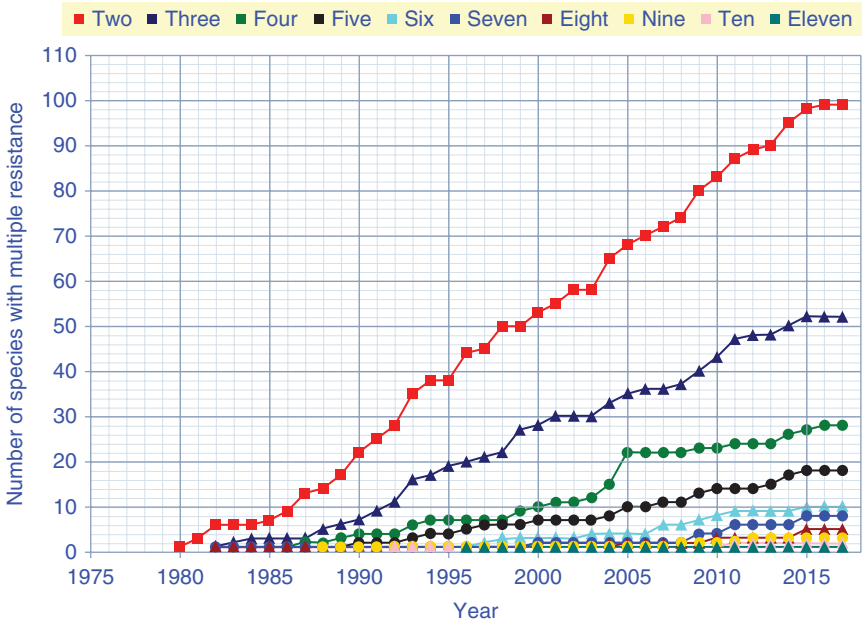

Figure 1.4 The recent chronological evolution of species for which populations showing resistance to multiple sites/modes of action. *Source:* Reproduced with permission of Heap 2017 [4].

chlorsulfuron) is the *L. rigidum* population WLRl. As the main mechanism of resistance, this population had an ALS with reduced sensitivity to chlorsulfuron, sulfometuron, and imazethabenz and as additional mechanism an enhanced metabolism of chlorsulfuron [115]. Extreme cases of multiple resistance, due to an application history of many herbicides, were reported from Australia for several *L. rigidum* populations. For example, population 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 [40].

The selection of multiple resistance following the sequential use of different herbicides has been described for a population of *K. scoparia* from North America. In this case, many years of triazine usage resulted in the selection of a population with target-site resistance of the Dl 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 population target site resistant also to SUs and IMIs [66].

Some *Lolium* populations from Australia and South Africa have shown both target site and a reduced translocation to glyphosate [76]. Further examples of weed species and populations with multiple resistance mechanisms have been described in various reviews and also in the database of the International Survey of Herbicide-Resistant Weeds [4, 116]. Clearly, multiple resistance leads to complex patterns of broad HR, particularly in cross-pollinating weed species. This

can cause a serious restriction on the remaining options for chemical weed control in agricultural practice.

1.3.4 Global Herbicide Resistance Action Committee (HRAC)

1.3.4.1 Missions and Goals

The Global HRAC (http://hracglobal.com/who-we-are/about) is an international body founded by the agrochemical industry, which helps to protect crop yields and quality worldwide by supporting efforts in the fight against herbicide-resistant weeds.

Herbicides are the primary economic means to control weeds, and they play a crucial role in helping humanity feed itself. The evolution of herbicide-resistant weeds is a serious problem facing the global agricultural community – they threaten the regions, economies, and livelihoods of farming families. But HR can be managed, and HRAC provides the information necessary to take a stand against herbicide-resistant weeds.

HRAC is dedicated to a cooperative approach to the management of herbicide-resistant weeds. By collecting, assessing, and sharing information on weed resistance, HRAC acts as a comprehensive and reliable source for the people who feed our growing world. The work done by the Global HRAC contributes to sustainable crop practices worldwide, which allow farming families to grow more food on less land and help preserve and protect our natural resources, in particular soils, for generations to come.

From rural communities to agriculture experts, HRAC provides the knowledge to protect the planet while winning the fight against HR.

1.3.4.2 Members, Organization, and Tasks

The Global HRAC is an industry-based group administrated by CropLife International (https://croplife.org/). The organization is operated by important members of the agrochemical industry:

- Arysta LifeScience
- BASF
- Bayer CropScience Division
- Corteva Agriscience
- FMC
- Makhteshim Agan/ADAMA
- Syngenta Crop Protection
- Sumitomo Chemical Company.

The Global HRAC supports the work of regional offices around the world. Global HRAC equips them with the resources they need to bring education on herbicide-resistant weeds to farmers, agronomists, industry members, and officials. Global HRAC also identifies and organizes working groups that tackle key HR challenges.

HRAC is supporting the International Survey of Herbicide-Resistant Weeds (http://weedscience.org/) and has set and is updating the Global Classification of

Herbicides (http://hracglobal.com/tools/classification-lookup) and the World of Herbicide Map (http://hracglobal.com/tools/world-of-herbicides-map).

Working groups are dedicated to provide comprehensive information on HR and management on particular topics (e.g. synthetic auxins, HPPD inhibitors) in order to propose the best strategies to mitigate the evolution of resistance. In addition, HRAC is working on the development of information on labels, so growers have the resources they need to make responsible herbicide decisions on their farms. In particular HRAC is proposing to any herbicide registrant to include the site/MoA numbers and guidelines in herbicide labels in the United States and other countries as appropriate. Moreover HRAC is recommending to follow best weed management practices as edited (http://hracglobal.com/files/Management-of-Herbicide-Resistance.pdf). Finally HRAC is working to develop and propose weed resistance mitigation strategies as well as resistance survey and diagnostics (http://hracglobal.com/files/Monitoring-and-Mitigation-of-Herbicide-Resistance.pdf).

HR is evolving because of economic pressure (simplified agronomic systems), higher regulation standards (less herbicides and sites/MoA registered), and less innovation reaching the market. In that context, Global HRAC has the task to become a reference body related to weed control and HR management.

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