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Herbicide Resistance in Weeds

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Historical Perspective

The first recorded case of evolved herbicide resistance was to the auxinic herbicide 2,4-D in *Daucus carota* L. (wild carrot) in Canada in 1957 (Whitehead & Switzer, 1963; Mithila *et al.*, 2011). Although the number of cases of *insecticide* resistance was increasing rapidly at that time, with an average of 17 new cases per year between 1954 and 1960 (Forgash, 1984), herbicide resistance was not expected to become a major problem. The longer life-cycle of weeds, with usually only one generation per year, the lack of mobility and the buffering effect of persistent soil seed-banks meant that weeds were viewed as fundamentally different to insects in terms of the risk of evolution of resistance (LeBaron & Gressel, 1982). However, this view was challenged when the first serious case of herbicide resistance to the triazine herbicides, atrazine and simazine, was detected in 1968 in *Senecio vulgaris* L. (common groundsel) in the USA (Ryan, 1970). Subsequently, numerous cases of triazine resistance were reported worldwide and resistance to other herbicide modes of action occurred.

The number of cases of herbicide resistance increased particularly rapidly after 1975 (Fig. 7.1) and continues to increase, with about 11 new cases being reported annually (Heap, 2014). Herbicide resistance has now been reported to 22 of the 25 known herbicide modes of action, which were introduced mainly in the 50-year period between the 1930s and 1980s (Heap, 2014). However, no new mode of action has been marketed since the 1980s (Duke, 2012), which is the key reason why herbicide resistance poses an increasing threat worldwide. Weeds with resistance to several different modes of action commonly occur and, in such situations, resistance cannot be countered simply by using an alternative herbicide. Increasingly, there is a recognition that farmers have become over-reliant on herbicides in the past few decades and that this 'dependency culture' will have to change. Greater use of non-chemical methods of weed control in combination with herbicides within an integrated weed control strategy will be essential.

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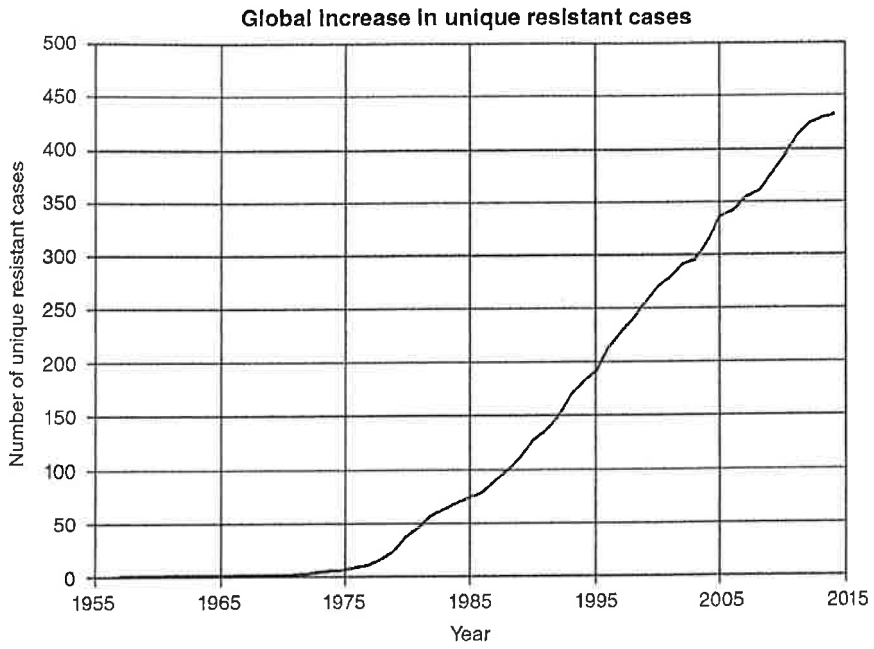


Fig. 7.1 The chronological increase in unique cases of herbicide-resistant weeds (Heap, 2014). A unique case is a species with resistance to a specific herbicide class. So if a weed species becomes resistant to one herbicide class, it is listed as one unique case; if another population of the same weed species becomes resistant to a different herbicide class, then it is counted as a separate 'unique' case.

What is Herbicide Resistance?

Herbicide resistance can be defined as the *inherited* ability of a weed to survive a dose of herbicide that would normally result in effective control. Resistance, in this context, is an *evolutionary* process whereby a *population* changes from being susceptible to being resistant. There is no evidence that herbicides directly change a plant genetically (i.e. by causing mutations that confer resistance); rather the *proportion* of resistant individuals within the population increases over time in response to repeated selection for plants with some level of natural genetic resistance to that herbicide (Vencill *et al.*, 2012). It is important to recognise that such a selection process will operate on *any* heritable mechanism that gives some plants an advantage in terms of survival. Thus, in a large weed population, several different mechanisms may be selected simultaneously, and there is no reason to assume that these will occur at the same frequency in geographically diverse populations (Powles & Matthews, 1992).

From an agronomic perspective, evolved herbicide resistance becomes a problem when control of a weed becomes 'unacceptable' to a farmer or grower. This will be typically when about 10 to 15% of weeds, normally considered susceptible, survive herbicide application. However, there are many other reasons why herbicides fail in the field (e.g. poor application technique, unfavourable climatic or soil conditions, weeds too big or excessively high infestations) and new cases of resistance can rarely be confirmed by field observations alone. Resistance rarely results in a sudden, unexpected, total failure to control weeds in the field, either because only a small proportion of the population is resistant or because individual weeds are only partially resistant rather than completely

insensitive. Resistance at the field level is best considered as 'shades of grey', with a continuum from complete susceptibility through to complete resistance. This poses a considerable challenge in detection and in assessing the severity and impact of resistance (Beckie *et al.*, 2000; Burgos *et al.*, 2013).

The term 'cross-resistance' is often used to describe cases in which weeds are resistant to two or more herbicides (of the same chemical class, or different ones) due to the presence of a single resistance mechanism. The term 'multiple resistance' is used where more than one resistance mechanism occurs within individual plants and is usually a consequence of sequential selection of resistance mechanisms by herbicides with different modes of action (Heap, 2014). Recent advances in molecular diagnostics have identified many different mutations conferring resistance to some of the major herbicide modes of action (e.g. ACCase and ALS inhibitors) (Kaundun, 2014; Yu & Powles, 2014). Consequently, it has become increasingly difficult to define what a single distinct resistance mechanism is. It has to be acknowledged that no universally accepted definitions of 'resistance', 'cross-resistance' and 'multiple resistance' exist. Such distinctions are not always helpful as weed infestations in the field may consist of a complex mix of individual plants with different types of resistance.

The Worldwide Occurrence of Resistant Weeds

The incidence of resistance to insecticides and fungicides increased rapidly after 1950 and 1960 respectively (Georghiou, 1986). In contrast, herbicide resistance is a more recent phenomenon, with few cases being reported prior to 1975 (Fig. 7.1).

By January 2017, the International Survey of Herbicide Resistant Weeds database (Heap, 2017) had recorded:

- a total of 478 unique (weed species with resistance to a specific herbicide site of action) cases of herbicide-resistant weed biotypes worldwide;
- 252 distinct resistant weed species, of which 147 are dicotyledonous and 105 monocotyledonous;
- resistant weeds in 91 crops (especially wheat, maize, soya and rice) and other situations (e.g. roadsides and orchards) in 67 countries;
- weeds had evolved resistance to 23 of the 26 known herbicide sites of action classes and to 161 different individual herbicides.

The relationship between plant families and their tendency to evolve resistance is shown in Table 7.1. Although some families (e.g. Poaceae) appear to be slightly over-represented in the list of resistant species, relative to their frequency as weeds in general, there is little evidence of a strong bias at the family level (Heap, 1999).

However, at the individual genus level, there is good evidence that some weeds are more prone to evolve resistance than others. According to Heap (2014), weed species from the genera *Lolium*, *Amaranthus*, *Conyza* and *Echinochloa* are some of the worst herbicide-resistant weeds worldwide (Table 7.2). Weeds in these genera are a particular problem as they have a proven capacity to evolve resistance to a wide range of different herbicide modes of action and most have worldwide distribution. *Lolium rigidum* Gaudin (rigid rye-grass) is the world's worst herbicide-resistant weed, having evolved resistance to 11 different herbicide modes of action in 12 countries

Table 7.1 The number and percentage of resistant species by family and the percentage of species considered principal weeds by Holm *et al.* (1977, 1997) for the ten plant families most frequently associated with resistance. *Source*: Based on Heap (1999); updated 2015.

Family	Number of resistant species	Resistant species (% of total)	Weed species (% of world's principal weeds)[a]
Poaceae (Gramineae)	77	31	25
Asteraceae	38	16	16
Brassicaceae	21	9	4
Amaranthaceae	12	5	3
Cyperaceae	11	4	5
Scrophulariaceae	9	4	1
Chenopodiaceae	8	3	2
Polygonaceae	7	3	5
Alismataceae	6	3	1
Caryophyllaceae	5	2	2
All other families	51	21	–
Total	245	100	–

[a] The number of species within a family, as a percentage of the total, reported by Holm *et al.* (1977, 1997) as being among the approximately 200 principal weeds of the world.

Table 7.2 The major herbicide-resistant weeds listed in order of the number of herbicide modes of action (MOA) to which resistance has been detected (Heap, 2014).

Species	Common name	No. of MOA to which resistance has been detected	No. of countries with resistant populations
Lolium rigidum	Rigid rye-grass	11	12
Echinochloa spp.	Barnyardgrass	9	34
Amaranthus spp.	Pigweeds	9	19
Eleusine indica	Indian goosegrass	7	8
Other *Lolium* spp.	Rye-grass	6	14
Alopecurus myosuroides	Black-grass	6	14
Avena spp.	Wild-oats	5	19
Conyza spp.	Fleabanes	5	23
Chenopodium album	Fat hen	4	14
Sorghum halpense	Johnson grass	4	8
Kochia scoparia	Kochia	4	3

worldwide. The closely related species *Lolium multiflorum* Lam. (Italian rye-grass), *Lolium perenne* L. (perennial rye-grass) and *Lolium persicum* Boiss. & Hohen. ex Boiss. (Persian darnel) are also resistance prone. In Europe, the most important herbicide-resistant weed is *Alopecurus myosuroides* Huds. (black-grass) (Moss *et al.*, 2007) and this has been shown to have a similar capacity to *Lolium rigidum* in its ability to evolve resistance to multiple herbicides.

The 66 countries in which resistant weeds have been recorded are: the USA (most states); Canada (most provinces); 24 European; 16 South and Central American; 16 Asian; 5 African; and 3 Australasian countries (Heap, 2017). Hence herbicide resistance is now truly a global problem. The 12 countries with the greatest number of different resistant cases (in parentheses) are: the USA (153); Australia (76); Canada (61); France (45); China (41); Japan (36); Israel (36); Spain (34); Brazil (34); Germany (32); Italy (30); the UK (27). The number of cases reported by China, Brazil and other South American and Asian countries has increased considerably in recent years, largely as a consequence of greater herbicide use, more research and reporting. In contrast, the database contains only a single record of a herbicide-resistant weed in Russia (from 2000), which seems improbable given the large areas devoted to agricultural production. Hence, those countries with active research programmes for detecting and reporting resistance are well represented, although the survey undoubtedly under-represents the actual occurrence of herbicide-resistant weeds (Heap, 2014).

Herbicide Mode of Action and Risk of Resistance

Herbicides kill weeds by many different means – for example, some inhibit photosynthesis (e.g. triazines), others inhibit amino acid or lipid biosynthesis (e.g. ALS and ACCase inhibitors, respectively), while others disrupt mitosis and hence cell division (e.g. dinitoanilines). For a comprehensive review of herbicide modes of action see Cobb and Reade (2010). Herbicides have been classified into 25 main classes, based on their mode of action, by the International Herbicide Resistance Action Committee (HRAC) (Schmidt, 1997; Herbicide Resistance Action Committee, 2015a).

Herbicide resistance has evolved to 22 of these 25 known herbicide mode of action classes (Table 7.3), although not necessarily to all individual herbicide members within a class. Some modes of action are more vulnerable to herbicide resistance than others, but the worldwide usage of the different herbicide classes will also be an important factor. The ALS, PSII and ACCase inhibitors can be considered 'high risk' classes from a resistance perspective as they each account for 10% or more of resistant species worldwide. The four classes each accounting for 5 to 10% of species can be considered as 'medium risk', the nine classes each accounting for 1 to 5% of species as 'low risk' and each of the other six modes of action classes to which only one or two species have evolved resistance as 'very low risk'. This is a somewhat arbitrary assessment of resistance risk, but it is based on a considerable worldwide body of data and does highlight the importance of considering the herbicide mode of action when developing strategies for preventing and managing herbicide-resistant weeds.

The evolution of herbicide resistance is a dynamic process and this is demonstrated in Fig. 7.2 which shows the chronological increase in the number of species that have evolved resistance to five of the major herbicide classes. In the 1980s the majority of

Table 7.3 Occurrence of resistant weeds by HRAC herbicide mode of action (MOA) class. *Source*: Based on Heap (2014); Herbicide Resistance Action Committee (2015a).

	HRAC MOA class	Code	Example	No. of resistant species	Percentage of total	Resistance risk
1	ALS inhibitors	B	Chlorsulfuron	153	34	
2	PSII inhibitors (triazines)	C1	Atrazine	72	16	High
3	ACCase inhibitors	A	Cycloxydim	46	10	
4	Synthetic auxins	O	MCPA	32	7	
5	PSI electron diverters	D	Paraquat	31	7	
6	EPSP synthase inhibitors	G	Glyphosate	31	7	Medium
7	PSII inhibitors (ureas/amides)	C2	Isoproturon	26	6	
8	Microtubule inhibitors	K1	Trifluralin	12	3	
9	Lipid synthesis inhibitors	N	Tri-allate	10	2	
10	Protoporphyrinogen oxidase inhibitors	E	Actifluorfen	6	1	
11	Carotenoid biosynthesis (unknown target)	F3	Amitrole	5	1	
12	Very long chain fatty acid inhibitors	K3	Metolachlor	4	1	Low
13	PSII inhibitors (nitriles)	C3	Bromoxynil	4	1	
14	Carotenoid biosynthesis inhibitors	F1	Diflufenican	3	1	
15	Cellulose inhibitors	L	Dichlobenil	3	1	
16	Inhibitors of microtubule organisation	K2	Flamprop	3	1	
17	Six other MOA	–	–	8	2	Very low
			Total	449		

cases involved the PSII inhibiting herbicides, notably the triazines (e.g. simazine, atrazine) which were very widely used, especially in maize crops. The use of both simazine and atrazine has been prohibited in EU member states since March 2004 due to concerns regarding contamination of groundwater. Global usage has also declined as a consequence of more glyphosate-resistant GM crops being grown and the greater use of alternative herbicides. Hence resistance to the triazine herbicides is relatively unimportant now.

Since the early 1980s, resistance to both the ALS- and ACCase-inhibiting herbicides has increased rapidly and, by 2000, the number of ALS cases exceeded those for the PSII inhibitors for the first time. ALS-inhibiting herbicides control both broad-leaved weeds and grass-weeds, whereas the ACCase inhibitors only have activity on grass-weeds. This is the major reason why there is a difference in the number of resistant species to these two classes.

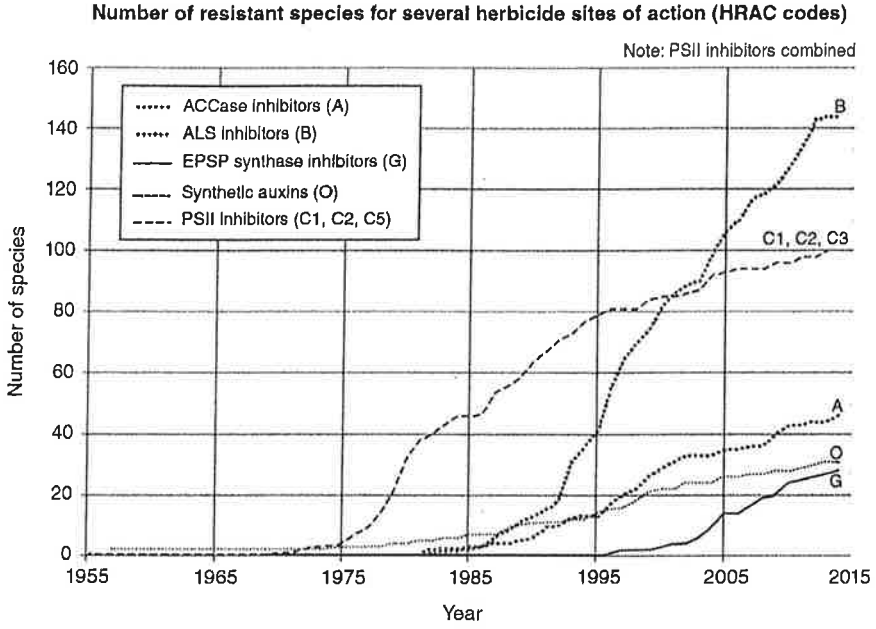


Fig. 7.2 The chronological increase in the number of herbicide-resistant weed species for five herbicide mode of action classes (Heap, 2014). The letters refer to the Herbicide Resistance Action Committee (HRAC) code to identify herbicide sites of action (see Table 7.3).

Synthetic auxin herbicides (e.g. MCPA, 2,4-D) were first introduced in the 1940s, but despite extensive global use for over 60 years, the incidence of resistance is relatively low compared with some other herbicide classes. Resistance to this class has increased gradually over a period of years but without any sudden increase. This low incidence of resistance has been attributed to the rarity and potential fitness penalties in alleles conferring resistance and the complex mode of action of auxinic herbicides, which is still not completely understood (Mithila *et al.*, 2011).

Glyphosate, the most widely used herbicide worldwide, was first commercialised in 1974 (Nandula, 2010), but it was over 20 years before the first case of evolved resistance to glyphosate in a weed was recorded, in 1996, in *Lolium rigidum* in Australia (Pratley *et al.*, 1999). It was thought that evolution of glyphosate resistance was unlikely, on the basis of partly its unique mode of action and partly the lack of cases during its first 20 years of extensive global use (Bradshaw *et al.*, 1997). However, the number of cases of glyphosate resistance has increased considerably, especially since 2000, and now 36 species in a total of 27 countries show resistance. Most cases of resistance to glyphosate are associated with the growing of glyphosate-resistant genetically modified (GM) crops, especially soya, cotton and maize, first commercialised in 1996, 1997 and 1998 respectively (Feng *et al.*, 2010). Growers initially relied almost exclusively on glyphosate for weed management, but this over-reliance on the herbicide applied an unprecedented selection pressure on weeds to evolve resistance (Dill *et al.*, 2008; Powles, 2008; Green, 2014). Interestingly, the known glyphosate resistance mechanisms, which include a target-site mutation, target-site gene duplication, reduced translocation as a consequence of active vacuole sequestration, limited cellular uptake and a rapid necrosis response, now exceed those described for any other herbicide (Sammons & Gaines, 2014).

Glyphosate resistance has also been recorded in situations where conventional crops (non-GM) are grown, but these are mainly perennial crops such as orchards or vineyards where glyphosate may be applied several times annually. In Europe, glyphosate-resistant weeds have evolved in perennial cropping situations (mainly orchards) in France, Greece, Italy, Portugal and Spain or on railways in Poland and the Czech Republic. However, the first case of a glyphosate-resistant weed (*Lolium* spp. in Italy) in a European annual arable cropping system has recently been reported (Collavo & Sattin, 2014). The lack of more cases of glyphosate-resistant weeds in Europe is due largely to the absence of commercially grown glyphosate-resistant GM crops. However, it should not be assumed that glyphosate-resistant weeds will not evolve more widely in arable cropping situations in Europe, even with the current restrictions on growing GM crops. Paraquat is no longer available as an alternative, as its approval for use in the EU was revoked in 2007. Consequently, there is a now an even higher dependency on glyphosate for control of weeds pre-sowing or inter-row in arable cropping systems, as well as in perennial crops, forestry and amenity situations, and this is likely to result in more cases of resistance.

Resistance Mechanisms

Resistant weeds can survive herbicide application by a variety of mechanisms, which are now divided into two broad categories (Powles & Yu, 2010; Délye *et al.*, 2013b). First, **target-site resistance (TSR)** mechanisms can occur by gene mutations conferring amino acid changes in a target enzyme that prevents or reduces herbicide binding, with a consequent reduction in herbicide activity. Alternatively, target-site resistance can be conferred by regulatory mutations (gene amplification or changes in a gene promoter) causing overproduction of a target enzyme that compensates for the herbicide inhibitory action. Second, **non-target-site resistance (NTSR)** can be due to mechanisms that reduce the amount of herbicide reaching a target site. Mechanisms include enhanced rates of metabolism or sequestration and decreased uptake or translocation. Such mechanisms minimise the amount of herbicide reaching the target site and mimic, at least to some degree, the selectivity mechanisms in many crops, which survive treatment due to their ability to metabolise a herbicide before it reaches the target site (e.g. wheat and diclofop-methyl).

Target-Site Resistance

Herbicides have distinct target sites where they act to disrupt biochemical processes leading to cell, tissue and plant death. The majority of herbicide target sites are enzymes and the interaction between herbicide and target site can be disrupted if there is a change in the primary structure of the enzyme protein molecule. Where this occurs, the herbicide may no longer be effective in blocking the action of the target site and the weed will survive, exhibiting target-site resistance (Cobb & Reade, 2010) (Fig 7.3). This mechanism is particularly common in the PSII (triazines – class C1), ALS (B) and ACCase (A) inhibiting herbicides, which are all classed as having a high resistance risk (Table 7.3). Target-site resistance can confer very high degrees of resistance, enabling weeds to survive many times the recommended

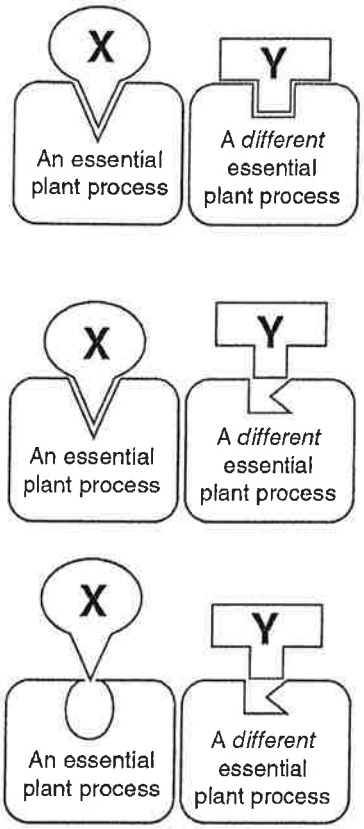


Fig. 7.3 Target-site resistance mechanisms: a diagrammatic representation. X and Y are two herbicides that inhibit different essential plant processes (= target sites) within a weed. These could, for example, be associated with photosynthesis, cell division, amino acid or lipid biosynthesis. *See* Table 7.3 for more information on the range of modes of action. *Source*: James 1995. Reproduced with permission of John Wiley and Sons.

herbicide rate. However, it is increasingly evident that the degree of resistance can vary considerably depending on the specific amino acid changes, the number of resistant alleles, weed species, plant growth stages and recommended field rates of herbicide (Kaundun, 2014).

PSII (Triazines)

Triazine resistance evolved independently in many different species worldwide as a consequence of prolonged use in maize, horticultural crops and non-crop situations. Triazine herbicides are photosystem II (PSII) inhibitors, and in the majority of triazine-resistant weeds studied worldwide, resistance is due to a mutation in the chloroplastic *psb*A gene which encodes for the D1 protein. Molecular analysis has shown that resistance is usually due to a point mutation causing a Ser-264-Gly amino acid substitution in the PQ binding site protein (Cobb & Reade, 2010). Virtually all evolved triazine-resistant weeds species have this mutation, which usually confers a very high degree of resistance, although additional mutations have been identified (e.g. Ala-251-Val, Asn-266-Thr, Phe-255-Ile, Ser-264-Thr and Val-219-Ile) which, unlike the more common Ser-264-Gly mutation, confer cross-resistance to some other non-triazine PSII

herbicides (Beckie & Tardif, 2012). The Ser-264-Gly mutation of the *psb*A gene causes a reduction in photosynthetic capacity, resulting in a significant reduction in relative ecological fitness of resistant plants (Gronwald, 1994).

ALS Inhibitors

Acetolactate synthase (ALS), also referred to as acetohydroxyacid synthase (AHAS), is the first enzyme in the biosynthesis pathway for the branched-chain amino acids valine, leucine and isoleucine. ALS is the common target for inhibition by five chemically different groups: sulfonylureas (SU), imidazolinones (IMI), triazolopyrimidines (TP), sulfonyl-aminocarbonyl-triazolinones (SCT) and pyrimidinyl-thiobenzoates (PTB). ALS herbicides have been widely used globally since their introduction in the early 1980s due to their ability to control many weed species at low rates of application (often less than 25 g ha^{-1}), low mammalian toxicity and good selectivity in major crops (Yu & Powles, 2014). However, resistance was soon reported and there are now more cases of resistance to ALS inhibitors than to any other chemical class (Table 7.3). Since 1995, a large number of target-site resistance-endowing mutations have been identified which are due mainly to single point mutations in the target ALS gene. Recent reviews show that over 20 amino acid substitutions conferring resistance at eight codon positions (Ala-122, Pro-197, Ala-205, Asp-376, Arg-377, Trp-574, Ser-653, Gly-654) of the ALS gene have been identified, with over 12 amino acid substitutions recorded at the Pro-197 position alone (Powles & Yu, 2010; Yu & Powles, 2014; Tranel *et al.*, 2015). This large number of substitutions is more than for any other major herbicide class, indicating that ALS is the most resistance-prone target site. Pro-197-Ser and Trp-574-Leu mutations are the most common, each being recorded in over 20 weed species. The former confers resistance to sulfonylureas but not to the imidazolinones, whereas the latter confers resistance to both groups. However, recent studies have shown that ALS cross-resistance patterns are very complex, as they are dependent not only on the position and amino acid substitution but also on the specific herbicide and species under consideration (Yu & Powles, 2014). Most, but not all, major ALS target-site resistance mutations are associated with low, or no, fitness costs and this also helps explain why resistance to ALS-inhibiting herbicides is so common worldwide (Vila-Aiub *et al.*, 2009). It should be noted that non-target-site, metabolism-based resistance is also a common and widely occurring resistance mechanism to ALS-inhibiting herbicides, especially in grass-weeds.

ACCase Inhibitors

Acetyl-CoA carboxylase (ACCase) is a key enzyme in lipid biosynthesis and this is the target for three chemically different herbicide groups: aryloxyphenoxypropionates ('fop'); cyclohexanediones ('dim'); and phenylpyrazolins ('den'). The terms 'fop', 'dim' and 'den' are used as a convenient shorthand version based on the last three letters of the common names of herbicides in each group (e.g. clodina**fop**, cycloxy**dim** and pinoxa**den**). Two forms of ACCase are found in the plastids of higher plants and this plays an important role in the selectivity of these herbicides. In dicotyledonous plants, a heteromeric form is present which is insensitive to ACCase herbicides. In contrast, in monocotyledonous grasses, a different, homomeric, form is present which is sensitive to ACCase-inhibiting herbicides (Cobb & Reade, 2010). Consequently, ACCase herbicides are widely used for controlling grass-weeds in broad-leaved crops such as oilseed rape

and legumes. However, they are also commonly used in monocotyledonous crops (e.g. cereals) where selectivity is often conferred by the use of safeners (e.g. cloquintocet-mexyl, mefenpyr-diethyl) which enhance the level of detoxification in the crop (Kaundun, 2014).

Many grass-weeds have evolved resistance to the ACCase-inhibiting herbicides since they were commercialised in the late 1970s (Délye, 2005; Powles & Yu, 2010). The first ACCase target-site mutation was identified in the early 2000s and research since then has identified mutations at seven ACCase codon positions (Ile-1781, Trp-1999, Trp-2027, Ile-2041, Asp-2078, Cys-2088, Gly-2096), with 14 allelic variants so far identified (Délye, 2005; Kaundun, 2014). The frequency of the mutations varies according to the weed species and location. For example, the Ile-1781-Leu mutation is the form most commonly detected in *Alopecurus myosuroides* (black-grass) in the UK, while Gly-2096-Ala is relatively much more common in Germany. The reasons for this difference are unclear but may be determined by the local herbicide selection pressure applied (Délye *et al.*, 2010). As with ALS target-site resistance, recent studies have shown that ACCase cross-resistance patterns are complex for similar reasons (Yu & Powles, 2014). Most, but not all, major ACCase target-site resistance mutations are associated with relatively low, or no, fitness costs, and in many grass species, non-target site, mainly enhanced metabolic resistance, is also a common and widely occurring resistance mechanism (Vila-Aiub *et al.*, 2009).

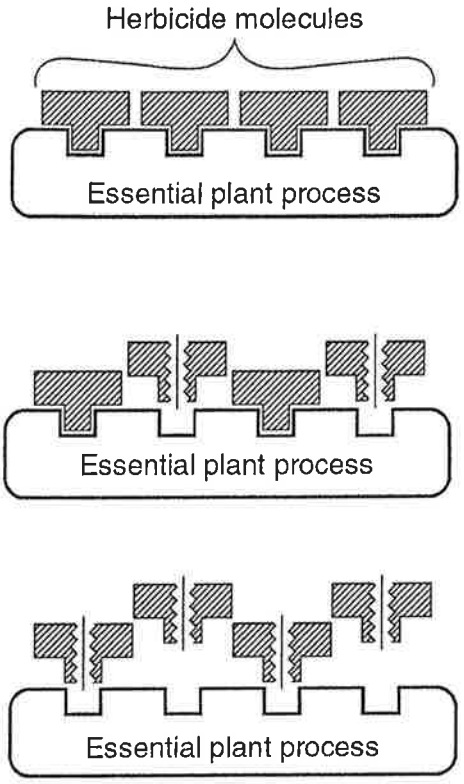
Other Herbicide Classes

As well as the three classes above, target-site resistance has been identified to microtubule inhibitors (class K1), inhibitors of microtubule organisation (K2), protoporphyrinogen oxidase (PPO) inhibitors (E), carotenoid biosynthesis inhibitors (F1) and the EPSP synthase inhibitors (G) (Beckie & Tardif, 2012; Délye *et al.*, 2013b), and is a probable mechanism to the synthetic auxins (O) (Mithila *et al.*, 2011) (Table 7.3). EPSP synthase target-site mutations, at the Pro-106 position, conferring resistance to glyphosate have been recorded in six weed species (Sammons & Gaines, 2014). In addition, resistance to glyphosate conferred by EPSP synthase gene amplification, better described as gene duplication, was first reported in *Amaranthus palmeri* S. Watson (palmer amaranth) and reported subsequently in four additional species (Gaines *et al.*, 2010; Sammons & Gaines, 2014).

Non-Target-Site Resistance

The importance of non-target-site resistance is being recognised increasingly (Fig 7.4). In the past, there was an assumption that non-target-site resistance generally conferred partial resistance and was of importance mainly in grass-weeds. However, it is now recognised as being of major significance in resistance to the ACCase (class A), ESPS synthase (G), ALS (C) and urea PSII (C2) inhibitors, as well as other classes of herbicides (Délye *et al.*, 2013b). Although best documented in grass-weeds, its importance in broad-leaved weeds is probably underestimated (Délye *et al.*, 2011). Enhanced metabolism and other non-target-site resistance mechanisms are relatively unimportant with the triazine PSII inhibitors (C1), with target-site resistance much more significant (Yuan *et al.*, 2007; Délye, 2013).

An important practical aspect of non-target-site resistance, especially that based on enhanced metabolism, is that cross-resistance patterns are complex, unpredictable and



Susceptible weed: herbicide molecules reach the binding site and inhibit the essential plant process (typically an enzyme function), and consequently the weed dies.

Partial herbicide resistance: some herbicide molecules are metabolised, and hence detoxified, prior to reaching the binding site. Consequently the essential plant process is only partially inhibited, so the weed may be damaged, but survive.

High degree of resistance: most herbicide molecules are metabolised prior to reaching the binding site. Consequently the essential plant process is not, or only slightly, inhibited, so the weed survives.

Fig. 7.4 Non-target-site resistance mechanism: a diagrammatic representation of enhanced metabolism. A herbicide inhibits an essential plant process within a weed but may be metabolised, and hence detoxified, before reaching the target site. This is a quantitative process (in contrast to the qualitative process in target-site resistance) and hence variable degrees of resistance can occur.

can occur to many different herbicide classes (Beckie & Tardif, 2012). This contrasts with target-site resistance that only affects herbicides within the same chemical class. Non-target-site cross-resistance may even occur to a herbicide with a different mode of action, and not to a herbicide with the same mode of action (Fig. 7.5). The critical factor is the degree to which herbicides can be metabolised, and hence detoxified, and this is more dependent on the molecular structure of individual herbicides than on their mode of action (James *et al.*, 1995).

One current hypothesis for induced non-target-site resistance is that herbicide application is a stress that triggers response pathways in all weed individuals (Délye, 2013; Délye *et al.*, 2013b). This, as yet unproven theory, predicts that genetic variation in the magnitude of the response among individuals results in variation in sensitivity, and this is the basis for the evolution of induced non-target-site resistance. Many different forms of non-target-site resistance would be expected, given that the selection process operates on *any* heritable mechanism that gives some plants an advantage in terms of survival. Non-target-site resistance mechanisms identified so far include decreased uptake or translocation and enhanced rates of metabolism or sequestration.

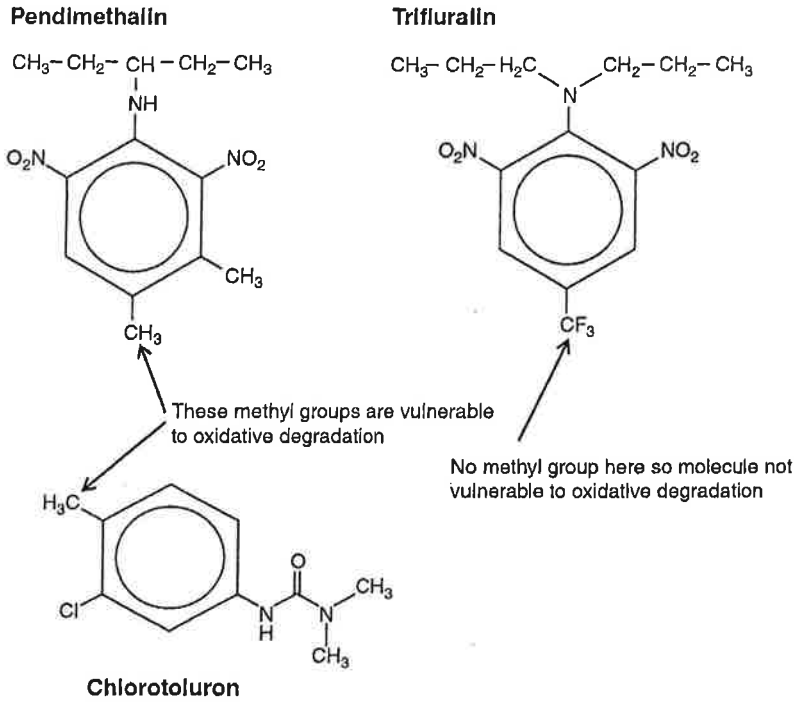


Fig. 7.5 Molecular structures of the microtubule inhibitor (K1) herbicides pendimethalin and trifluralin and the PSII inhibitor (C2) chlorotoluron. The presence of ring-methyl groups in both pendimethalin (which inhibits cell division) and chlorotoluron (a photosynthetic inhibitor) explains cross-resistance between these two herbicides with contrasting modes of action. The absence of ring-methyl groups in trifluralin explains why enhanced metabolic resistance does not occur to this herbicide, despite it having the same mode of action as pendimethalin (James *et al.*, 1995).

Reduced Herbicide Uptake

Reduced herbicide uptake has been reported to herbicides in several different classes and can be due to differences in the physical and chemical properties of the leaf cuticle resulting in reduced retention or penetration through the cuticle (Délye, 2013). This mechanism tends to confer only low levels of resistance and is generally considered to be of minor importance.

Reduced Herbicide Translocation

Reduced herbicide translocation involves a restriction on the movement of the herbicide within the plant, often as a consequence of compartmentation or sequestration in the cell vacuoles. This is now recognised as a major mechanism of resistance to glyphosate (ESPS inhibitor, class G) and paraquat (PSI electron diverters, D) (Hawkes, 2014; Sammons & Gaines, 2014). Neither herbicide is prone to metabolism and target-site resistance is uncommon, which is why resistance conferred by reduced translocation appears to be more significant. Inheritance of this trait appears to be by a single nuclear semi-dominant gene in both cases. However, restricted glyphosate and paraquat translocation are independent mechanisms and resistance to one of these herbicides does not confer cross-resistance to the other, although both mechanisms can exist within the same plant (Powles & Yu, 2010).

Enhanced Herbicide Metabolism

Enhanced herbicide metabolism is the most important, and is certainly the most studied, type of non-target-site resistance, especially in grass-weeds such as *Lolium rigidum* and *Alopecurus myosuroides*. Susceptible plants of these species often have some capacity to metabolise herbicides into non-toxic, or less toxic, compounds, but to a limited degree. Resistant weeds survive because they have an *enhanced* ability to metabolise herbicides, and differences between resistant and susceptible plants are quantitative ('shades of grey') rather than qualitative ('black and white'). This can make detection of this type of resistance and interpretation of its practical significance problematic, especially when other resistance mechanisms are also present.

Several enzyme systems have been identified as being important in enhanced metabolism in resistant weeds, especially the cytochrome P450 monooxygenases and the glutathione-S-transferases (GST) (Yuan *et al.*, 2007; Powles & Yu, 2010; Délye, 2013). Studies of the inheritance of P450 resistance have shown that more than one P450 gene is usually involved, which is not surprising given that hundreds of different P450s isozymes have been identified in plants. GST enzymes may have a direct role (herbicide conjugation) or an indirect role (stress response). Herbicide metabolism is a complex, multi-step process involving the co-ordinated action of several types of enzyme.

Other enzyme systems have been implicated in enhanced metabolic resistance. For example, *Echinochloa* spp. have evolved resistance to propanil due to increased aryl acylamidase activity (Leah *et al.*, 1994) and glycosyltransferases have been implicated in chlorotoluron resistance in *Alopecurus myosuroides* (Brazier *et al.*, 2002).

Although target-site gene mutations that confer resistance have been precisely identified, our current understanding of non-target-site resistance is more limited. There is substantial biochemical evidence for the role herbicide-degrading enzymes play in enhanced metabolic resistance, but knowledge of the genetic and molecular basis is lacking, although the genes encoding two GST and one esterase have been identified (Cummins *et al.*, 1999; Cummins & Edwards, 2004; Cummins *et al.*, 2013). Cytochrome P450 is also clearly involved in metabolic resistance to herbicides, but the genetic control is complex, and often polygenic. A major aim of research must be to identify the genes conferring non-target-site resistance, including both enhanced metabolism and reduced translocation mechanisms, and much progress can be expected in the future due to new genomic approaches (Délye, 2013).

It is clear that weeds can survive herbicide applications by a wide variety of resistance mechanisms; some confer a high degree, others a low degree of resistance. To add to the complexity, weed populations, and even individual plants, may possess several different resistance mechanisms. The greatest challenge posed by herbicide-resistant weeds is the accumulation in individuals of many resistant mechanisms, both target site and non-target site. Multiple resistance mechanisms will contribute to the overall degree of resistance seen at the whole plant level.

Evolution of Herbicide Resistance

There are two main ways in which resistance may arise within a weed population (Maxwell & Mortimer, 1994). First, a major gene, or genes, conferring resistance to a herbicide may be present at a very low frequency because of natural random mutations.

Such mutations, which often confer target-site resistance, may have occurred before the herbicide was used (standing variation) or after introduction of the herbicide (arisen variation) (Délye *et al.*, 2013a). The degree of resistance in the weed population will largely be a function of the proportion of resistant to susceptible individuals. Second, selection may act on continuous (quantitative) variation that pre-exists within a weed population, achieving a gradual, progressive increase in resistance over several generations. Many different genes may be involved, each conferring only a small degree of resistance, but selection and genetic recombination over several generations may result in what has been described as 'creeping' resistance (Gressel, 2009). In this case, the whole population shifts to a higher mean level of resistance, but there is likely to be considerably greater variation in the response of individual plants within the population than occurs with major gene resistance.

Weeds may become resistant to more than one class of herbicide because they possess independent mutations as a consequence of selection by two or more different modes of action (e.g. ACCase and ALS inhibitors). Alternatively, selection by one group of herbicides may confer cross-resistance to herbicides with different biochemical modes of action as a consequence of enhanced metabolic non-target-site resistance.

The development of resistance as a problem on a field scale is dependent on the increase in the proportion of the resistant genotype within the population. The following factors are generally considered to be of particular importance in the development of herbicide resistance (Diggle & Neve, 2001):

- Initial frequency of the resistance trait and size of weed population
- Genetic basis of resistance
  a) Number of genes/alleles involved
  b) Degree of dominance of resistance alleles
  c) Mode of inheritance
  d) Reproductive/breeding characteristics
  e) Fitness cost of resistance genes
- Selection pressure
- Seed-bank in the soil

Initial Frequency of the Resistance Trait and Size of Weed Population

Conventional resistance theory indicates that gene mutations conferring resistance to a specific herbicide class are not induced by application of herbicide, but are believed to occur spontaneously and are continually being generated in natural populations of weeds. Mutations at some loci, particularly those encoding herbicide sites of action, may confer resistance. Typical spontaneous mutation rates are often cited as 10^{-5} to 10^{-9} for an allele carrying a mutation at a given nucleotide position (Jasieniuk *et al.*, 1996). Detecting resistant plants at such low frequencies in unselected populations is clearly very difficult. However, weed seed-banks in the soil can be very high – a seed-bank of 10^3 seed m^{-2} for an individual weed species, which is not atypical, represents 10^7 seed ha^{-1}. Thus even with very low estimates of initial frequency of resistant individuals, there is a high probability of resistance occurring in a relatively small area as a consequence of the large number of weed seeds that exist in the seed-bank (Jasieniuk *et al.*, 1996).

Clearly, the probability of resistance occurring is greater at higher than at lower weed infestations, but it still seems hard to explain how resistance to ACCase, ALS and triazine PSII inhibiting herbicides has evolved as a field problem after only three to ten generations under continuous selection (Maxwell & Mortimer, 1994). Recent research suggests that the conventional theory is over-simplistic.

Délye *et al.* (2013a) evaluated herbarium plant specimens of *Alopecurus myosuroides* and detected one Ile-1781-Leu ACCase heterozygous mutant in a sample collected in France in 1888, 90 years before ACCase-inhibiting herbicides were first used. This represents the first demonstration in a weed species that a mutation endowing resistance to herbicides pre-dated herbicide discovery. Critically, the observed frequency of one heterozygous resistant mutant in 685 specimens assayed corresponded to a frequency of 7.3×10^{-4}, which is at least four orders of magnitude greater than that expected in unselected populations on a spontaneous mutation frequency basis. This indicates that at least some resistance mutations can be present at much higher frequencies than expected, although the underlying genetic processes that facilitate this are unclear.

In addition, recurrent selection acting on continuous or quantitative variation can result in the evolution of resistance even when the initial population is very small. For example, at Rothamsted, we have selected for enhanced metabolic resistance to the dinitroaniline (K1) pendimethalin using a baseline population of only 200 *Alopecurus myosuroides* plants grown from seeds collected from the section of the Broadbalk field experiment never treated with herbicides (Moss *et al.*, 2004). In outdoor containers, pendimethalin was applied pre-emergence at 900 g ha^{-1} and the survivors grown on in isolation to produce seed, which was subsequently re-sown in new containers and the process repeated for 7 years. The differences in percentage reduction in plant numbers between the baseline population, which was re-sown each year, and the selected populations are shown in Fig. 7.6. The good conditions for herbicide activity in year six explain the apparent slight 'loss' of resistance seen in that year. There was a progressive reduction in pendimethalin activity as a consequence of selection over 7 years,

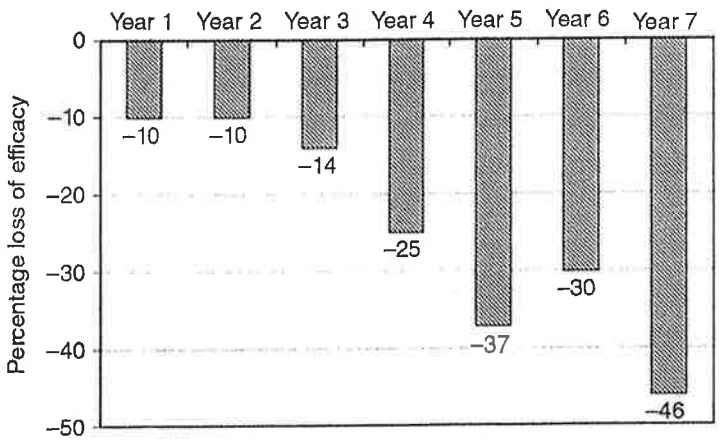


Fig. 7.6 Loss of efficacy of pendimethalin relative to the baseline population of an *Alopecurus myosuroides* population never previously treated with herbicides. Results are based on number of surviving plants per container, relative to untreated, after annual treatment with 900 g pendimethalin ha^{-1} in outdoor containers for 7 years (least significant difference (LSD) $P_{\leq 0.05} = 13.6$).

averaging about 6% per year. This reduction was statistically significant after 3 years of selection and agronomically significant after 4 to 5 years.

These two examples, both involving *Alopecurus myosuroides*, the most important herbicide-resistant weed in Europe, highlight the fact that it may well be misleading to think that the potential to evolve resistance is only present in rare individuals in unselected populations. A similar situation is likely to occur with *Lolium rigidum*, which causes major resistance problems in many countries. However, both these grass-weed species seem particularly prone to multiple herbicide resistance, so it should not be assumed that other weeds have similar characteristics.

The degree to which mutation rates differ between weed species and populations is largely unknown but might explain why resistance appears much more common in some species than others. It has also been suggested that 'stress', which could well include pesticide applications, increases the mutation rate through the action of mutator genes (Gressel, 2002; Gressel, 2011). This is controversial, and there is, at present, no direct evidence that mutator genes are associated with the more rapid evolution of herbicide resistance.

There is still much that is unknown about the factors that determine the rate of evolution of herbicide resistance in the field, and why some species and herbicides appear much more resistance-prone than others. What is clear is that herbicide resistance, at least in some species, can develop as a practical field problem remarkably rapidly, and much faster than conventional theory would suggest.

Genetic Basis of Resistance

Enhanced metabolic resistance, the most important non-target-site resistance mechanism, is generally considered to be mainly polygenic, but its genetic basis is complex and currently poorly understood. In contrast, most target-site resistance is conferred by a single nuclear gene with resistance alleles showing a variable degree of dominance. Generally, resistant alleles are dominant over susceptible alleles at the recommended field application rates of herbicide, which means that heterozygous resistant plants survive normal field rates, facilitating the rapid increase in resistant alleles (Jasieniuk *et al.*, 1996; Yu & Powles, 2014). However, the degree of resistance exhibited by heterozygous individuals can vary, depending on weed species and specific herbicide, and may often be over-estimated owing to the presence of additional non-target-site resistance mechanisms (Kaundun, 2014).

There are sound theoretical reasons why, in most cases, resistance is conferred by dominant, or semi-dominant, alleles. A rare dominant resistance allele is much more likely to become established in an outcrossing weed population, following herbicide treatment, than a recessive resistance allele (Jasieniuk *et al.*, 1996). This is because heterozygous individuals will survive if resistance alleles are dominant, but die if they are recessive. Resistance conferred by recessive alleles is more likely to occur in self-pollinating than cross-pollinating species, as the progeny of homozygous recessive resistant individuals will not be subject to dilution into heterozygous individuals by cross-pollination with plants with susceptible alleles. This theory is supported by the fact that relatively few cases of resistance conferred by recessive alleles have been detected, and most of these are in self-pollinating species, such as *Setaria viridis* (L.) P. Beauv. (green foxtail) (Jasieniuk *et al.*, 1994), *Eleusine indica* (L.) Gaertn. (Indian goosegrass) (Zeng & Baird, 1997) and *Avena fatua* L. (wild-oat) (Kern *et al.*, 2002). However, resistance conferred by a single nuclear recessive gene was found in

Centaurea solstitialis L. (yellow star-thistle) (Sabba *et al.*, 2003), despite this species being primarily an out-breeder, which shows that this trait is not an absolute barrier to resistance evolution.

In contrast to all other documented cases of resistance, in which conventional nuclear inheritance occurs, resistance to the triazine (PSII inhibitors C1) herbicides is not inherited via chromosomal DNA in the nucleus but by maternal inheritance of non-nuclear DNA in the chloroplasts. An important consequence of this is that resistant genes are not transmitted in pollen, but that all seeds of a resistant plant will produce resistant individuals (Darmency, 1994). With all other forms of resistance, the extent to which a species is an obligate cross-pollinator, compared with being self-pollinated, can strongly determine the genotypic structure of a population (Maxwell & Mortimer, 1994). A key factor is that pollen exchange means cross-pollinated plants can rapidly share and accumulate resistance genes (Powles & Yu, 2010). In theory, the spread of herbicide resistance will occur more rapidly in cross-pollinated populations. In general, most resistant gene flow via pollen is likely to occur over relatively short distances because pollen is only viable for a few days, at most (Vencill *et al.*, 2012). In contrast, resistant gene flow via seeds has probably been underestimated and is important regardless of pollination system. It is also a means by which resistance genes can spread globally, in contaminated crops or seed, for example (Jasieniuk *et al.*, 1996).

Fitness is generally defined as reproductive success, or the proportion of genes an individual leaves in the population. The two fundamental components of fitness are survival and reproduction (Silvertown & Charlesworth, 2001). Resistance mechanisms confer an obvious fitness advantage in plants treated with the herbicide to which there is resistance. However, it is now widely accepted that many resistance mechanisms have associated pleiotropic effects which confer a fitness cost in the absence of the selecting herbicide (Délye *et al.*, 2013b). For a comprehensive review see Vila-Aiub *et al.* (2009). Many older studies demonstrated that the Ser-264-Gly mutation, which is the main mechanism of resistance to triazine (PSII inhibitors, C1) herbicides, conferred a significant fitness penalty, resulting in reduced biomass, competitive ability and seed production in resistant plants (Holt, 1990). More recent studies, with other forms of resistance, have shown that the degree of any fitness penalty varies considerably. For example, the Ile-1781-Leu mutation, conferring resistance to ACCase inhibiting herbicides (Class A) in *Alopecurus myosuroides* and *Lolium rigidum*, has no detectable fitness penalty in terms of plant productivity, which may explain why it is the commonest mutation. In contrast, the Asp-2078-Gly mutation is associated with a fitness penalty, demonstrating that the expression of fitness is specific to the amino acid substitution conferring resistance (Menchari *et al.*, 2008).

Fitness penalties are not necessarily large, but an understanding of the fitness consequences of herbicide resistance alleles in the presence or absence of herbicides is important for predicting the evolutionary dynamics of herbicide resistance (Neve *et al.*, 2003). Such studies can explain why certain mutations are common, while others remain rare. However, if resistance has evolved to become a practical problem in the field, then, by definition, fitness penalties in the resistant weeds must be relatively insignificant. Hence, studies conducted in realistic field situations, over several years, are essential in order to quantify the practical impact of fitness costs and to improve management in agro-ecosystems (Maxwell & Mortimer, 1994; Vila-Aiub *et al.*, 2009).

Selection Pressure

A unique characteristic of the evolution of herbicide resistance in weeds, in contrast to most evolutionary processes occurring in natural plant populations, is the intensity of selection imposed by herbicides (Jasieniuk *et al.*, 1996). Selection pressure is the most important factor determining the evolution of resistance, and has been defined as the proportion of resistant plants surviving divided by the proportion of susceptible plants surviving a herbicide treatment (Gressel & Segel, 1982). Thus, if all resistant plants but only 5% of susceptible plants survive a herbicide application, the selection pressure (SP) would be $1/0.05 = 20$. The higher the intensity of selection, the faster the expected rate of evolution of resistance. The highest selection pressure will exist when all resistant plants survive but all susceptible plants are killed. No selection pressure will exist in the absence of a differential kill of resistant and susceptible individuals.

Most herbicides are applied at rates that kill 90 to 99% of susceptible weeds, so repeat herbicide applications can result in the rapid selection of initially rare resistant individuals until they come to dominate the population. However, selection pressure, as defined above, is based on a ratio of proportions rather than numbers, so it is independent of both the size of the initial weed infestation and the relative proportion of resistant and susceptible individuals pre-treatment. In practical management situations both these factors are of critical importance. Consequently, selection pressure is a very useful theoretical concept, but has limitations practically and is difficult to quantify in real field situations.

Selection pressure depends on:

- frequency of herbicide use;
- persistence of the herbicide and pattern of weed emergence;
- intrinsic activity of the herbicide and degree of resistance conferred by the resistance mechanism(s);
- specificity of the herbicide – the number of species the herbicide controls.

Frequency of Herbicide Use

Repeated use of the same herbicide, or one with the same mode of action, is likely to impose a greater selection pressure than the use of rotations of herbicides with different modes of action. If there is no resistance to the alternative herbicides, there will be no differential selection, and hence the evolution of resistance is likely to be delayed. However, where cross-resistance to herbicides with different modes of action occurs, as with enhanced metabolic resistance, rotating different herbicides may not reduce the selection pressure. The outcome will depend on the degree to which the different herbicides are affected by enhanced metabolism.

Persistence of the Herbicide and Pattern of Weed Emergence

Applications of herbicides with considerable residual activity (e.g. atrazine, a PSII inhibitor, C1) were considered to impose a high selection pressure because successive flushes of germinating weeds were continually exposed to the herbicide. This was considered a major reason for the increase in resistance to the triazine (PSII C1) herbicides in the late 1970s and 1980s (Gressel & Segel, 1990). However, resistance has also evolved to many herbicides that have little or no residual activity in the soil, such as the ACCase inhibitors (Class A), synthetic auxins (O), PSI electron diverters (D) and ESPS synthase

inhibitors (G). These comprise four of the seven classes in the high or medium resistance risk categories in Table 7.3. Consequently, the residual activity of herbicides appears to be less important than predicted, probably because many selective foliar-acting herbicides are applied after most weeds have emerged and non-selective herbicides (e.g. paraquat and glyphosate) are often applied several times a year, thus imposing repeated selection on successive cohorts of germinating weeds. Residual activity may be more important in total weed control situations (e.g. industrial sites, railways) where high rates of persistent herbicides may be used.

Intrinsic Activity of the Herbicide and Degree of Resistance Conferred by the Resistance Mechanism(s)

A highly active herbicide will kill all susceptible plants, leaving only resistant individuals, whereas a herbicide with no activity on an individual species will impose no selection pressure. This 'black and white' resistance scenario does exist, but, in practice, the situation is usually more complex as herbicides vary in efficacy, weeds vary in their susceptibility to herbicides and many resistance mechanisms confer only partial resistance. Consequently, in many situations, not all susceptible individuals are killed and not all resistant plants survive. The use of mixtures or sequences of herbicides with different modes of action coupled with the presence of multiple resistance mechanisms further complicates the issue, making quantifying and interpreting the effects on selection pressure very difficult.

Primarily for financial reasons, many farmers use reduced rates of herbicides to control weeds. There has been a longstanding debate ('the dose rate debate') regarding the degree to which different herbicide rates affect the rate of resistance selection (Gressel, 2011; Neve *et al.*, 2014; Renton *et al.*, 2014). Higher rates might be expected to select for monogenic target-site resistance, where the mutation confers a high degree of resistance, as the selection pressure will be higher due to the greater kill of susceptible individuals (Gressel & Segel, 1978). In contrast, lower rates might favour polygenically inherited traits conferring partial resistance, as a greater number of partially resistant individuals will survive and cross with other individuals with partial resistance conferred by other polygenes, producing progeny with higher resistance as a consequence of this 'stacking' of minor genes (Renton *et al.*, 2014). Consequently, there can be a progressive increase in the overall level of resistance over a period of years.

There is good experimental evidence, supported by modelling, that low doses encourage resistance to diclofop-methyl, glyphosate and pyroxasulfone in *Lolium rigidum* in Australia (Neve & Powles, 2005; Busi & Powles, 2009; Renton *et al.*, 2011; Busi *et al.*, 2012). However, there is a danger that the concept of low rates encouraging resistance becomes accepted as a general principle. This is particularly dangerous as agrochemical companies and distributors have an obvious vested interest in promoting the idea that the use of low doses increases the resistance risk and this concept has been promoted in agrochemical company technical literature. There may well be individual cases where either lower, or indeed higher, than recommended rates of herbicides increase the risk of resistance in a specific weed, but this should not be interpreted as being indicative of a general concept. The term 'low dose' is unhelpful when herbicides vary so much in their intrinsic efficacy on different weed species – quantifying in efficacy terms would be better. In addition, if a herbicide has relatively low efficacy

against a target weed, whether that be at full or reduced rate, additional herbicides with other modes of action may be used. This is generally considered a good anti-resistance strategy.

Many other factors are involved and some researchers view the 'dose rate debate' as a distraction from more important concepts, namely: reduced rates increase the risk of ineffective weed control regardless of resistance issues; some herbicides pose a higher risk than others; some weeds are more resistance-prone than others; and a need for greater use of non-chemical weed control methods in order to reduce reliance on high-risk herbicides (see Melander *et al.*, Chapter 9).

Specificity of the Herbicide: Number of Species the Herbicide Controls

Herbicides that are only active on a very limited range of weed species (e.g. difenzoquat on *Avena* spp., wild-oats) will only impose a selection pressure on those few species that are susceptible. In contrast, broad-spectrum herbicides (e.g. glyphosate) will impose a selection pressure on a much wider range of species. A key question, still largely unanswered, is why resistance evolves in some weed species in such situations, but not others, despite all being under the same intensity of selection.

Seed-Bank in the Soil

The weed seed-bank in the soil can, in theory, exert a strong 'buffering' influence which can delay the rate of enrichment for resistance (Gressel & Segel, 1982). The importance of this reserve of unselected, or less selected, genetic material depends largely on the persistence of the seeds in the soil, the germination characteristics of the weed and the cultivation system used prior to establishing the crop. However, despite its sound theoretical basis, there is surprisingly little evidence that this is a significant factor in practice. In contrast, once resistance has evolved, species with more persistent seeds are likely to pose a greater threat due to long-term emergence of resistant plants from seeds in the seed-bank.

Resistance Risk

The evolution of herbicide resistance as a practical problem in an individual field is dependent on the interaction of three risk factors (Vencill *et al.*, 2014). Figure 7.7 highlights some of the various elements that contribute to the risk associated with each of these three factors.

- **Herbicide risk** – specific to the herbicide under consideration. Most herbicides are vulnerable to resistance, although some are more vulnerable than others. The risk posed by a herbicide can be estimated from the number of cases of resistance that have evolved to that class worldwide, relative to other modes of action (Table 7.3).
- **Weed risk** – specific to an individual weed species. Among the thousands of weed species that occur in cultivated fields worldwide, only a relatively small number have evolved resistance to herbicides, and of these, only a proportion cause major problems. Currently, the best predictor is the occurrence and severity of resistance in the same or a closely related species growing in similar agronomic systems and climatic conditions.

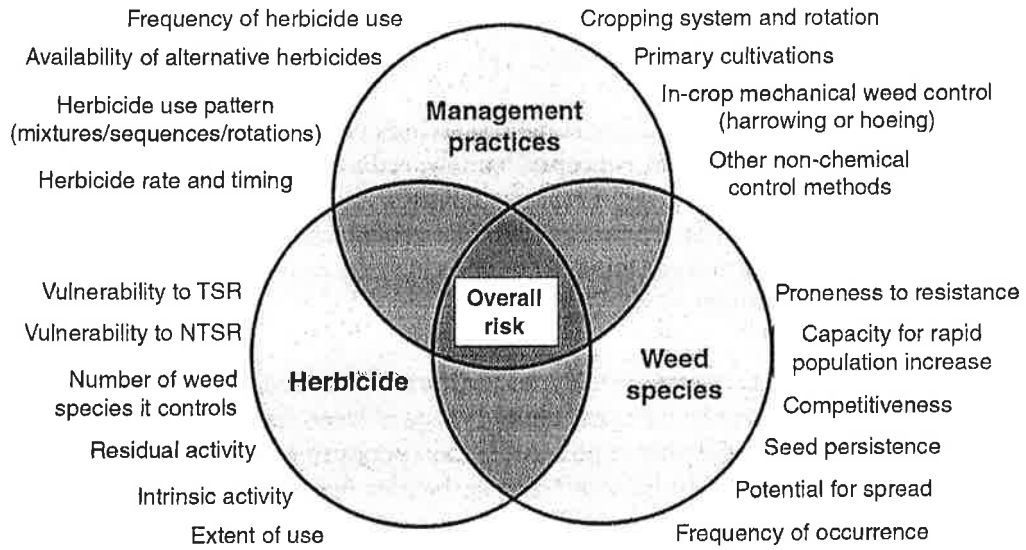


Fig. 7.7 Three key components determining the overall risk of herbicide resistance.

- **Management practices** – includes the way herbicides are used as well as the cropping system and non-chemical methods of weed control. Management is the one aspect of the herbicide–weed–management triangle where the grower's decisions can greatly influence herbicide resistance development. Growers who use non-chemical control measures, such as good crop rotations, cultivations, delayed sowing, fallowing, mechanical weed control, increased crop competition and preventing seed return, can reduce their reliance on herbicides and so reduce the threat of resistance compared with growers who rely solely on herbicides for weed control. Non-chemical control methods are unlikely to totally replace herbicides, but the way these are used will influence the risk of resistance. If the herbicide is likely to be used more than once a year (e.g. in some total weed control situations or in herbicide-tolerant crops) the risk is likely to be higher than where it is used once annually (e.g. in successive cereal crops). The risk is likely to be even lower where the herbicide (and others with the same mode of action) is used less than once a year (e.g. as in rotational crops such as oilseed rape). If the herbicide is used alone, then the risk is likely to be higher than if it is used in mixture or sequence with other herbicides with activity on the same target weed, as long as the alternative herbicides have different modes of action. When the herbicide is the only one available for controlling a specific weed (an increasing issue, especially in minor crops, with loss of herbicides due to EU regulatory action), the resistance risk is likely to be higher than if many alternative modes of action are available.

Resistance is not inevitable – it is the interaction of these three factors that is critical in determining the overall risk and whether resistance will become a problem in a specific situation. Note that both the herbicide and weed risk are based largely on the detection, evaluation and reporting of cases of resistance rather than on theoretical considerations. This highlights the importance of having a comprehensive, constantly updated, global database such as that maintained by Heap (2017).

Prevention and Management of Herbicide Resistance

Detection of Resistance in the Field

Early detection of resistance is very important so that effective management strategies can be introduced over the whole farm to minimise the impact and spread of resistance. Indicators of herbicide resistance in the field are:

- a gradual decline in weed control over several years;
- healthy plants besides dead plants of the same species;
- poor weed control leading to discrete patches;
- poor control of one susceptible species when other, equally susceptible, species are well controlled.

However, there can be many reasons for poor weed control in the field other than resistance (e.g. inappropriate dose or timing, adverse soil or environmental conditions, poor application), so an essential pre-requisite for confirmation of resistance is a reliable diagnostic test that should be rapid, accurate, cheap and readily available. Many diagnostic tests have been developed, including glasshouse pot assays, Petri-dish germination assays, molecular tests for specific mutations and laboratory tests using radiolabelled herbicide to determine metabolism (Beckie *et al.*, 2000; Burgos *et al.*, 2013; Herbicide Resistance Action Committee, 2015b). Glasshouse pot assays, in which plants are grown from seeds collected from suspect fields, sprayed with the herbicide and then survival or plant biomass recorded, are the most widely used tests, as they mimic field applications and generate results that are independent of herbicide mode of action. The value of any diagnostic test is greatly enhanced by the inclusion of known susceptible and resistant standard reference populations.

Seeds or plants collected for resistance testing often represent a biased sample, as they have usually been collected from survivors of a herbicide treatment and may comprise only a small proportion of the total population. Hence, resistance test results will tend to exaggerate the level of resistance and this can result in a lack of credibility by farmers, especially if they achieve good control with the same herbicide in the field the following year. However, this bias in resistance testing should not be viewed as a limitation, but as a very positive attribute, as confirmation of resistance provides an early warning sign of greater potential problems ahead if no changes are made to the current herbicide strategy.

Integrated Weed Management

Most farmers are over-reliant on herbicides to control weeds, but this approach is not sustainable due to increasing resistance (Shaner & Beckie, 2014). Even when a range of herbicides with different modes of action is available, resistant biotypes with multiple resistance traits are likely to be selected. The lack of herbicides with new modes of action means that an increase in the number of cases and severity of resistance is inevitable if there is over-dependency on herbicides. Diversity is the key to successful long-term weed management, with greater use of non-chemical methods of control and less reliance on herbicides (Shaner, 2014; see also Melander *et al.*, Chapter 9). An integrated weed management (IWM) approach is needed, in which as many tactics as possible are used to combat weeds. In this way, less reliance is placed on herbicides and so selection pressure for resistance should be reduced (Table 7.4).

Table 7.4 Agronomic factors influencing the risk of herbicide resistance development.

Factor	Low risk	High risk
Cropping system	Good rotation	Crop monoculture
Cultivation system	Annual ploughing	Continuous minimum tillage
Weed control	Non-chemical only	Herbicide only
Herbicide use	Many modes of action	Single mode of action
Control in previous year	Excellent	Poor
Weed infestation	Low	High
Resistance in vicinity	Unknown	Common

Non-Chemical Control Methods

Many non-chemical methods of weed control are available, including crop rotation, primary and in-crop cultivations, delayed drilling, more competitive crops or cultivars and fallowing. Individual circumstances will dictate which are most appropriate to use within any given agronomic system. Provided susceptible and herbicide-resistant plants respond similarly, there should be no selection in favour of resistant individuals. Consequently, the control achieved by non-chemical methods may be more durable than that achieved by herbicides, even if it is poorer. Hence, investigating how best to use non-chemical methods at an individual farm level should pay dividends long term. Recent reviews that include a lot more information on non-chemical methods and IWM are: Beckie (2006); Beckie & Gill (2006); Norsworthy *et al.* (2012); Vencill *et al.* (2012); Harker and O'Donovan (2013); and Melander *et al.* (2013). See also Shaw and Hatcher, Chapter 8, and Melander *et al.*, Chapter 9, this volume.

Herbicidal Control

Non-chemical methods of weed control are unlikely to completely replace herbicides as a means of combating herbicide-resistant weeds. Herbicide resistance often results in reduced herbicide activity, rather than no activity at all, especially at a field scale where only part of the weed population may be resistant.

Alternative Herbicides

When resistance is first confirmed, the efficacy of alternatives is likely to be the first consideration. Their effectiveness will be dependent on the extent of cross-resistance, so if resistance is confined to a specific mode of action, then simply using a herbicide from another mode of action class may offer a simple and effective solution. For example, in the UK, ALS (Class B) target-site resistant *Papaver rhoeas* L. (common poppy) can be controlled effectively by using pendimethalin (K1), a herbicide with a different mode of action unaffected by the target-site mutation conferring ALS resistance. However, the success of this strategy depends on the availability of effective alternatives. The situation is more complex if resistance extends to more than one herbicide class, as occurs in populations with multiple target-site resistance or enhanced metabolic non-target-site resistance.

It should not be assumed that resistance automatically extends to all herbicides with the same mode of action, although it is wise to assume this until proved otherwise. With both ACCase and ALS target-site resistance, cross-resistance patterns are very complex and dependent not only on the mutation position and amino acid substitution but also on the specific herbicide and species under consideration (Yu & Powles, 2014). For example, the ACCase inhibitor clethodim is much more effective than cycloxydim on target-site resistant *Alopecurus myosuroides* despite both herbicides having the same mode of action (Class A) (Moss *et al.*, 2012). Clethodim poses a lower resistance risk than other ACCase-inhibiting herbicides, as only a few of the many target-site-resistant mutations confer resistance (Beckie & Tardif, 2012).

Mixtures, Sequences and Rotations

The use of two or more herbicides that have differing modes of action is considered a key method of reducing selection for resistance (Gressel & Segel, 1990). Herbicide mixtures, sequences and rotations generally have the greatest effect in delaying resistance when the mechanisms conferring resistance are target-site based and the target weed species is self-pollinated (Beckie, 2006). This is because the probability of plants with multiple target-site resistance is the product of the probabilities of mutations at each site of action, and thus very low (Délye *et al.*, 2013b). Ideally, each component should:

- be active at different target sites;
- have a similar and high level of efficacy against the target weed;
- be detoxified by different biochemical pathways;
- have similar persistence in the soil, if a residual herbicide.

There is some evidence that herbicide mixtures are more effective at combating resistance than herbicide rotations (Diggle *et al.*, 2003). To combat non-target-site enhanced metabolic resistance, mixing partners must be degraded by different biochemical pathways; otherwise there will be no benefit. Such information is lacking for most herbicides, making the outcomes difficult to quantify, although using a range of different modes of action is still likely to be beneficial. However, in the long term, use of herbicides with different modes of action may provide continuous strong selection for broad-spectrum non-target-site resistance, especially enhanced metabolism, and hence increase the risk of resistance evolution, rather than reduce it (Délye *et al.*, 2013b).

Regardless of theoretical concepts, herbicides should be used to their greatest potential by ensuring that the timing, dose, application method, soil and climatic conditions are optimal for good activity. Crop rotations will dictate which herbicides can be used; some cannot be used in mixtures due to antagonism and some effective programmes require herbicides to be used in sequence, such as residual pre-emergence followed by foliar-acting post-emergence herbicides.

Managing Resistance in *Alopecurus myosuroides* (Black-grass): A Case Study

Alopecurus myosuroides is the most important herbicide-resistant weed in Europe (Moss *et al.*, 2007) and the UK currently has the most serious problem, where it is estimated that resistance occurs on virtually all of the estimated 20,000 farms where herbicides are applied regularly for its control (Hull *et al.*, 2014). Some degree of resistance occurs to virtually all herbicides currently available, so there is increasing emphasis on non-chemical control methods. Table 7.5 summarises the major control

Table 7.5 Mean and range in levels of control of *Alopecurus myosuroides* achieved by a range of non-chemical methods based on a review of over 50 field experiments (Lutman *et al.*, 2013).

	Percentage control of *A. myosuroides* achieved		
Method	**Mean**	**Range**	**Comments**
Ploughing	69	−82 to 96	Rotational ploughing has considerable benefits
Delayed autumn drilling (by ≈ 3 weeks from mid-September)	31	−71 to 97	The later the better – but increased risk
Higher seed rates	26	+7 to 63	The higher the better – but lodging issues
More competitive cultivars	22	+8 to 45	Useful, but marginal effects
Spring cropping	88	+78 to 96	Effective, but challenging on heavy soil and limited herbicide options
Fallowing/grass leys	70–80 per year (of seed-bank)	–	Absence of new seeding critical

methods available in winter cereals and the mean and range in levels of control, based on a comprehensive review of over 50 field experiments (Lutman *et al.*, 2013).

The mean level of control for each method is modest compared to what would be expected from herbicides, and this is likely to be true for non-chemical methods used against many other weeds worldwide. The wide range for each method shows how variable non-chemical control can be – in some cases negative control was achieved. The aim must be to integrate the use of *several* non-chemical control methods, in combination with herbicides, to improve overall control (Fig. 7.8).

In this example, based on values taken from Table 7.5, the four non-chemical methods give a combined level of control of 90% (note that this is not simply the sum of the control from the individual methods). Then, assuming that a herbicide programme gives 90% control, the overall control in this example is 99% – the sort of level of control needed to prevent *Alopecurus myosuroides* increasing in an intensive arable system. However, if control from herbicides continues to decline as a consequence of increasing resistance, greater control will be required from non-chemical methods to maintain the same overall high level of control. That is the challenge for the future.

Farmer Psychology: An Under-Recognised Component of Resistance Management

Over the years, a variety of resistance management strategies employing mixtures, sequences and varying rates of herbicides have been promoted globally. However, there is no good evidence that any of these have successfully stopped herbicide resistance from evolving. At best, resistance has been delayed (Preston, 2013). IWM, which

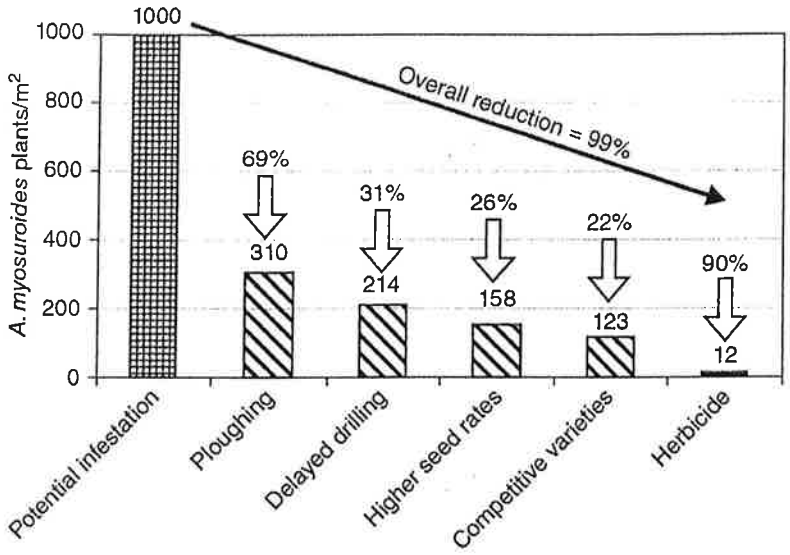


Fig. 7.8 Potential benefit of integrating the use of several non-chemical methods with herbicides for control of *Alopecurus myosuroides*. The percentage figures above each arrow are taken from Lutman *et al.* (2013) and give a combined 90% reduction in weed population. Assuming herbicides give 90% control, an overall reduction in weed population of 99% is achieved.

includes non-herbicidal weed management strategies, has been actively promoted as a means of reducing herbicide use and the risk of herbicide resistance, but the uptake of these technologies by most growers has been poor (Sanyal, 2008; Shaner & Beckie, 2014).

A major barrier to implementing herbicide resistance management practices has been the reluctance of growers to proactively manage herbicide-resistant weeds. There is often an assumption that resistance simply will not occur, the farmer's own decisions have little influence on the selection process or that a new herbicide will be introduced that will solve any resistance problem (Doohan *et al.*, 2010). Global experience has been that little is done to prevent or manage herbicide-resistant weeds until they start to cause a significant problem at a field scale (Shaner, 2014). Perversely, surveys of growers often show a good awareness of the need to proactively manage herbicide resistance, although there is a difference in the perception of the need to manage herbicide resistance and the reality of actually doing so (Vencill *et al.*, 2012). Compared with herbicides, non-chemical methods are often more complex and time-consuming to manage, less effective, more variable, less predictable, more risky (to advisor as well as farmer) and more expensive for the level of control achieved and there is often little visible evidence of success (Moss, 2010). Clearly, these are broad generalisations and will not all be relevant to every weed control situation, but they are major reasons why farmers are reluctant to use more non-chemical methods in place of herbicides.

It is, therefore, not surprising that farmers are reluctant to embrace IWM and continue to place greater reliance on herbicides as a simpler, more reliable and cost-effective method of weed control – at least until they fail. Greater adoption of IWM may be achieved by greater attention to the farmer's perspective, and by framing messages in a manner that coincides with the farmer's experience and belief structure (Wilson *et al.*, 2009). Morse (2009) stressed that, while research is an important part of the

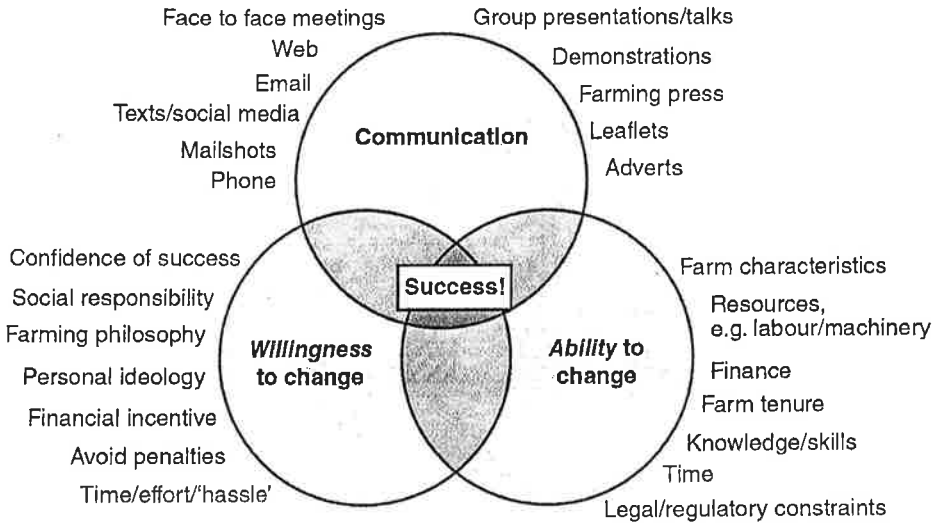


Fig. 7.9 The complex relationship between advice and resulting behaviour.

overall strategy, there must be greater recognition of what farmers are able and willing to 'do'. This consideration needs to be central to the whole process, rather than added in, as so often happens, as an afterthought. A key aim must be a greater recognition of the part played by farmers and their advisors in decision making.

Figure 7.9 highlights the complex relationship between communicating advice (e.g. promoting anti-resistance strategies) and behaviour (e.g. active adoption of those strategies). Advice may be provided to farmers in many different ways, but the method that achieves the biggest impact will vary considerably between individual farmers. It is important to recognise that the advice will only be successfully implemented if the farmer both is *willing* and has the *ability* to change.

Other key aspects of farmer psychology that are important in communicating advice are:

- 'repetition for retention' – patience is needed, as gaining acceptance of major changes in agronomic practices is usually a slow process;
- if the perceived benefit is greater than the 'effort' involved, farmers will accept the advice, but they will not if the 'effort' is more than the benefit;
- it is important to 'let the farmer make the final decision' as 'preaching' can be counter-productive.

Perhaps the critical point is that opinions are amenable to change. For example, if convinced of the benefits of ploughing, a farmer may change from being an 'unwilling/unable' to a 'willing' adopter. If a farmer accepts that major changes in agronomic practices could make a big difference to his weed population, he may well consider the 'effort' (in terms of time, money and 'hassle') worthwhile.

Herbicide resistance and farmer psychology may seem unrelated bedfellows. However, if strategies for resistance prevention and management are not implemented, the very considerable amount of research on which they are based will have been a waste of time,

effort and money. It is clear that successful resistance management will not happen until more attention is paid to the farmer's perspective, and a better appreciation gained of the impact of farmer psychology on decision making.

Conclusion

Herbicide resistance needs to be kept in perspective. Among the thousands of weed species that occur worldwide, only a relatively small proportion have evolved resistance and pose serious problems. Those that have evolved resistance to multiple herbicides pose a particular threat, and these include weed species from the genera *Lolium*, *Amaranthus*, *Conyza*, *Echinochloa* and, in western Europe especially, *Alopecurus* (Heap, 2014). Herbicide resistance used to be considered as largely a problem of developed countries, but increasing use of herbicides worldwide means that the problem is now truly global, affecting 66 countries. The fact that there has been no new herbicide mode of action for over 20 years and the loss of herbicides due to regulatory action means that the threat from resistance is likely to increase due to increased dependency on a more limited number of active substances from long-established, and often resistance-prone, chemical classes.

Herbicide resistance has become a problem because too much reliance has been placed on herbicides for weed control. It is clear that reliance on herbicides alone for weed management is not sustainable and more diversified strategies are required with greater emphasis on non-chemical control methods (Shaner & Beckie, 2014). There has, perhaps, been too much emphasis on academic studies into characterising resistance, at the expense of research and extension activities more directly related to tackling the problem in the field. Strategies for resistance prevention and management are of no use unless they are implemented. It is vital that the very considerable research effort worldwide produces sound, practical herbicide-resistant weed management strategies solutions that are easily implemented by farmers (Shaner, 2014).

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