Experience with herbicide resistance in Europe: global implications

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Summary Herbicide-resistance occurs in 55 weed species in 21 European countries. 91% of cases are associated with just four herbicide groups: ACCase and ALS inhibitors, and triazine and urea/amide photosynthetic inhibitors. There are also a few cases of resistance to bypiridiliums, dinitroanilines and synthetic auxins. Resistance to ALS inhibitors tends to be less prevalent in Europe than elsewhere, but is likely to increase. The major resistance problems are the grass-weeds *Alopecurus myosuroides* Huds. and *Lolium* spp. which show multiple resistance to at least eight different herbicide groups. Within EU countries there is a requirement to submit baseline sensitivity data with submissions for new active ingredients where resistance is a risk. The aim is to establish the variation in herbicide response between weed populations, prior to the introduction of the new herbicide. There has been little progress on pesticide mode of action labelling and there is no general agreement on this at present. Virtually no commercial GM crops are grown in Europe although there are numerous research projects, including extensive field trials. The main issue preventing the commercialisation of GM crops within Europe is public acceptance. It is far from clear what will happen in the future regarding the growing of GM crops in Europe.

Keywords Herbicide resistance, weeds, Europe.

HERBICIDE-RESISTANT WEEDS IN EUROPE

Herbicide-resistant weeds have been identified in 21 European countries, with the highest number of resistant biotypes found in France (30), Spain (24), United Kingdom (23), Belgium (17) and Germany (17) (Table 1). Consequently, herbicide-resistance is mainly a problem in western-Europe. These 21 European countries represent 40% of the 53 countries worldwide in which herbicide resistant weeds have so far been detected (Heap 2002).

Within Europe, triazine-resistance occurs in 16 countries, ALS resistance in 12 countries, urea/amide resistance in 10 countries, ACCase resistance in eight countries and resistance to other mode of action groups in seven countries.

Table 1 also presents the total number of resistant biotypes by major mode of action groups, and summarises the comparative information for the 32 non-European countries with resistance. The per cent values allow comparison of the relative incidence of resistance to different mode of action groups within Europe compared with countries in the rest of the world. Compared with the 32 non-European countries in which resistance has developed, resistance to ALS inhibitors tends to be less prevalent (9% vs. 32%), and resistance to triazines more prevalent (63% vs. 18%) in Europe. The incidence of resistance to ACCase inhibitors and ureas/amides is similar in European and non-European countries.

In Europe, 91% of cases of resistance are associated with just four herbicide mode of action groups: ACCase and ALS inhibitors, and triazine and urea/amide photosynthetic inhibitors. In the 32 non-European countries, the comparative figure is 71%, showing that resistance to other herbicide groups is comparatively more important.

Resistance has evolved in 55 species in Europe and those in which resistance has evolved most frequently are listed in Table 2. Seven of these (Numbers 1, 3, 4, 7, 9, 10, 12) are listed by Heap (2002) as being in the top 10 most important herbicide-resistant weeds worldwide. Half of the species both in his worldwide list, and in the European list in Table 2 are grass-weeds. A small number of species, especially of the *Poaceae* (*Gramineae*) appear to be particularly prone to evolve resistance worldwide. While there is no clear relationship between plant families or genera and their tendency to evolve resistance, grass-weeds tend to be over-represented in the list of resistant biotypes both within Europe and worldwide (Heap 1999). While grass-weeds accounted for 33% of all resistant species and 40% of all resistant biotypes, they account for only 25% of the world's major weeds.

Resistance usually evolves in one, or at most a few, species in a weed community, despite all being exposed to the same intensity of herbicide use. The reasons for this are poorly understood at a fundamental level, which is why our ability to predict the risk of resistance evolving in any given species is poor. For example, throughout Europe, two of the commonest weeds are *Poa annua* L. and *Sinapis arvensis* L. yet few cases of resistance have been reported in either species. However, once a species is known to be prone to evolve resistance, then that information can be used to predict the likelihood that resistance will evolve subsequently in other locations or countries.

Table 1. Occurrence of herbicide-resistant weed biotypes in Europe by country and mode of action, and % in each category for 21 European and 32 non-European countries. Based on Heap (2002).

	Country	Total resistant biotypes	A ACCase inhibitors	B ALS inhibitors	C1 Triazines	C2 Ureas/ amides	Other
1	France	30	5	1	22	1	1
2	Spain	24	1	1	18	3	1
3	United Kingdom	23	4	3	8	2	6
4	Belgium	18	2	1	7	1	7
5	Germany	17	1	0	13	3	0
6	Switzerland	14	0	0	11	3	0
7	Czech Republic	13	0	1	12	0	0
8	Italy	10	3	4	3	0	0
9	Poland	9	0	1	8	0	0
10	The Netherlands	7	1	0	5	1	0
11	Greece	5	1	1	2	1	0
12	Bulgaria	4	0	0	2	1	1
13	Norway	4	0	0	3	1	0
14	Austria	2	0	0	2	0	0
15	Sweden	2	0	1	0	0	1
16	Yugoslavia	2	0	0	2	0	0
17	Denmark	1	0	1	0	0	0
18	Hungary	1	0	0	0	0	1
19	Ireland	1	0	1	0	0	0
20	Portugal	1	0	1	0	0	0
21	Slovenia	1	0	0	1	0	0
	Total in 21 European countries	**189**	**18**	**17**	**119**	**17**	**18**
	Total in 32 non-European countries	270	40	86	49	15	80
	Total in 53 countries worldwide	459	58	103	168	32	98
	As % of totals						
	European countries	100	10	9	63	9	10
	Non-European	100	15	32	18	6	30
	Worldwide	100	13	22	37	7	21

Note: Some biotypes of the same weed species were recorded in several different countries. Consequently the totals do not represent unique biotypes. The total number of unique herbicide-resistant biotypes recorded worldwide was 258 (Heap 2002).

IMPACT OF HERBICIDE-RESISTANT WEEDS

The number of species that have evolved resistance and the number of countries in which they occur is a useful indication of the frequency of occurrence of resistance. However, such information does not in itself give any indication of the scale of the resistance problem. An overview of the impact of resistance to the major mode of action groups is given below.

Triazines (Group C1) The repeated use of herbicides such as atrazine and simazine in maize, orchards, horticultural crops and on non-cropped land such as railways led to numerous reports of resistance between 1978 and 1990. Virtually all reported cases are due to a herbicide-insensitive target site (the D1 protein) as found in most cases of triazine resistance worldwide. Resistance to triazines accounts for 63% of the total

Table 2. The 12 most common herbicide-resistant weed species in Europe listed in order of number of records (Heap 2002).

	Weed species	Records	Countries
1	*Amaranthus* spp. (mainly *retroflexus* and *hybridus*)	22	10
2	*Alopecurus myosuroides*	15	7
3	*Chenopodium album*	15	14
4	*Conyza canadensis*	10	7
5	*Solanum nigrum*	10	7
6	*Poa annua*	10	7
7	*Lolium* spp.	9	5
8	*Senecio vulgaris*	9	8
9	*Setaria* spp.	8	3
10	*Echinochloa crus-galli*	7	6
11	*Epilobium* spp.	7	5
12	*Avena* spp.	6	4

number of herbicide resistant biotypes recorded in Europe. However, it has not become a major, long term problem as alternative herbicides with different modes of action have been used successfully, although there is a lack of information on the impact of triazine resistance in Eastern Europe. Relatively few new records of triazine resistant biotypes in Europe were submitted to the herbicide resistance database after 1990 (Heap, 2002).

ALS inhibitors (Group B) Resistance to ALS inhibitors has occurred much less frequently in Europe than in many other areas. Resistance to ALS herbicides has been confirmed in 72 biotypes worldwide, but in only nine species in Europe (Heap 2002). The first case of high degree resistance in Europe occurred with *Stellaria media* (L.) Vill. in Denmark in 1991. ALS-resistant *S. media* has subsequently been recorded in Sweden (1993), Ireland (1996), Scotland (2000) and England (2001), but so far on only very few farms (<5) in each country. ALS-resistant *Papaver rhoeas* L. was first found in Spain in 1993 and has subsequently been identified in Portugal, Italy, Greece and England. Many farms are affected in Spain, and some populations also show resistance to synthetic auxin herbicides such as 2,4-D. ALS-resistance has also evolved in *Alisma plantago-aquatica* L. and *Scirpus mucronatus* L., which are weeds of rice in Italy and Portugal.

The mechanisms of resistance have not been fully documented in all the ALS-resistant weeds in Europe, but appears to be due mainly to the presence of mutated forms of ALS, as found in many broad leaved weeds worldwide. Some populations of *A. myosuroides*, *Lolium rigidum* Gaudin and *Avena sterilis* ssp. *ludoviciana* (Durieu) Gillet & Magne show resistance to sulfonylureas and imidazolinones as well as to several other mode of action groups. It appears that resistance to ALS-inhibiting herbicides in these grass weeds is due, at least at present, mainly to enhanced metabolism rather than to target site resistance due to a mutated ALS.

ACCase inhibitors (Group A) Resistance to ACCase inhibitors has been reported in six species in Europe: *A. myosuroides*, *Avena fatua* L., *A. sterilis* ssp. *ludoviciana*, *Lolium multiflorum* Lam., *L. rigidum* and *Phalaris paradoxa* L. Resistance was first reported in *A. myosuroides* in Germany and the UK in the early 1980s, and since 1990 many more instances have been documented. These grass-weeds cause major resistance problems within many countries of western Europe: Belgium, France, Germany, Italy, Netherlands, Spain and UK. There is evidence that resistant *Lolium* spp. in particular are becoming increasingly important in many other European countries. Studies into the mechanisms of resistance in *A. myosuroides*, *Avena* and *Lolium* spp. have demonstrated both enhanced metabolic detoxification of herbicides and target site resistance due to an insensitive ACCase (De Prado and Menendez 1996). Both glutathione S-transferases (GSTs) and P450 monooxygenase enzymes have been implicated in enhanced metabolism. Other mechanisms of resistance to ACCase inhibitors, such as recovery of plasma membrane potential, lack of translocation and lack of conversion of the ester to the herbicidally active acid form of aryloxyphenoxypropionate herbicides have been investigated. However, the importance of these mechanisms, and others such as the over expression of ACCase, remains unclear. In Europe, *A. myosuroides* and *Lolium* spp. are the two best examples of weeds exhibiting multiple resistance.

Recent investigations into the molecular basis of target site resistance in *A. myosuroides* in the UK showed that sethoxydim resistance was associated with the substitution of an isoleucine in susceptible with a leucine in resistant plants, which has also been found in three other resistant grass-weed species (*Setaria viridis* (L.) P. Beauv., *A. fatua*, *L. multiflorum*) (Brown *et al*. 2002, Delye *et al*. 2002). Given the varying cross-resistance patterns seen in resistant populations of many grass-weed species, it is likely that other mutations exist which have yet to be identified.

Ureas/Amides (Group C2) Nine weed species have been identified which show resistance to ureas and one species, *Echinochloa crus-galli* (L.) P.Beauv., to the amide propanil (Heap 2002). Most of the species showing resistance to ureas are grass-weeds of cereals; *A. myosuroides*, *Apera spica-venti* (L.) P.Beauv., *Bromus tectorum* L., *L. rigidum*, *L. multiflorum*. Many populations of *A. myosuroides* show resistance to the substituted ureas, chlorotoluron and isoproturon, in several countries, but particularly in the UK.

The mechanism of resistance to the substituted urea herbicides in all the grass-weeds studied so far, is due to enhanced metabolism involving P450 monooxygenases or, in the case of *E. crus-galli*, elevated aryl-acylamidase activity against propanil (De Prado and Menendez 1996). The enhanced ability to metabolise herbicides in *A. myosuroides* and *Lolium* spp. confers cross-resistance to many other herbicides with different modes of action.

Other herbicide groups Resistance has been also demonstrated to the bypiridiliums (D), dinitroanilines (K1) and synthetic auxins (O), but to a limited extent. However, grass-weeds with multiple resistance, especially *A. myosuroides* and *Lolium* spp. show at least partial resistance to some herbicides in at least eight different herbicide groups.

TARGET SITE VS. ENHANCED METABOLISM

Resistance conferred by an insensitive target site is the mostly widely recognised resistance mechanism worldwide. Most, but not all, cases of resistance to triazines, ALS and ACCase inhibitors are considered to be due to target site insensitivity. This is usually characterised by a high degree of resistance which can build up rapidly in response to repeated applications of herbicides with the same mode of action.

Enhanced metabolism is also recognised as a mechanism in several species, such as *L. rigidum* in Australia, *Phalaris minor* Retz. in India and *A. myosuroides* in Europe. This often gives partial, rather than absolute resistance, although this is often sufficient to cause substantial reductions in herbicide efficacy in the field. The genetics of enhanced metabolism is poorly understood in most species but is generally considered to be polygenic.

Worldwide there has been a tendency to place most research emphasis on highly resistant populations, as these can result in spectacular failures in the field. In many such cases, resistance is due to an insensitive target site which, while giving a high degree of resistance, affects only a single herbicide group. Such populations may be controlled easily by alternative herbicides, assuming they are available.

Mechanisms such as enhanced metabolism, which usually result in reduced, rather than no herbicide activity, tend to be overlooked and are probably under-recorded worldwide. Although the degree of resistance may be lower, resistance can extend to many different herbicide groups. Cross-resistance within and between herbicide groups is largely unpredictable, because the degree to which herbicides are metabolised is dependent on molecular structure, and not related in any simple manner to the conventional grouping of herbicides by modes of action. Enhanced metabolism tends to evolve slowly but may ultimately be of greater significance because of the effects on a wider range of herbicides.

In UK populations of *A. myosuroides*, *Avena* spp. and *L. multiflorum*, enhanced metabolic detoxification of ACCase and other herbicides is the commoner mechanism of resistance at present (Table 3), although target site resistance conferred by an insensitive ACCase has also been detected in all three species (Cocker *et al*. 2001).

Enhanced metabolic detoxification has also been demonstrated to be a major mechanism of resistance in *L. rigidum* in other European countries (De Prado and Menendez 1996). In Europe a substantial research effort has been made in studying enhanced metabolism as a resistance mechanism in the major grass-weeds.

It should be born in mind that many other, as yet unrecognised or under recognised mechanisms, may exist and these deserve detailed investigation. This point was also made by Powles and Matthews (1992).

'A herbicide exerts strong selection pressure favouring individual plants possessing genes endowing *any* mechanism enabling survival in the presence of that herbicide. When a herbicide is applied to a large, polymorphic population there are plants which survive because they possess one or more (different) mechanisms enabling survival at the dose of herbicide used. This means that there can be survivors because of *different* resistance genes. There is no reason to believe, *a priori*, that only one resistance mechanism will be exclusively selected from a panmictic population. Similarly, there is no *a priori* reason to believe that mechanisms selected from geographically diverse populations will be the same, or present at the same frequency before, during or after the selective process.'

BASELINE SENSITIVITY

Baseline sensitivity (or background monitoring) is now specifically referred to in the resistance risk

Table 3. Number of UK populations evaluated for resistance mechanisms at the biochemical level.

	Suscept.	Metabolism	Target site
A. myosuroides	2	7	2
Avena spp.	2	2	1
L. multiflorum	2	3	1

analysis section of the European and Mediterranean Plant Protection Organisation's (EPPO) 'Guideline for the Efficacy Evaluation of Plant Protection Products' (EPPO 1999a). The guideline sets out to communicate to both pesticide registration authorities and applicants, what their obligations are with respect to assessing resistance risk and developing appropriate management strategies. Although it is unlikely that all 43 EPPO member countries, (mainly but not exclusively European), will adopt a unified approach, registrants will be required to adhere to the EPPO Guidelines unless they can give good reasons for not doing so. Consequently, there will be a *requirement* to submit baseline sensitivity data with submissions for new active ingredients where a resistance risk has been identified.

The aim of baseline sensitivity testing is to establish the scale of variation in herbicide response between weed populations, prior to the introduction of the new herbicide. Any subsequent changes in sensitivity of a weed to the herbicide, after it is introduced commercially, should then be detected more reliably. A good baseline will enable any cases of evolved herbicide resistance to be identified promptly.

Companies will increasingly need to analyse resistance risk, and modify use patterns or develop management strategies, at an early stage in product development within Europe. Resistance risk evaluation will become an integral part of the registration decision making process.

Baseline sensitivity is only one aspect of resistance risk assessment but has been one of the most controversial aspects due to widely different views on how best to satisfy registration requirements. Moss (2001) suggested procedures which should assist registrants in satisfying the requirement for baseline sensitivity data, although these do not constitute an EPPO agreed protocol.

PESTICIDE MODE OF ACTION LABELLING

There has been little progress on pesticide mode of action labelling within Europe. Companies are reluctant to include such information on pesticide labels. The arguments against mode of action labelling are the same as those raised in other countries: codes are too simplistic, complications with herbicide mixtures, labelling does not address metabolism-based/multiple resistance, too much information on labels already, farmers do not read labels (Orson 1999a). There is a wider acceptance within companies of the inclusion of mode of action information in technical literature, although there is no Europe or company wide agreement on this at present.

GM HERBICIDE RESISTANT CROPS

The estimated global area of transgenic (GM) crops grown in 2001 was 52.6 million ha grown by 5.5 million farmers in 13 countries worldwide (James 2002). Four countries grew 99% of the global transgenic area (USA, Argentina, Canada, China). Between 1996 and 2001, herbicide tolerance, especially in soyabean, maize and cotton, has been consistently the dominant trait occupying 77% of the global GM area.

Virtually no commercial GM crops are grown in Europe, although there is a very intensive programme of research. Many field trials are being conducted especially on GM maize, oil-seed rape and sugar beet. In the UK there has been a voluntary moratorium on the growing of GM crops commercially until the completion of an extensive series of field scale evaluations, due for completion in 2002/03. A similar situation exists in other EU countries.

The main issue preventing the commercialisation of GM crops within Europe is public acceptance, and it is clear that most Western European governments have accepted delays in the regulatory system for marketing GM crops due to the concerns of potential voters. Recent surveys indicate that the acceptance of GM technology is increasing slightly, but the majority of the 'general public' are still 'anti-GM'. The public's main concerns are about food safety and genetic contamination via pollen. The shift from 'input' agronomic traits (such as herbicide resistance) to 'output' (quality) traits may increase public acceptability of GM crops in Europe.

Some countries consider that Europe's unwillingness to grow or import GM crops is protectionist and a barrier to free trade. This is likely to be a major issue in talks within The World Trade Organisation (WTO). It is far from clear what will happen in the future regarding the growing of GM crops in Europe. Glyphosate-resistant crops have potential benefits for the management of herbicide-resistant weeds, especially those with multiple resistance such as *A. myosuroides* and *L. multiflorum*. No glyphosate-resistant weeds have been found in Europe yet. Whether the introduction of glyphosate-resistant crops is a benefit to resistance management, or simply increases the potential problem, is debatable.

CONCLUSIONS

Herbicide resistance needs to be kept in perspective. Whereas some resistant weeds unquestionably cause major problems in Europe, as elsewhere, many are of minor significance and easily managed. The decreasing use of triazine and urea herbicides, due to regulatory restrictions related to groundwater contamination and availability of better alternatives, means that resistance to these two groups is likely to continue to decline in importance in Europe. Although resistance to ALS inhibitors is less common in Europe than elsewhere, it is likely to increase, especially in grass-weeds as more active herbicides are introduced. Resistance to ACCase inhibitors is also likely to increase. Resistance to glyphosate has not yet been recorded within Europe, and although it is probable that instances will be detected soon, the scale of any problem is uncertain.

The major resistance problems, at least in the short term, are likely to be *A. myosuroides* and *Lolium* spp. These grass-weeds are very competitive, well adapted to Western European cropping systems, capable of rapid population increase, so high levels of weed control are needed to contain them. This is difficult to achieve as both species exhibit cross- and multiple resistance to a wide range of herbicides. It is unlikely that many herbicides with new modes of action will become available.

Attempts have been made to calculate the cost to farmers of herbicide resistance in Europe (Orson 1999b). Resistance has a cost in terms of more expensive herbicides and cultural control measures, but there is also a cost attached to preventing resistance. The challenge is to persuade farmers to spend money on preventing resistance, which may of course, never happen on their individual farm.

It is clear that a broader based approach to weed control is needed in which herbicide use is integrated with non-chemical methods of weed control. It is vital that the very considerable research effort within Europe and elsewhere produces sound practical solutions. Strategies for resistance prevention and management are of no use unless implemented. It is essential that technology transfer initiatives are developed to ensure the effective communication of practical advice to farmers and growers.

ACKNOWLEDGMENTS

Financial support from DEFRA and the Home-Grown Cereals Authority (HGCA) is gratefully acknowledged. IACR-Rothamsted receives grant-aided support from the Biotechnology and Biological Sciences Research Council.

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