The Ultimate Guide to Herbicide Resistance

The herbicide resistance challenge

Herbicide resistance is a major threat to the sustainability of UK arable farming – it is a problem no farmer or agronomist can ignore.

Understanding the types and mechanisms of resistance, with how it develops at the cellular and field scale should be the first step in any effective control strategy.

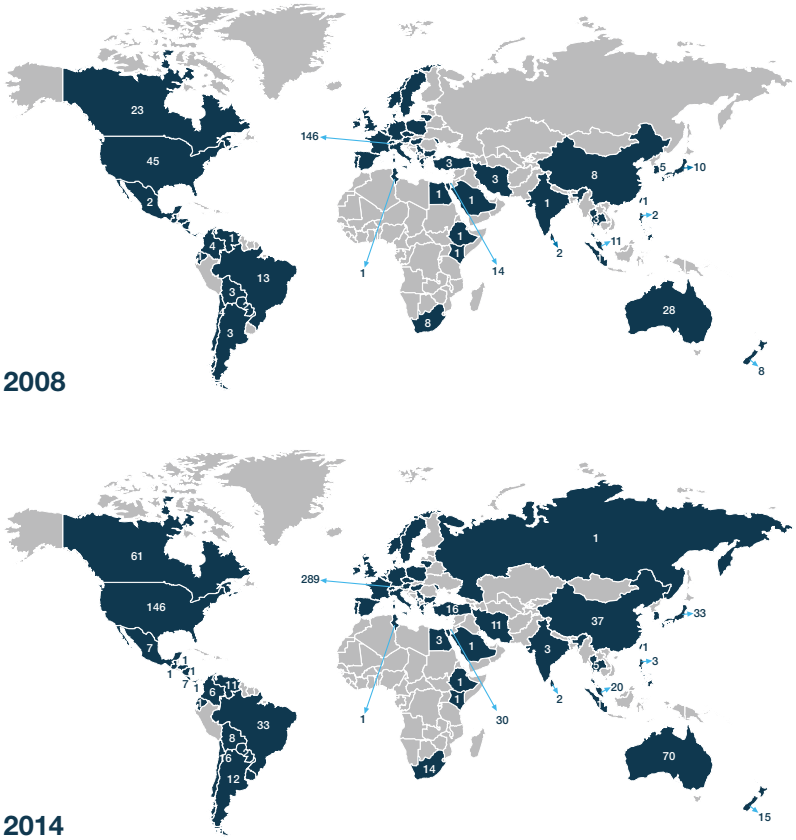
This booklet explains the science behind weed resistance and provides information which can be incorporated into your weed control management plan. ▸

A global threat

Our focus is on herbicide resistant black-grass (Alopecurus myosuroides), which has become the most widespread problem for UK arable farmers since resistance to acetyl-CoA carboxylase (ACCase) inhibitors ('fops' & 'dims') was discovered in 1982, but many principles apply to other weed species.

Indeed, there are currently around 250 herbicide resistant weed species in 86 crops and 66 countries around the world (see figure 1 below). Globally, weeds have evolved resistance to 23 of the 26 known herbicide sites of action and 160 different herbicides[1].

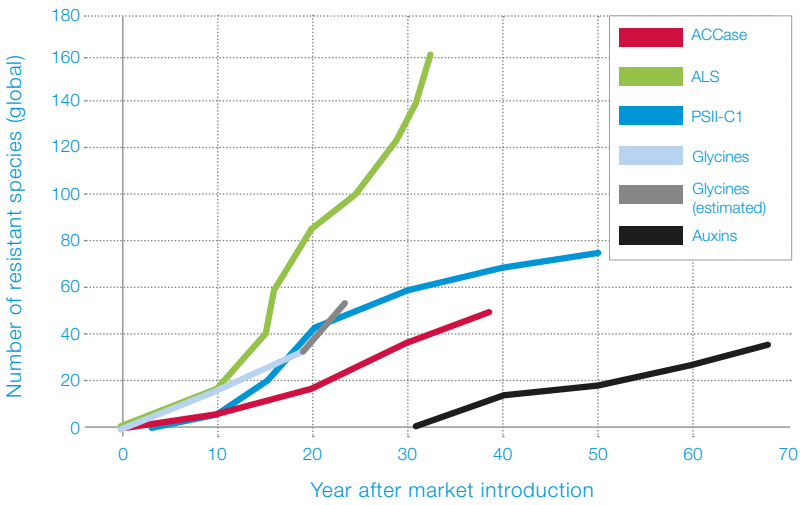
Figure 1:
Global resistance trends: confirmed resistant biotypes



Drawn from data from Heap, 2015

There have been significant differences in the speed of resistance development among different modes of action (MoA) (see figure 2 below), with the most rapid increase among ALS-inhibiting herbicides (e.g. sulfonylureas). However, there remains an increasing resistance trend across all key MoA.

Figure 2:
Number of resistant species for selected MoA



Drawn from data from Heap, 2015

The situation is being compounded by the number of weed species exhibiting multiple resistance to more than one MoA. This has increased steadily over the past 40 years and further limits the control options available.

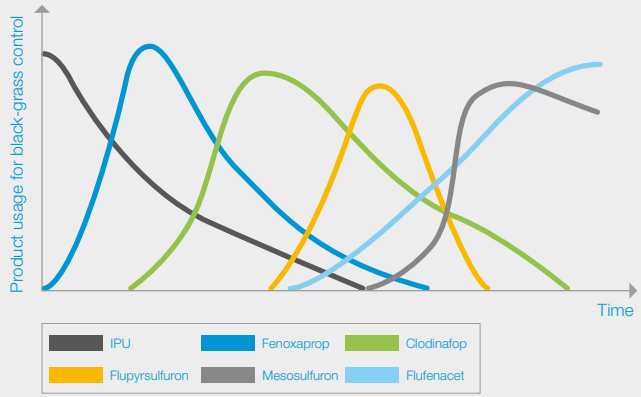
Total losses to weeds worldwide are estimated to be around 13.2% of agricultural production, worth more than €55 billion a year. This could feed around 1 billion people.

No silver bullet

Historically, herbicide manufacturers have always been able to combat resistance by introducing new chemistry that can overcome resistance to a particular active ingredient or MoA.

The discovery of new actives has declined considerably since the period between 1990 and 2004 (see figure 3 below), due to a variety of reasons, not least longer development cycles, higher costs and tighter regulation.

Figure 3:
UK black-grass herbicide life cycles
Product usage



Source: Bayer, 2015

Quite simply, we are not registering new herbicides fast enough to replace those that have been lost, which puts greater pressure on the remaining chemistry.

The long-term benefits to UK farmers of tackling herbicide resistance far outweigh the potential losses to their own business and the industry as a whole from doing nothing.

Herbicide resistance is here to stay and we all have a duty to tackle it at the same time as safeguarding existing chemistry. Fully understanding the problem is just the first step in this process.

What exactly is resistance?

Herbicide resistance is defined as:

‘the inherited ability of a weed to survive a rate of herbicide that would normally kill it[2].’

Three key criteria must be fulfilled for a plant to be classified as ‘resistant’:

- Resistance must be heritable (passed on to offspring)
- The plant must occur naturally and not be the result of deliberate/artificial selection
- Resistance must be confirmed using acceptable scientific protocols (see Chapter 3)

Central to the definition is ‘inherited ability’. Resistance development is a natural evolutionary process resulting from a genetic mutation within plants that is selected for by the use of herbicides.

Contrary to some misconceptions, gene mutations are not generally caused by the application of a herbicide[3], but occur naturally in plants. They can be caused by a variety of factors, including cosmic and solar radiation, and through the natural DNA repair process.

So-called spontaneous mutation rates vary massively[4]. Estimates suggest a point mutation involving a single amino acid exchange conferring target-site resistance (TSR), for example, occurs in many weeds at a frequency of 1 in 1 million, although more conservative estimates suggest 1 in 10 million[5]. Inevitably the bigger the starting population, the more chance there is of a mutation occurring.

How this mutation is exhibited in the field depends on which gene is affected. Some mutations show no discernible impact on the plant, while others could be detrimental or give the plant an evolutionary edge by making it better able to survive new threats.

Given enough time, any weed will try to adapt to chemical, culturalor mechanical selection pressure.

Nature favours individuals with a competitive advantage – weeds that are more tolerant to any selection pressure (such as moisture or disease) have the best chance of survival.

How does it spread?

Once a mutation has occurred, the trait (e.g. herbicide resistance) is embedded in the genetic make-up of the plant and can be passed to offspring. Only one plant has to survive and produce viable seed for the traits conferring resistance to be passed on to the next generation.

Repeated application of any herbicide, for which a resistant gene is present will eventually select for yet more plants with reduced susceptibility, resulting in a build-up of the resistant population (see figure 4 in Chapter 1).

Resistant plants may come to dominate the weed population, resulting in herbicides having little or no impact on weed control. Again, the size of the starting population and annual seed return has a big impact on how quickly resistant populations develop.

Beware the false alarm

It is worth remembering that several other factors could cause a plant to survive herbicide treatment and it may not always be due to resistance.

Other factors which must be eliminated first include:

- Application problems
- Rain after application
- Too cold or too dry for herbicides to work
- Germination from outside the zone of activity (pre-ems.)

How resistance develops

The repeated use of herbicides with the same mode of action (MoA) has undoubtedly led to the evolution of resistant weed populations, but herbicides themselves are not to blame for causing resistance in the first place.

We have already discussed how resistance initially develops from a natural spontaneous genetic mutation within the plant, populations of which are then selected for by the application of a selection pressure such as a herbicide of a given MoA or a specific cultural practice.

Indeed, an investigation of 734 black-grass specimens collected between 1788 and 1975 found one sample from 1888 which contained a mutation giving resistance to acetyl-CoA carboxylase (ACCase)-inhibiting herbicides; clearly demonstrating herbicides do not fabricate resistance within a plant[6].

The process of how resistance develops and is passed on through subsequent generations is summarised in the following sections.

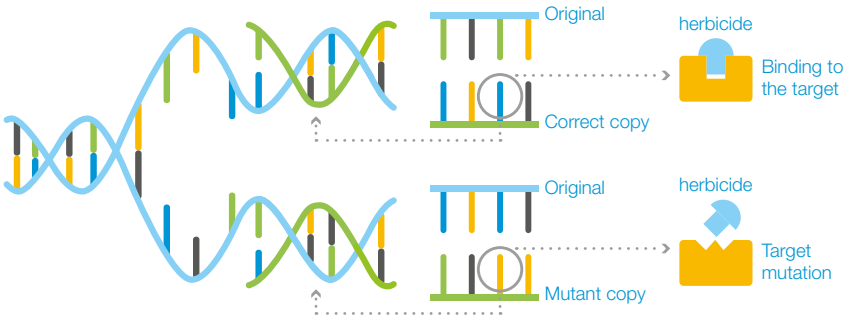
Resistance development at the genetic level

The four main resistance mechanisms described in Chapter 2 initially develop in a similar way at the genetic level.

Genes are the blueprint which living things are built on. The code which genes carry is used to create proteins and it is proteins which carry out the functions in the cell. Natural gene mutations occur spontaneously in all plants and have the potential to change how proteins function. It is largely down to chance where any mutation strikes and how it alters that gene. Some have no impact on the plant's characteristics (phenotype); others have a detrimental effect, while some can give it an evolutionary advantage.

When a mutation occurs in the specific gene which encodes a protein which a herbicide acts on, resistance can develop (see figure 4 below). This can result in a structural change in the herbicide binding site (see Chapter 2).

Figure 4:
Resistance development in the plant



Source: Bayer, 2015

This type of resistance is known as target-site resistance (TSR) and it is usually inherited via a single gene and has been relatively easy to study by scientists.

However, more genetically complex non-target-site resistance (NTSR) mechanisms are also widespread and are currently less well understood. A number of projects are underway to better understand the evolution of inheritance patterns for NTSR[7].

The main form of NTSR is enhanced metabolism resistance (EMR). EMR works by inactivating a herbicide through a degradation mechanism in the plant that minimises the amount of the herbicide reaching the target site (see Chapter 2 for more).

Furthermore, multiple resistance mechanisms (e.g. both TSR & EMR) can be present within resistant individuals, often involving complex genetic linkages[8].

Herbicide resistance controlled by two or more genes is referred to as being polygenic.

Cross resistance refers to resistance to two or more herbicides caused by a single resistance mechanism. Conversely, multiple resistance is when resistance to several herbicides is due to two or more resistance mechanisms in the same plant[9].

Inheriting resistance

The evolution and spread of herbicide resistance through subsequent generations is best explained by the Mendel theory of inheritance[10] (see figure 5 on p.16).

A genetic mutation in the parent generation results in different versions of a gene being formed, which are called alleles. Plants have two alleles for any particular gene and these can either be an identical pair (homozygous), or two different alleles (heterozygous).

During the breeding process the two alleles segregate during gamete production, with one allele going to the male part (pollen grain) and the other to the female ovum. If different alleles are present then 50% of gametes receive the dominant resistant allele while 50% receive the recessive susceptible allele.

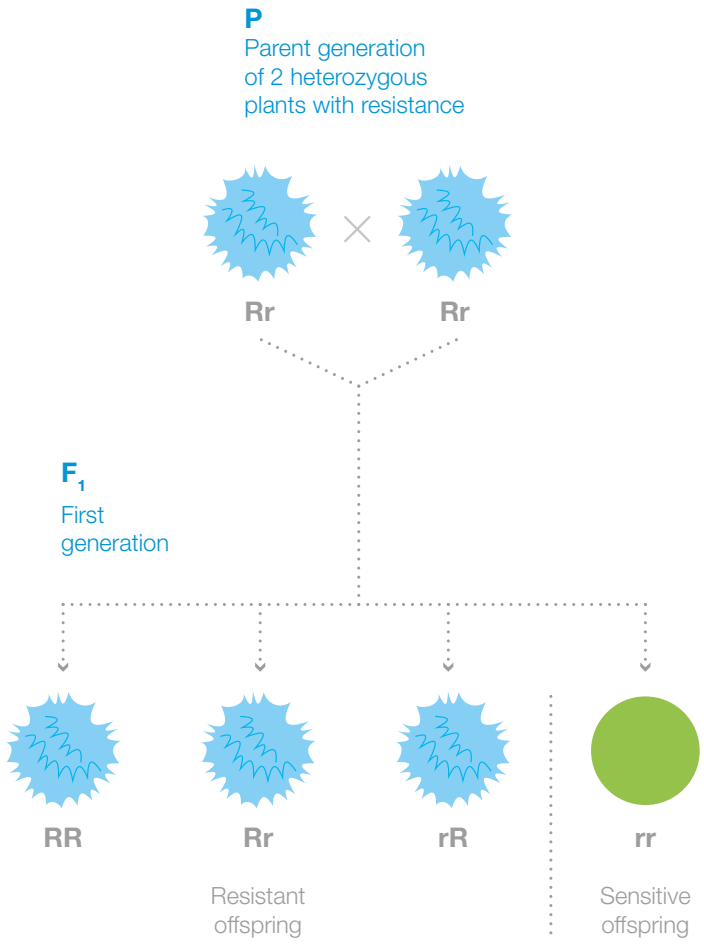
The offspring resulting from cross-pollination (the F1 generation) therefore inherit two alleles, one from each parent.

If the inherited alleles differ (i.e. one confers herbicide resistance and the other is normal), it is the dominant resistant allele that will be fully expressed in the phenotype of the offspring.

Because the resistant allele is dominant, three quarters of the offspring from this cross will be resistant, but there remains one quarter which will still be susceptible to the herbicides having inherited the susceptible allele from both parents.

Therefore even if 100% of seed present in a population is classed as ‘resistant’, it does not necessarily mean that every seed shed by this population is resistant. In reality there will be a slow build-up of resistant individuals within a population over several seasons.

Figure 5:
Development of hereditary resistance



Source: Bayer, 2016

Field-scale development and spread

During herbicide treatment, the active ingredient will only be effective against those individuals which are susceptible. Those which are resistant will survive treatment and be free to set seed and reproduce in following seasons.

Because black-grass is a cross-pollinating species, resistant genes can therefore be spread aerially in pollen, as well as via seed return in the soil.

Studies have shown around 70% of pollen dispersal occurs within 1m of the donor plant, although fertilisation can occur 60m away in the absence of physical barriers[11].

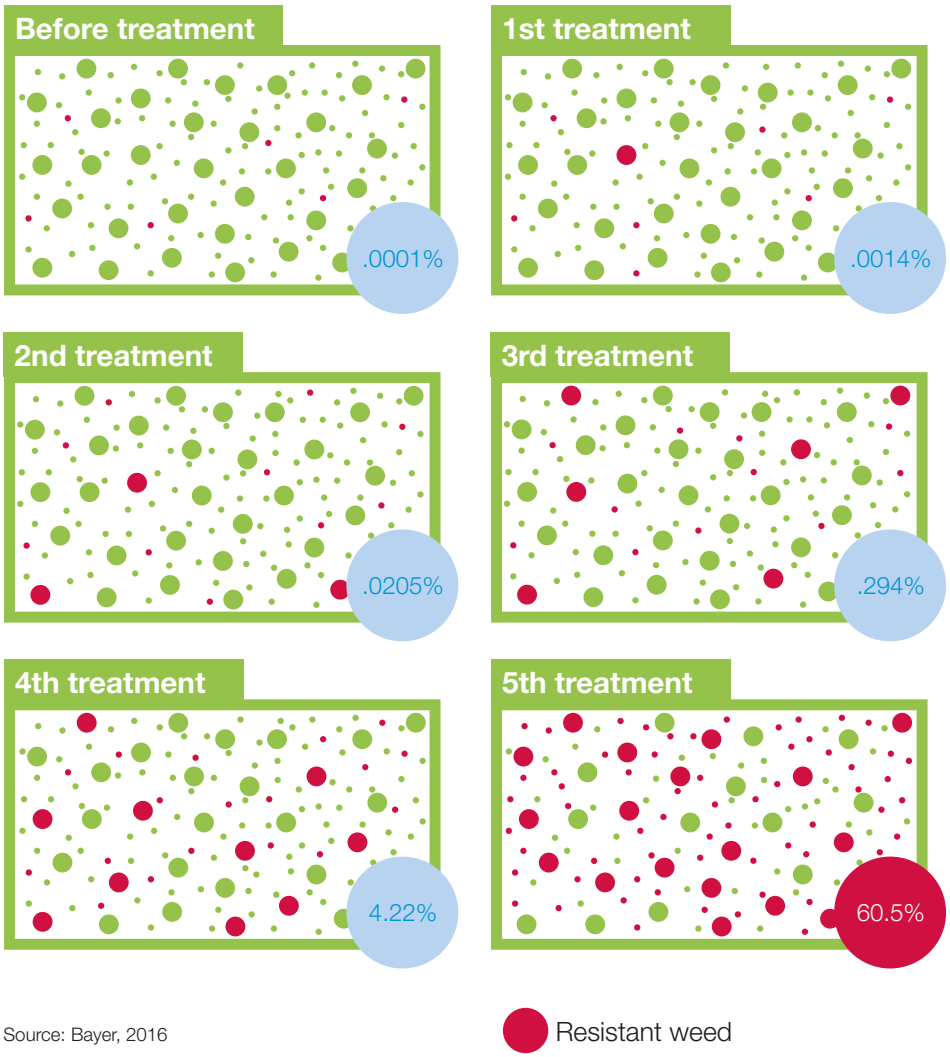
Seed spread is an equally significant factor given that black-grass seed return[12] can easily exceed 50,000/m^2. This makes managing seed accumulation in the soil profile one of the biggest challenges for growers and agronomists alike.

Repeated use of a particular herbicide gradually selects for plants with the resistance gene, leading to a build-up of the resistant population in the field, as illustrated in figure 6.

Figure 6:

Development of herbicide resistance through a field

% Resistant weeds in population



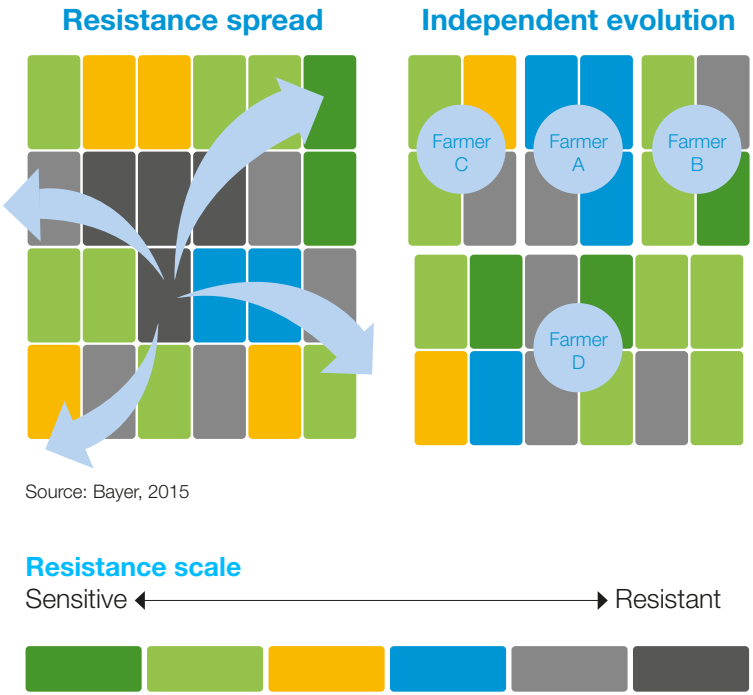
Source: Bayer, 2016

Once resistant plants have established within a field, it can be relatively easy for seeds from these plants to be picked up and moved within the field by cultivation, harvesting or baling equipment.

It is also possible for seeds from resistant plants to be picked up and transported longer distances by machinery, straw and manure, slurries[13] or digestate, moving between fields or farms, resulting in a wider geographic spread of resistant individuals (see figure 7 below).

Although this does occur, resistance can also develop independently in fields. For example, farmers may use weed resistance control measures correctly but still observe the development of resistance in their fields, after enjoying excellent weed control.

Figure 7:
Spread of resistance from field to field



Source: Bayer, 2015

It is widely believed that resistance appears on farms having been transported there (on kit, in straw etc.) and then spreads from field to field as in figure 7. However, it is becoming increasingly evident that independent evolution plays a very important role in promoting or slowing the occurrence of resistance.

When black-grass appears in a field, especially in areas where it has not traditionally been a problem, it can be easy to assume herbicide resistant seeds have been imported from a neighbouring field or elsewhere.

While this may be the case in some instances, do not assume a weed is resistant just because it survives spraying.

As mentioned previously, a number of other application factors could be responsible and these must be eliminated first. The only way to be sure resistance is to blame is to conduct an appropriate resistance test on the surviving population (see Chapter 3).

It is possible seed has been brought into a field from elsewhere, but equally, it may be due to several other reasons, including:

- An independent mutation within the field population which triggers resistance. This is then selected for by repeated applications of certain chemistry
- Resistant populations may have been present for some time at low background levels, but incorrectly put down to poor spray application conditions or performance
- Resistant weeds may have only become evident when the appropriate selection pressure (i.e. herbicide with a certain MoA) has been applied

Resistant seed may also have been ploughed up from depth after laying dormant for several years – black-grass typically persists in the soil for up to five years, but there are suggestions it can remain viable for longer.

Just because excellent control has been achieved over a number of years and black-grass has never been a major issue, it does not mean the problem hasn't been evolving in the background.

Indeed, it may take just a single season of poor control, for any reason, for weed levels to reach such a point where the problem is finally noticeable.

Resistance risk assessment

Understanding how resistance develops is the first step in controlling the problem, but knowing the factors which exacerbate resistance pressure is equally important.

Although active ingredients have different MoA, nearly all are affected by herbicide resistance to some extent (see figure 8 in Chapter 2).

The risk of developing resistance is increased (or decreased) by a combination of factors that increase the selection pressure on a given herbicide.

Essentially it is a 'numbers game' where higher weed infestations combined with few modes of action and limited control methods within a cropping system leads to higher resistance risk.

The main factors that speed up or delay the onset and future development of resistant populations are summarised below:

Increase resistance risk

- Chemistry focused on a single MoA
- Same MoA used several times within a single cropping season or rotation
- Poor efficacy from applied herbicides (e.g. applied in sub-optimal conditions, below an effective label rate[14] or poor timing)
- Reliance on chemical weed control only
- Crop rotation limited to one or two crops (monoculture)
- High background levels of weed infestation
- Poor weed control in previous seasons and increased weed seed bank
- Virulent weeds allowed to thrive due to poor control
- Unknown resistance status due to lack of testing

Delay resistance risk

- Varied chemistry, including mixes or sequences of products with different modes of action (>2)
- Same MoA only once in a season
- Maximise efficacy by following application guidelines and label recommendations correctly
- Use of a variety of cultural, mechanical and chemical weed control options
- Varied rotation, incorporating winter and spring-sown crops
- Maintaining low weed levels
- Resistance tests and plant counts carried out regularly to monitor changes in resistance status and weed population

Types of resistance

Herbicide resistance mechanisms are very diverse but can be broadly divided into two classes; target-site and non-target-site resistance.

Three mechanisms dominate UK grass-weed populations[15], the most common being enhanced metabolism resistance (EMR), a type of non-target-site resistance (NTSR).

The other two most common resistance mechanisms are acetyl-CoA carboxylase (ACCase) target-site resistance (ACCase TSR) and the less widespread, but increasing, target-site resistance (TSR) to acetolactate synthase inhibitors (ALS TSR).

It is these that we will focus on in this chapter along with a look at another increasingly important type of resistance and how different mechanisms can interact within the same plant.

Target-site resistance

To understand how resistance works it is worth remembering how herbicides function within the plant cell.

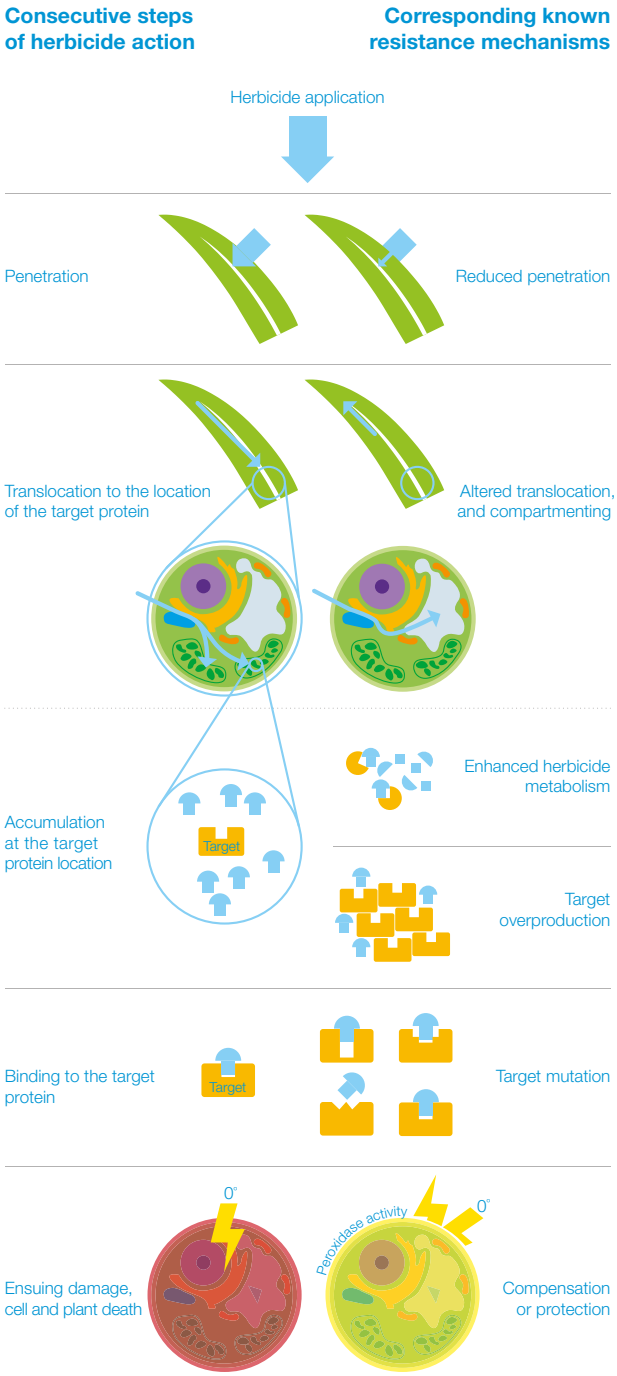
Selective herbicides generally work by targeting and binding to a protein catalyst (enzyme) required for growth of that weed species, disrupting its activity and eventually leading to death of the plant. In non-target species (such as the crop), the herbicide is typically broken down (metabolised) by the plant so that it is no longer active.

A target-site mutation causes a structural change in the protein's binding site, such as a subtle amino acid substitution, which means the herbicide can no longer bind tightly and is unable to exert its phytotoxic effect[16] (see figure 8). The enzyme therefore either remains partially or fully active and the weed survives treatment.

In ACCase TSR, the mutation only blocks the site of activity specific to 'fop', 'dim' or 'den' herbicides, while ALS TSR is specific to sulfonylurea and other related herbicides.

Although both types of TSR only affect their respective herbicide groups, they often result in very poor herbicide efficacy and can increase quickly within resistant populations[15].

Figure 8:
Overview of different resistance types and normal herbicide function



Non-target-site resistance: Enhanced metabolis

There are many known forms of NTSR including: reduced uptake, modified translocation and compartmentalisation within a plant (see figure 8). However, globally these are relatively rare and certainly within UK black-grass populations EMR is by far the most widespread.

EMR is caused by complex mechanisms involving multiple genes and works by enhancing the natural ability within every plant to detoxify foreign compounds, such as herbicides. Therefore, most herbicides are affected to varying degrees[17].

In resistant weeds with EMR, the herbicide is structurally altered into biologically inactive molecules by various degradation mechanisms before it can reach the target site, allowing the weed to continue growing after treatment.

The speed of detoxification is what ultimately matters most with EMR and influences whether the herbicide is structurally altered quickly enough so that it does not reach the target site intact.

EMR incidence tends to increase more slowly than TSR and generally results in a decline in herbicide efficacy over time rather than a complete loss of control – although the latter is possible in very severe cases.

Cross resistance

EMR can also lead to cross resistance, where the higher levels of plant enzyme are able to detoxify more than one type of herbicide group[18].

For example, resistant plants may be able to detoxify both acetolactate synthase (ALS) and ACCase inhibitors as well as other herbicide groups, including substituted ureas, triazines etc. This is known as non-target-site cross resistance.

Target-site cross resistance is also possible where herbicides bind to the same target site. Any mutation affecting that binding site therefore impacts on all herbicides which have activity at this location.

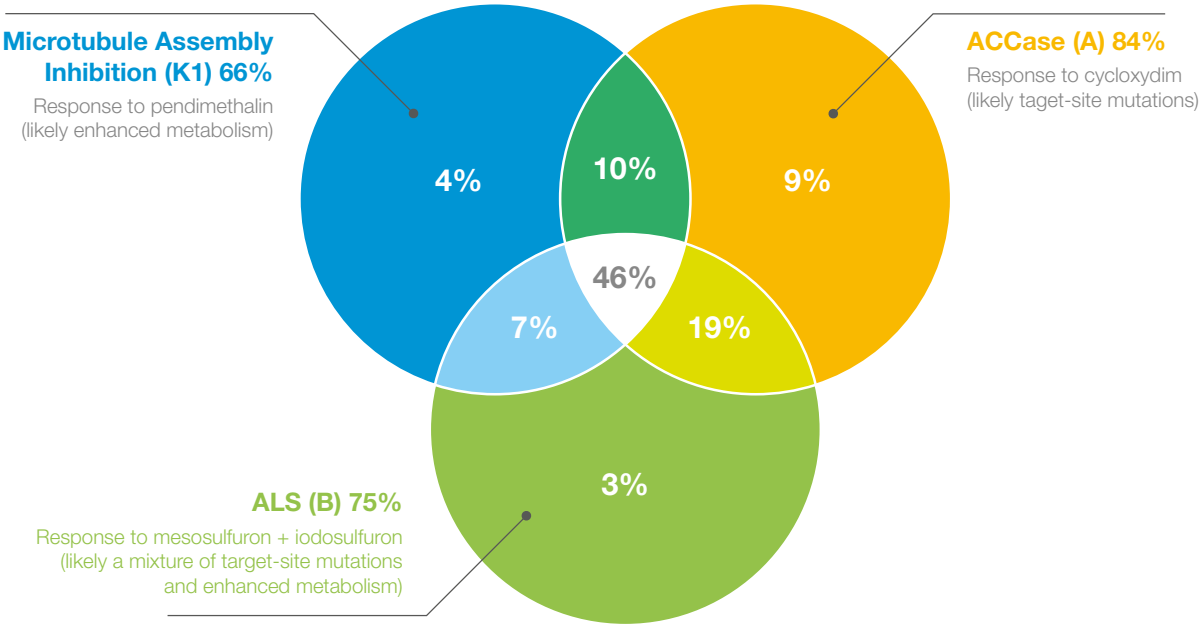
When any type of cross resistance is present weeds are much more difficult to manage.

Multiple resistance

Although some weeds may only be affected by a single resistance mechanism, recent years have seen an increase in plants exhibiting multiple (two or more) resistance mechanisms to different herbicide classes or modes of action (MoA).

Estimates suggest nearly half of UK herbicide resistant black-grass has multiple resistance to three MoA (see figure 9 below).

Figure 9:
Proportion of *Alopecurus myosuroides* samples resistant to three herbicides representing different MoAs, alone and in combination, in the UK:



Source: Hull et al, 2014, based on 213 non-random samples

The genetic interactions involved in multiple resistance are complex and less well understood than TSR alone, which complicates management decisions.

The simplest multiple resistance cases are where a plant (or population) has two or more different resistance mechanisms to a single herbicide, or class of herbicides. However, it can be much more complex, with the worst cases having a number of TSR and NTSR resistance mechanisms present within the same plant[19].

The rise of multiple resistance is thought to be due to two possible factors[20]:

- Over-reliance on a particular herbicide until a weed population displays resistance, followed by repeated use of another herbicide without proper resistance management until the same weed population develops resistance to the second herbicide
- Transfer of pollen (cross-pollination) between sexually compatible individuals carrying different resistance genes

Studies[21] suggest varied and persistent application of selective herbicides can favour the development of multiple resistance, especially where alternative selective herbicides have been employed as the sole means of controlling an already resistant population.

In contrast, resistant biotypes with less varied herbicide histories typically only exhibit one or two resistance mechanisms[21].

Controlling weed populations with multiple resistance is a major challenge for the industry as it drastically reduces the already limited chemical options available, placing more emphasis on cultural remedies, such as crop choice, cultivation techniques and rotation.

New resistance threats

Herbicide resistance is evolving all the time so it is worth being aware of other resistance types that have already been identified and could become more widespread in future.

The resistance mechanisms discussed so far are initially due to a mutation that alters the DNA structure, causing some form of herbicide resistance to develop.

However, another important type of resistance-causing mutation also exists, which alters the expression of one or several genes in resistant plants compared to sensitive plants[22]. This change in DNA sequence can cause an increase in the amount of the protein targeted by the herbicide.

Increased gene expression has not yet been found in herbicide resistant black-grass populations and identifying mutation(s) responsible for changes in gene expression is not straightforward due to their diverse nature[22].

But scientists have discovered it in weeds that have evolved resistance to glyphosate, where some plants have shown up to 160 extra copies of a gene called EPSPS; the enzyme that glyphosate interferes with to stop plant growth[23].

Given the importance of glyphosate within farming systems and some black-grass control options, such as stale seedbeds, it is vital growers protect its future use by following the latest stewardship guidelines.

Side effects of resistance

As with anything in nature, the development of herbicide resistance mechanisms affects plants and weed populations differently.

One potential impact of the gene mutation responsible for herbicide resistance is the fitness penalty seen in some resistant weeds compared with their 'wild' counterparts, which can potentially mitigate effects of resistance.

There are only a few cases where herbicide resistance causes a fitness penalty and the extent to which different weeds are affected varies considerably depending on the genetic background and environment.

There are three possible causes of a fitness cost[24]:

1. The target-site mutation responsible for resistance also interferes with normal plant function or metabolism (e.g. the structural modification of the target enzyme prevents herbicide binding but also compromises enzyme function in the process)
2. A trade-off within the plant, whereby the development of resistance such as EMR diverts energy and resources away from other important plant functions, such as growth and reproduction
3. Resistance alters the normal ecological interactions, for example, making resistant plants less attractive to pollinators or more susceptible to diseases

Currently there is some limited evidence of fitness penalties in rye-grass[25] however measuring impacts can be tricky given the inherent variability across growing seasons.

One study[26] examining two mutant ACCase alleles (Gly-2078 ACCase and Leu-1781) did confirm a resistance cost associated with Gly-2078, but no cost to plant growth from the presence of Leu-1781. Where a penalty was seen, resistant plants showed lower enzyme activity than wild types, resulting in poorer growth and seed production.

More noticeable impacts have been seen in other weed species and herbicides.

For example, triazine (atrazine/simazine) – resistant groundsel and black nightshade have been found to not use sunshine as efficiently as wild types, while Australian research has found rye-grass with metabolic resistance produced 20% less seed than susceptible plants.

Although the presence of a fitness penalty may mitigate the impact of herbicide resistance to a certain extent, natural evolutionary processes are likely to eventually select for stronger resistant plants over those that are weaker or less productive.

Large plants gain more resources (water, light, nutrients) and produce more seeds than smaller ones, which tend to be eradicated in plant populations, reducing the visible fitness cost seen in the field over time[26].

Once a resistant population has established, it is therefore vital growers and agronomists reduce the population as quickly and effectively as possible. To do this requires knowing exactly what type of resistance is present within field populations.

Resistance testing and results

If herbicide resistance is suspected within a field population then testing is the only way to confirm the presence, type and severity of any problem.

Understanding resistance status allows problems to be managed effectively using both chemical and cultural control methods.

Equally, a negative test result can eliminate resistance as a cause of lower-than-expected herbicide efficacy and may instead indicate other agronomic factors that are to blame, such as poor application conditions or timing.

Regular resistance testing can help monitor changes in field populations and provide an early warning of problems, providing results are interpreted correctly.

Improving accuracy

A variety of resistance tests are available, offering varying levels of information.

Most tests are based on seed samples collected from mature weeds that have survived herbicide treatment and set seed.

Seed samples are grown under controlled conditions before being exposed to different herbicides to see which are effective and which are not. An overall resistance rating can then be assigned, together with an indication of the type of resistance likely to be responsible (see p.39).

Although this approach is still widely used across the industry, advances in molecular biology and genetic diagnostics allow for a much more detailed analysis of resistance, showing the specific mechanism(s) and gene mutations responsible.

The Bayer shoot test for example (see p.41) uses genetic markers to detect target-site resistance (TSR) and radio labelling to analyse possible metabolic resistance.

The accuracy of any test result is only as good as the initial plant or seed sample provided, so it is crucial to follow the protocols required by testing centres.

Where testing is based on a seed sample, the established WRAG guidelines[27] should be followed.

See also Bayer's recommendations[28].

Testing should be done while reasonable herbicide control is still being achieved as waiting until products fail completely will limit the alternative chemistry available for managing resistance[28].

Testing types

The three main types of resistance test – seed (pot), Petri-dish test and genetic diagnostics – are described in the following pages.

Each has its own advantages and disadvantages, and in many cases testing centres can tailor tests to specific requirements, such as herbicide type or application timing.

Seed (pot) test

The glasshouse pot assay test remains the most widely used resistance test in the UK[29], as herbicide application and activity more closely mimic what happens in the field. Pot assays can also detect resistance regardless of mechanism[30].

1 Seed from suspect broad-leaf or grass-weeds is collected in the field when ripe and sent to Bayer's Weed Resistance Competency Centre for analysis.

2 Seed samples are recorded and cleaned. Some weed species have a dormancy period which must be broken by storing the seed at 0°C in special climate chambers which can take up to 2 weeks.

3 The seed is then sown in pots, covered with sand and germinated in the greenhouse under controlled climatic conditions.

4 The samples are then treated with herbicides from different active ingredient classes. Treatment with pre-emergence products takes place a few days after sowing. Approx. 3-4 weeks after germination, post-emergence active ingredients are applied.

5 After 3 weeks the effects of the different products are assessed. It is then possible to see which active ingredients are ineffective and which are still effective for each sample.

Most seed tests use a range of indicator herbicides to establish the likely types of resistance mechanism present. The main indicator herbicides used are:

- Pendimethalin: its primary mode of action (MoA) is to prevent plant cell division and elongation in susceptible species, so resistance to Pendimethalin typically indicates enhanced metabolism resistance is present
- Cycloxydim: an acetyl-CoA carboxylase (ACCase) inhibitor, resistance indicates ACCase target-site resistance (TSR) affecting 'fops' and 'dims'
- Mesosulfuron + iodosulfuron: this acetolactate synthase (ALS) inhibitor can indicate the presence of both ALS TSR and enhanced metabolic non-target-site resistance (NTSR) mechanisms[31]

Some test centres also offer glasshouse pot tests that use surviving young plants collected from the field. Plants are trimmed, transferred to pots, and then allowed to regrow before being sprayed and assessed. This can work for most herbicides, apart from those applied pre-emergence.

Shoot test (genetic diagnostics)

The shoot test developed by Bayer is one example of how advances in genetic diagnostics are improving the detection and management of herbicide resistance.

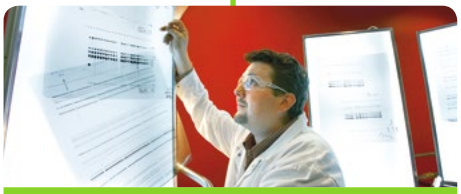
The test can be used to identify all forms of resistance, including enhanced metabolism, ALS target site and ACCase target site.

Plant samples are taken at the start of the season from plants at the 2 to 3 tillers stage – typically after treatments have been applied, but before spring growth begins.

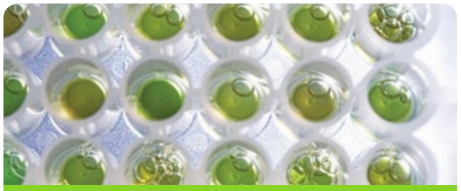
Different techniques are used to detect specific resistance mechanisms. These are summarised as follows:

Target-site resistance (TSR):

1 DNA (genetic material) is extracted from suspect plants and examined by polymerase chain reaction (PCR) analysis.



2 Markers are used to detect TSR.



3 If the sequence of amino acids on certain DNA segments differs from the genetic material of sensitive plants, this indicates TSR.

Extensive knowledge now exists regarding which changes on the DNA lead to inhibition of the effects of certain active ingredients.

Enhanced metabolism resistance (EMR):

1 Plants are incubated with herbicide for 1 day.

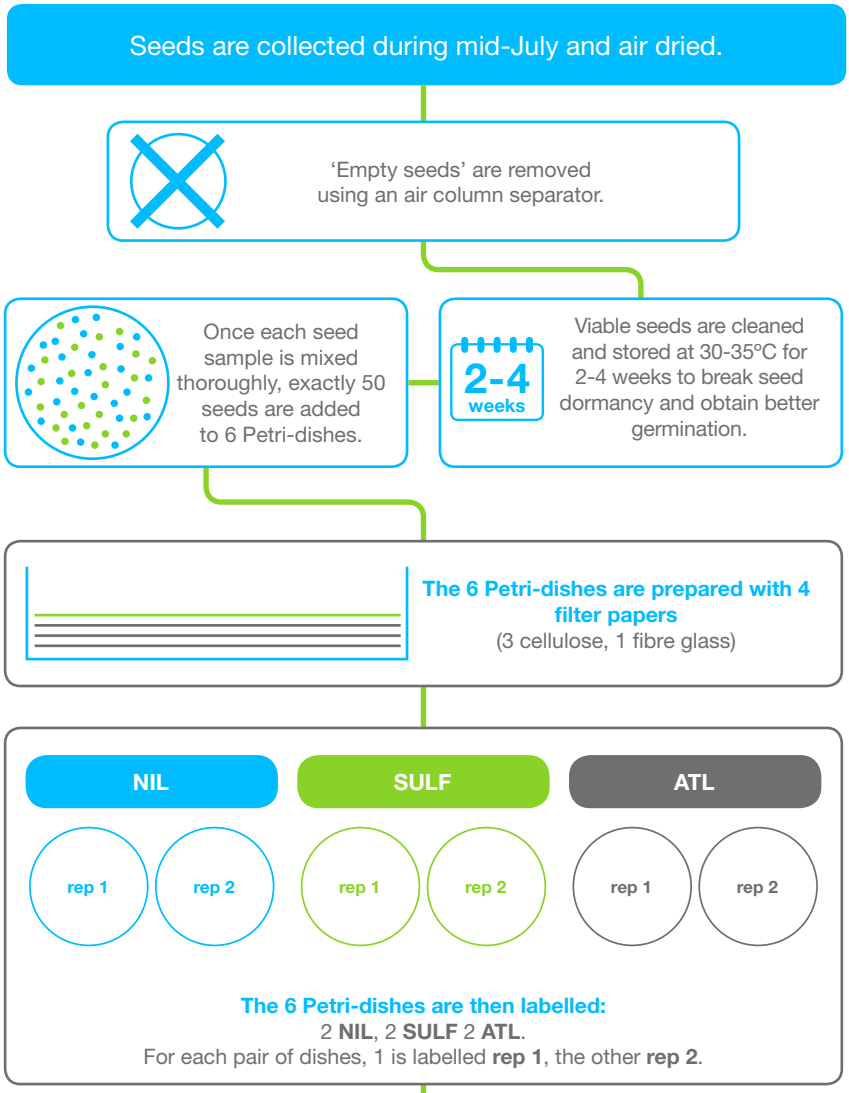
2 The concentration of the active ingredient and its breakdown products are measured using high performance liquid chromatography (HPLC).

A high concentration of metabolites indicates a rapid breakdown rate and therefore the presence of metabolic resistance.

Results are typically available in 1 to 2 weeks, and provide a detailed analysis of the severity of metabolic resistance and mutation(s) responsible for TSR (see p.47).

Petri-dish

Petri-dish tests, such as the 'Rothamsted Rapid Resistance Test[31]', are based on a similar principle to the pot test described earlier.



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Firstly, 1 litre solution of 2 g/litre potassium nitrate (KNO_3) is mixed in deionised water. Herbicides are then added to specific dishes.

NILS
control Petri-dishes

7ml of KNO_3 solution is added to the filter papers of the NIL Petri-dishes.

SULF
sulfometuron – Oust (1ppm)

7ml of 1ppm sulfometuron (Oust) solution is added to the filter papers of the SULF Petri-dishes using a syringe.

ALT
mesosulfuron and iodosulfuron – Atlantis® WG (0.1ppm*)

7ml of 0.1ppm mesosulfuron and iodosulfuron (Atlantis WG) solution is added to the filter papers of the ALT Petri-dishes using a syringe.

14h / 17°C

10h / 11°C

All Petri-dishes are placed in clear polythene bags and stacked in an incubator set at 17°C for 14 hours a day (with the lights on), and 11°C for 10 hours a night (with the lights off). The stacks are moved around the incubator every 2-3 days.

The % length reduction for shoot length of the germinated seeds is then calculated:

$$\left(\frac{\text{\% reduction = total shoot length in NIL dishes – total shoot length in treated dishes}}{\text{total shoot length in NIL dishes}} \right) \times 100$$

For black-grass, other Petri-dish indicator herbicides are[31]:

- Fenoxaprop – indicates resistance to this active and other 'fops'. Does not show the type of mechanism present as fenoxaprop is vulnerable to both EMR and TSR
- Sethoxydim – indicates TSR to all 'fops' and 'dims'. Sethoxydim is not affected by EMR
- Pendimethalin – indicates EMR

Results can be obtained more quickly and cheaply than the pot test as it is not necessary to wait for seeds to grow before treating with a herbicide.

However, Petri-dish tests are not as representative of field conditions, provide limited information on the resistance mechanisms and are not suitable for all herbicides.

	Glasshouse pots – seeds from field	Glasshouse pots – plants from field	Petri-dish germination	Molecular lab assays	Radio-labelled lab assays
Answer in the same crop year	No	Yes	No	Yes	Yes
Suitable for all weed species	Yes	Potentially	No	Potentially	Potentially
Mimics field conditions	Yes	Yes	No	No	No
Suitable for all herbicides	Yes	Most (not pre-em.)	No	No (ACCase and ALS only)	Potentially
Detects resistance regardless of mechanism	Yes	Yes	No	No (TSR only)	No (EMR only)
Test duration	Slow	Medium	Fast	Very fast	Very Fast
Cost	Medium	Medium	Low	High	Very High

Source: Rothamsted Research, 2012

Interpreting results

When interpreting results it is vital to remember an inherent level of bias is built into any resistance test which must be accounted for to avoid exaggerating problems at the field scale.

Regardless of the type of test used, the plant or seed samples tested usually come from weed populations that have survived herbicide treatment, while susceptible plants will have been killed and therefore not collected for testing.

This automatically biases the test towards resistant individuals.

How representative the results are of the entire field depends on the accuracy of sampling, weed density and distribution, and the proportion of plants that survived treatment across the field[29]. This last point is often hard to measure without having an untreated control area within the field.

It is also worth considering what herbicides (and associated actives) were applied before samples were taken to understand which types of resistant individuals may have been selected for.

Inherent bias does mean resistance can be detected at an early stage, so should be seen as a positive attribute rather than a reason for not testing.

What results mean

Most pot and Petri-dish resistance tests in the UK use the standard 'R' rating system to show resistance severity and likely impact on herbicide performance.

There are four grades of resistance severity:

S	R?	RR	RRR
Susceptible (i.e. no indication of resistance to applied herbicide)	Resistance not confirmed but early indications that resistance may be developing, possibly reducing herbicide performance	Resistance confirmed – probably reducing herbicide performance	Resistance confirmed – highly likely to reduce herbicide performance

An R-rating can be given for a single or range of active ingredients to show the presence (and severity) of resistance or multiple resistance.

Alongside the resistance rating, it is important to consider the type of resistance mechanism, as this has a major impact on herbicide performance and subsequent management.

TSR, for example, is often regarded as being absolute, meaning that the herbicide will either work or not.

In contrast, all plants have a natural ability to metabolise herbicides, so it is the speed of breakdown that is important when EMR is present. Severity differs, so herbicides may continue working to varying extents.

A typical range of herbicide control for each resistance rating is shown below. These thresholds are not definitive as they vary according to the susceptibility of the non-resistant reference population tested at the time.

RRR <40%

RR 40-80%

R? 80-90%

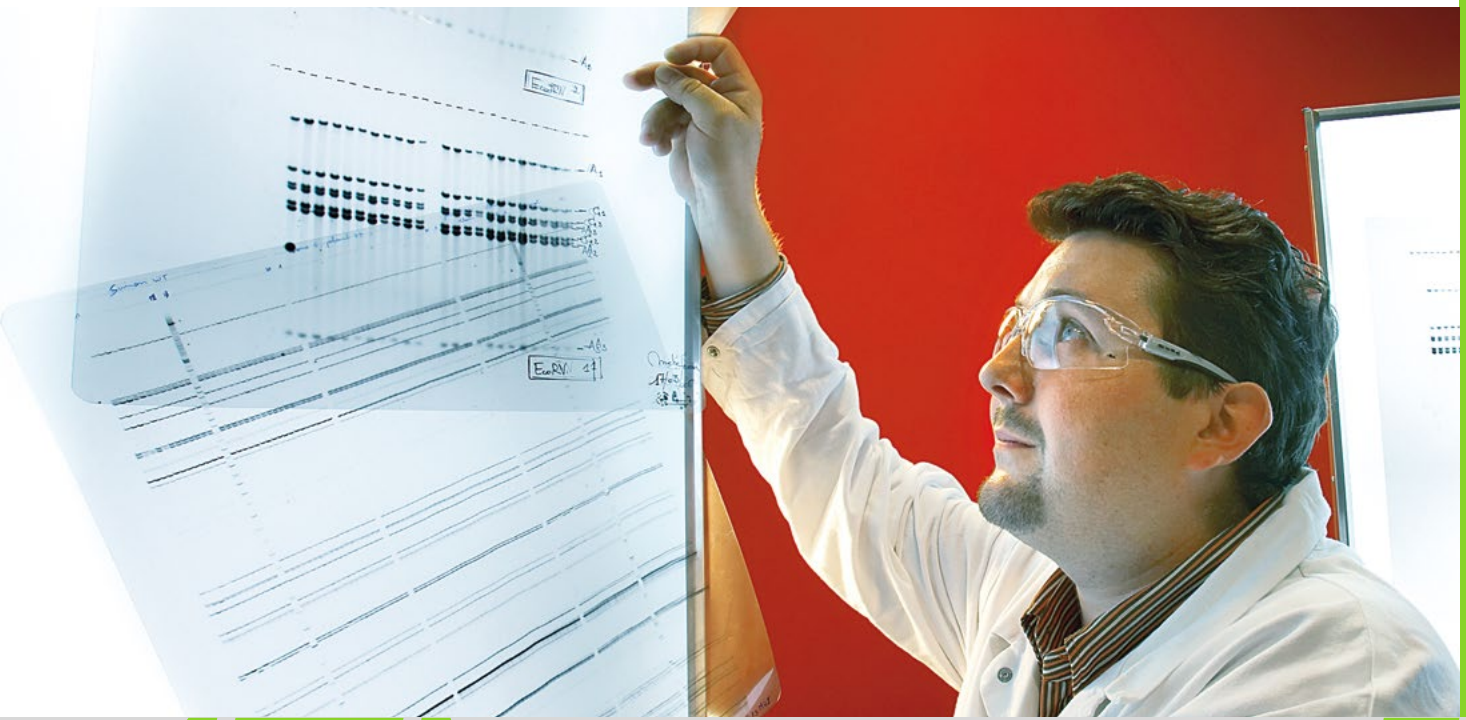
S >90%

The use of indicator herbicides in seed and Petri-dish tests provides an indication of the type of resistance present, but it does not show the exact cause.

Advanced genetic diagnostic tests, such as those offered by Bayer, do show the specific types and causes of resistance present (down to the mutation responsible) and/or the proportion of plants affected by different levels of enhanced metabolism (see example results sheet on p.49).

The following table summarises the key advice for determining the likely field impact and best course of action for each resistance rating.

Every farm and weed population is different though and the impact of resistance in the field depends on a host of factors, so talk to your local Bayer contact to discuss the best course of action for your situation.



Rating	Impact at field level	Action required
RRR	– The highest resistance rating and most likely to reduce field performance of herbicides affected – Significant potential for resistant populations to build-up quickly if no remedial action is taken – But, an RRR-rating is not the end of the world - the active may still have killed a significant proportion of the field population, leaving behind the few resistant individuals that were tested	– Avoid the sole use of the MoA where possible – especially if TSR is confirmed – If due to EMR, the herbicide may still be effective if applied to small weeds (up to three leaves max and before tillering) at a robust rate – Apply in optimum application conditions and use correct methods (nozzles, water volumes, etc.) – Do not rely on one alternative herbicide - rotate chemistry by using different MoA within crop and across the rotation – Monitor populations closely – Utilise anti-resistance measures (cultural and chemical)
RR	– Resistance is present, but less severe than RRR – Impact on herbicide performance likely to be most noticeable when other factors go against you (e.g. sub-optimal application conditions, poor spray timing)	– Focus on maximising application efficacy as described above – Vary MoA to reduce the likelihood of resistance increasing further – Monitor closely – Utilise anti-resistance measures (cultural and chemical) where possible
R?	– An R? is non-conclusive and means resistance may be developing in the field, although it may be hard to see any impact on herbicide performance	– Monitor weed populations and sample routinely to identify any changes in herbicide performance early – Consider anti-resistance measures to stop possible problems escalating
S	– A 'clean' resistance score means no resistance was found in the sample provided – but remember the result only applies to that sample	– Consider other factors potentially affecting herbicide performance (e.g. application equipment, conditions, timing, etc.) and address accordingly – Monitor situation for any changes in black-grass populations – Adopt a sensible anti-resistance strategy to prevent future problems arising

Testing frequency

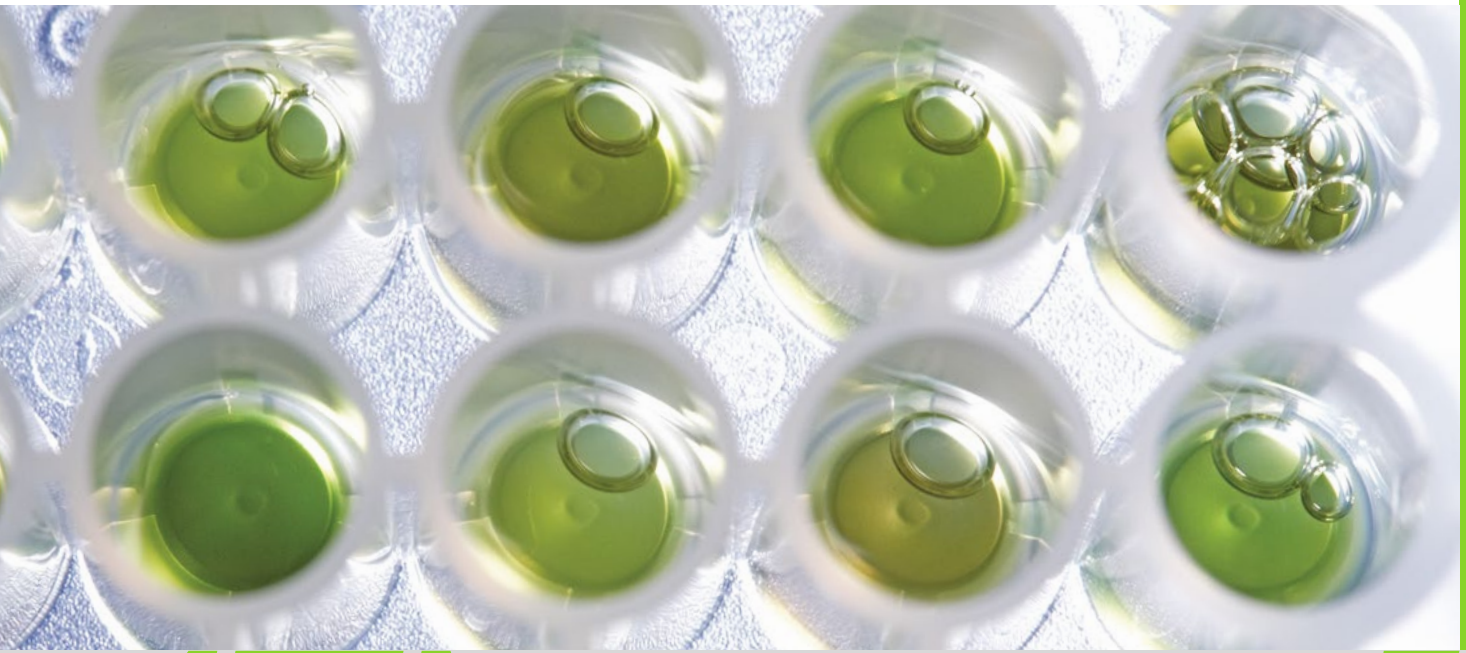
Testing is the only way to accurately determine whether resistance is present in a field population and repeated tests every few years can help monitor changes within the field population.

However, once resistance has developed it takes a long time to eradicate and repeated testing a few years down the line could well reveal no change in the overall resistance status.

What's more important is the impact of mitigation measures on the weed population in the field, which must be reduced to an acceptable level. In the case of herbicide resistant black-grass this should be a zero-tolerance policy.

Even just a few plants per square metre can reduce crop yield and will also provide a platform for resistant populations to increase.

Managing black-grass is all about reducing the plant population, but knowing the resistance status is an essential step in that process.



Managing resistance

The confirmation of resistance is certainly not the end of the road for herbicides, but it does require a step-change in how they are used.

Extra attention must be paid to managing resistant weeds across the entire rotation, utilising all chemical, cultural and biological controls available. There is no one-size-fits-all solution or ‘silver bullet’ in a can.

Some measures will be relatively simple, such as alterations to spray timing, products or rates, while others may require wholesale changes to cultivation techniques or cropping across several seasons.

This cannot be done ad-hoc in any one season so it is essential to take a ‘whole-farm’ approach and plan black-grass control over a number of years and crop types.

Black-grass planning

Below are some pointers on how to prepare a black-grass plan.

Key steps in black-grass planning:

- Identify the problem
  - Map weed infestations and prioritise the areas (or whole fields) to focus on
  - Test samples for resistance
  - Consider other causes of poor weed control – look at past cropping history, application conditions/ timing and agronomy
  - Assess current resistance risk (see p.22 for risk assessment)
- Set a realistic target e.g. reduce the black-grass population by 99% over x years or reduce to <5 plants/m^2
- Plan ahead for more than 1 season – at least cover a full crop rotation (typically 3-5 years)
- Examine all options available to help achieve this target (see p.55)
- Select those most appropriate options for each site – integrate as many chemical and non-chemical methods as possible
- Implement measures carefully and stick to the plan
- Monitor weed populations closely to gauge effectiveness of control measures
- Be prepared to amend the plan as necessary
- Maintain control measures indefinitely to avoid problems reoccurring – set a rolling 3-5 year plan
- Consider re-testing samples for resistance to identify change in status or mechanisms responsible

Control options to consider

Rotating chemistry is an essential part of resistance management, but this alone is not enough to prevent resistance development[32].

Chemical controls must be used responsibly in association with other non-chemical options to reduce the selection pressure herbicides are put under and give them the best chance of control without exacerbating resistance pressure.

Controlling black-grass, resistant or not, is all about reducing populations to a manageable level. Typically, more than 95% control is needed to prevent weed populations increasing[32].

Below is a summary of the main chemical and cultural options to consider within any black-grass management plan[33 & 37]:

Chemical

- Avoid repeated and continued use of herbicides with the same mode of action (MoA) in the same field, growing season and following year
- Use mixtures or sequential treatments that are active against the target weed but have different MoA
- Control weeds early, especially where enhanced metabolism resistance (EMR) is present
  - Use a robust rate as low rates may increase risk of resistance development
- Minimise the use of active ingredients affected by target-site resistance (TSR)
- Maximise pre-emergence control
  - Flufenacet is generally most effective
  - Adding other actives can give a 5-10% uplift in control[34]
  - Apply in optimum conditions (residuals need moist, level, clod and trash-free seedbeds) – consider delaying drilling for the right conditions (don't just delay the herbicide application)
- Minimise reliance on post-emergence chemistry – apply in optimum conditions if used
- Spray off bad black-grass patches with a non-selective herbicide before plants set seed
- Always follow label recommendations and application advice

Cultural

- Enhance crop competition
  - Sowing higher seed rates/narrower rows
  - Selecting more competitive crops or varieties e.g. ADAS work has shown hybrid barley is more effective than wheat at reducing black-grass head numbers and seed return[35]
  - Alter sowing date and/or fertiliser strategy to ensure the crop establishes quickly
- Delay drilling
  - Increases the window for controlling black-grass outside the crop with a non-selective herbicide (e.g. spraying-off stale seedbeds with glyphosate) – around 80% of black-grass emerges between August and October[36]
  - Use common sense to avoid drilling too late, achieving poor establishment and allowing black-grass to take hold
- Cultivation choice
  - Ploughing is useful to bury freshly-shed seed in high seed-return year, but will bring old seed to surface (average rate of decline is 74% a year[36])
  - Use shallow cultivations (25-50mm max) to create a 'kill zone' close to surface where weeds can germinate and pre-ems. work effectively
- Spring cropping
  - Allows time for black-grass control in autumn
  - Offers chance to introduce alternative chemistry and MoA
  - Some crops offer greater competition e.g. hybrid barley
- Cover cropping
  - Can disrupt weed life cycle and reduce seed bank, but must be integrated into cropping system to avoid unforeseen effects (e.g. volunteers, delayed following crop establishment)
  - May also help dry-out heavy land ahead of spring cropping
- Check field drainage and repair as necessary
- Cut heavily infested fields for wholecrop silage before seed set to prevent seed return
- Minimise the risk of spreading seed between fields by cleaning planting, cultivation, harvest and baling equipment

Appendix

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Glossary of terms

Allele	Alternative versions of a gene, found at a fixed spot on a chromosome. Organisms that inherit two alleles, one from each parent, are called diploid
Catalyst	A substance that increases the rate of chemical reaction without being permanently changed itself
Chromosome	A long threadlike association of Deoxyribonucleic acid (DNA) and protein found in the nucleus of living cells, carrying all genetic information
Cross resistance	Resistance to two or more herbicides caused by the same mechanism
Enhanced metabolism resistance (EMR)	EMR is a form of non-target-site resistance (NTSR) where herbicides are broken down by the plant before they reach the target site of activity
Gamete	An egg or pollen cell containing only one set of chromosomes (haploid). Gametes are formed when two alleles segregate during the reproduction process
Gene	A unit of hereditary information made up of DNA found on the chromosome. Provides 'instructions' for making specific proteins
Genotype	The genetic makeup of an organism
Metabolites	Small molecules produced when a substance is metabolised by chemical processes in an organism
Multiple resistance	Resistance to two or more herbicides caused by two or more different mechanisms in the same plant
Mutation	A change in the DNA of genes that creates genetic diversity
Non-target-site resistance (NTSR)	Resistance caused by any mechanism(s) other than altered target sites. EMR is the most common form of NTSR
Phenotype	The physical appearance of an organism, determined by the presence of dominant and resistant genes in its genotype
Phytotoxic effects	A toxic effect by a compound (e.g. pesticide) on plant growth
Polygenic	Where two or more genes influence a single characteristic
Resistance	The inherited ability of a weed to survive a rate of herbicide that would normally kill it
Selection pressure	Any cause that reduces reproductive success in a proportion of a population (can take various forms, both chemical and ecological)
Target-site resistance (TSR)	Resistance caused by a genetic mutation that alters the binding site for a specific herbicide, preventing it from working correctly
Trait	Different variants for a particular characteristic

Atlantis OD contains active substances: mesosulfuron-methyl and iodosulfuron-methyl-sodium. Atlantis is a registered Trade Mark of Bayer. All other brands listed may be Trade Marks of other manufacturers and proprietary rights may exist. Use plant protection products safely. Always read the label and product information before use. Pay attention to the risk indicators and follow the safety precautions on the label.

For further information, including contact details, visit www.cropscience.bayer.co.uk or call 0808 1969522.

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