MANAGING HERBICIDE RESISTANCE IN COTTON

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Herbicide resistance

Herbicide resistance occurs when a plant is able to tolerate a rate of a herbicide that kills other plants of the same species under the same conditions (both spray conditions and plant growing conditions).

Herbicide resistant individuals can occur at very low frequency in any natural plant population. Although these individuals may be present before a herbicide is first used in a field, their frequency is likely to remain low until a selection pressure is applied. This happens when a herbicide is applied. Individuals that are more tolerant of the herbicide survive the herbicide application and grow to set seed. This seed produces more individuals that tolerate the herbicide and set more seed, and so on. Eventually, the herbicide tolerant individuals represent a noticeable proportion of the weed population, and herbicide resistance is observed.

Genetic variability

Genetic variability is a characteristic of all populations. Even in a population where all individuals appear to be identical, there will be some genetic variability. Many of these genetic differences are of no obvious importance. Leaf shape and leaf colour in sow thistle, for example, are quite variable, especially in seedlings, but the differences do not appear to confer any difference in fitness or competitive ability.

Genetic differences that confer differences in the plant's tolerance to herbicides can exist in any plant population. Sometimes these differences are large enough that some individual plants may be able to tolerate quite high levels of herbicide without any apparent effect. These individuals are said to be herbicide resistant.

The level of herbicide resistance depends on the nature of the resistance and the genetic differences between resistance and susceptible individuals. Herbicide resistance could be as simple as the production of a waxy leaf surface that prevents the herbicide entering the leaf. Alternatively, resistance could be inferred by an individual over-producing a plant enzyme that was blocked by the herbicide, or producing a completely new enzyme that substitutes for the enzyme blocked by the herbicide, or by any number of other pathways.

The expression of herbicide resistance also depends on the genetics involved. Where herbicide resistance is caused by a single plant gene, this gene could be recessive and only expressed when the individual is homozygote (carries two copies of the gene). Alternatively, the gene could be dominant, expressing even when the plant only carries a single copy of the gene (heterozygote). In many cases, the heterozygote individual will express a lower level of herbicide resistance than homozygote individuals. A range of levels of herbicide resistance could occur when resistance is conferred by multiple genes.

Nevertheless, the selection for herbicide resistant individuals is the inevitable outcome of

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repeated use of a single herbicide or herbicide group. This selection pressure is greatly reduced when other weed management tools are used in combination with the herbicide.

Worldwide, 220 different weed species have been documented to have resistance to herbicides and some weeds have developed resistance to a range of different herbicides. Annual ryegrass in Australia, for example, is resistant to a wide range of herbicides from nine different herbicide groups.

Selection pressure

When applied correctly, a herbicide effectively controls its target weed. Repeated use of a herbicide has two effects. Firstly, the herbicide selects for the more tolerant weed species, resulting in a species shift in favour of those tolerant species. That is, the frequency of the species most susceptible to the herbicide declines most rapidly, while there is a relative increase in the frequency of species that are more tolerant of the herbicide. Species shift is the normal consequence of any selection pressure. Secondly, the herbicide selects out the more herbicide resistant individuals from within a species (if these are present) and the frequency of these individuals increases within the population, leading to the development of herbicide resistance.

The rate at which these changes occur depends on a number of factors, including:

- the selection pressure imposed, which is determined by herbicide efficacy, the frequency of herbicide application and the generation interval of the weed,
- the level of tolerance to the herbicide, the frequency of herbicide resistant individuals within the population, and the nature of the weed's reproductive mechanism,
- the relative fitness of resistant individuals,
- dilution of the population from the seed bank and external sources, and
- use of other weed management tools that reduce the population of tolerant and resistant individuals.

Herbicide groups

Every herbicide comes with detailed product information attached to the chemical container. Additional information may be included in an attached product booklet. This information includes details on the use of the product, the range of weeds controlled, the required application conditions, safety, and herbicide resistance (for the more recently registered products).

Included on the product label is information on the herbicide group to which the product belongs. This information is displayed prominently on the front of the product label.

The herbicide group information is essential for developing a weed management strategy which reduces the risk of selecting out herbicide resistant weeds. The herbicide groups are indicated by a lettering system, as shown in Table 1.

While all herbicides have the potential to cause a species shift in the weed population, they do not all have the same risk of developing a resistant weed population. Within the herbicide groups, there are two broad categories.

- herbicides with high risk (groups **A** and **B**).
- herbicides with moderate risk (groups **C** to **Z**).

The herbicide groups are based on the modes of action of the various herbicides, that is, the specific ways the herbicides work within a plant. There are many different modes of herbicidal action and a single herbicide may act on more than one plant process.

The herbicide risk categories have been developed from an understanding of the modes of action of these herbicide groups, and have been proven in practice.

The high risk herbicides (Groups **A** and **B**) target specific processes in the plant cell. Plants that are resistant to these herbicides occur relatively commonly in some weed populations. Herbicide resistant populations of weeds, such as ryegrass and black oats, for example, have been selected out after as few as two or three herbicide applications in extreme cases. This means that the herbicide completely fails to control the weeds by the third or fourth application because by this time the weed population is dominated by individuals that are resistant to the herbicide.

The post-emergence grass herbicides, Envoke® and Staple® are all in the high risk category. Resistance to these products is likely to occur within 3 to 5 years if they are used repeatedly without other weed management tools.

The herbicides in the moderate risk category (Groups C to Z) are less specific in their modes of action, targeting more general plant processes. Individual plants with resistance to these herbicides may still occur, but they are less likely. Some of these herbicides, such as 2,4-D and trifluralin, were used repeatedly over many years without any apparent resistance problems occurring. Nevertheless, resistance can occur and has now occurred to nearly all the herbicide groups. Resistance to 2,4-D and trifluralin have now been found and further resistance to 2,4-D is suspected.

Once herbicide resistance develops, an alternate management approach is needed, as the herbicide is no longer of any use for controlling the target weed. Loss of a broad-spectrum herbicide, such as glyphosate, has a major negative impact on the cotton farming system.

Further information on weeds that have developed resistance to herbicides in Australia is covered in the document Integrated Weed Management Systems for Australian Cotton Production in WEEDpak.

Over-use of glyphosate and a lack of residual grass herbicides in this system has resulted in species shift to weeds that are tolerant of or resistant to glyphosate. Failure to manage the feathertop Rhodes grass in this dryland field will result in years of problems.

Table 1. *The herbicide groups of the herbicides more commonly used in the cotton farming system. Examples of products containing these active ingredients are shown.*

	Herbicide group	Active ingredient	Example
High risk	A	butroxydim	Factor®
		clethodim	Select®
		fluazifop-P	Fusilade®
		haloxyfop	Verdict®
		propaquizafop	Correct®
		sethoxydim	Sertin®
	B	chlorsulfuron	Glean®
		halosulfuron-methyl	Sempra®
		imazapyr	Arsenal®
		metsulfuron-methyl	Ally®
		pyrithiobac sodium	Staple®
		trioxysulfuron	Envoke®
Moderate risk	C	atrazine	
		diuron	
		fluometuron	Cotoran®
		prometryn	Gesagard®
	D	pendimethalin	Stomp®
		trifluralin	
	F	norflurazon	Zoliar®
	G	flumioxazin	Valor®
		oxyfluorfen	Goal®
	H	isoxzflutole	Balance®
	I	2,4-D	
		dicamba	
		fluroxypyr	Starane®
		MCPA	
	K	metolachlor	Dual®
	L	diquat	Reglone®
		paraquat	Gramoxone®
	M	glyphosate	
		glyphosate-trimesium	Touchdown®
	N	glufosinate	Liberty®
	Q	amitrole	Amitrole T®
	Z	MSMA	Daconate®

Herbicide modes of action

Herbicides have their effects by disrupting specific plant processes. Group A herbicides, for example, inhibit the acetyl-CoA carboxylase enzyme, which inhibits fatty acid synthesis in the plant. Even though there are a large number of Group A herbicides that are all chemically different, they all target the same mechanism in the plant, all inhibiting fatty acid synthesis. Group H herbicides, as another example, inhibit the protoporphyrinogen oxidase enzyme, which inhibits the production of chlorophyll and heme, inhibiting photosynthesis and electron transfer and leading to the build up of protoporphyrin and toxic levels of oxygen. Consequently, the herbicides have been grouped according to their target site mechanisms or modes of action.

Similar herbicides often have similar modes of action. For example, all the post-emergence grass herbicides are Group As, with the same mode of action. Consequently, although six chemically distinct herbicides are listed in Group A in Table 1, they all act on the same plant pathway and have the same mode of action. In practice, a weed that develops resistance to any one of these herbicides will have some level of resistance to all six herbicides, even though it may never have been exposed to the other five herbicides. This is called cross-resistance. Conversely, any herbicide with grass activity from a different mode of action herbicide group will control Group A resistant and susceptible plants equally well, as resistance to Group A shouldn't confer any resistance to another mode of action group.

Apparently similar herbicides do not always have similar modes of action. Of the pre-emergent grass herbicides, trifluralin and pendimethalin are both group D herbicides, which inhibit tubulin formation, effectively inhibiting plant growth, while metolachlor is a group K herbicide, with multiple modes of action, inhibiting growth and root elongation. If a weed repeatedly exposed to trifluralin developed resistance to this herbicide, it may have cross-resistance to pendimethalin, but shouldn't have resistance to metolachlor.

Resistance mechanisms

Weeds develop resistance mechanisms that either block the target sites of the herbicides (target site mechanisms) or are more general, blocking the herbicide at some either point (non-target site mechanisms). Non-target site mechanisms include blocking the transport mechanism, over-expressing the target enzyme, demetabolising the herbicide, or sequesting the herbicide into less sensitive plant parts. The resistance mechanisms may be as simple as a waxy surface on the leaf, reducing the penetration of the herbicide into the plant.

Generally, the target site mechanisms confer much higher levels of resistance than the non-target site mechanisms and are conferred by simple substitutions in the plant's genetic code which can be detected by gene mapping.

Non-target site mechanisms are generally weaker, are not due to single gene substitutions and often appear to be polygenic. They are also the more common form of resistance and it is likely that resistant plants commonly have more than one non-target site resistance mechanism, with mechanisms stacking up over generations are selection pressure is continued. It also seems likely that most weeds that develop target site resistance also have non-target site resistance mechanisms.

The consequence of this is that even amongst a single resistant field population there are likely to be varying levels of resistance and that separate populations that develop resistance won't necessarily have exactly the same resistance mechanisms. Recent testing of eight populations of glyphosate resistant awnless barnyard grass collected from northern NSW, for example, found different levels of resistance in all eight populations. This result suggests that there are multiple resistance mechanisms involved, with different combinations of these mechanisms in the different populations.

Weeds around channels, roads and water storages can contribute large quantities of seeds to cotton fields. Using glyphosate as the main control tool on these weeds leads to high selection pressure for resistance.

Rotating herbicide groups

Where herbicides with similar weed spectrums have different modes of action, opportunity exists to rotate herbicides, reducing the risk of selecting weeds resistant to any one herbicidal mode of action. This strategy is difficult to implement in cotton, as many of the herbicides that could be readily substituted are from the same herbicide groups.

For example, as discussed earlier, although the post-emergence grass herbicides Correct®, Factor®, Fusilade®, Select®, Sertin® and Verdict® are chemically different, they are all group **A** herbicides with similar modes of action. A weed that develops resistance to one of these herbicides may be cross-resistant to all of them, even though the weed had not been exposed to the other herbicides.

Similarly, the residual, broad-leaf herbicides most commonly used with cotton production (diuron, prometryn and fluometuron) are all group **C** herbicides, with similar modes of action.

However, the pre-emergent grass herbicides belong to groups **D** (trifluralin and pendimethalin), **K** (metolachlor) and **F** (Zoliar®). Use of these herbicides in rotation allows an opportunity to expose weeds to totally different herbicide groups, greatly reducing the risk of developing herbicide resistance to any one of these herbicides.

Overall, the most effective approach to reduce the risk of the development of herbicide resistance and species shift to herbicide tolerant individuals, is to ensure that herbicides are used correctly, and to use an integrated approach to weed management, using as wide a range of herbicide groups as practical, and a variety of non-herbicidal weed management tools. Detailed information on the integrated weed management tools and developing an integrated weed management system in cotton is covered in the document Integrated Weed Management Systems for Australian Cotton Production in WEEDpak.

Special care needs to be taken when making repeated use of the high risk group **A** and **B** herbicides.

Multiple & cross-resistance

Herbicide resistance has become a very serious problem in many parts of the world over the last decade. Not only has resistance developed in a large number of plants, but many populations have developing resistance to multiple herbicides with different modes of action, and resistance has not always followed the same rules.

There have been examples where multiple resistance has crossed the modes of action groups. Resistance has developed in ryegrass, for example, to both glyphosate and glufosinate (Liberty), Group M and Group N herbicides, even thought the plants were never previously exposed to a Group N herbicide. This has happened because the resistance mechanism is a metabolism mechanism and it breaks down glyphosate and glufosinate equally well, with no regard to their modes of action. Similarly, it is likely that some of the other non-target site resistance mechanisms could confer resistance to herbicides with different modes of action.

Similarly, cross-resistance is likely to occur within a mode of action group, with resistant plants having some resistance to other herbicides within the same mode of action. However, there are examples reported where this has not been the situation. In the US, for example, hydrilla (a water weed) has developed resistance to fluridone, a Group F herbicide. The resistant plants are cross-resistant to norflurazon, another Group F herbicide, but have increased sensitivity to three other Group F herbicides.

Many of the early examples of herbicide resistance involved a fitness penalty, such that the resistant plants were less fit than the susceptible plants of the same species. This can occur because there is a cost to the resistance mechanism. Where resistance occurs due to a change in the enzyme that is targeted by the herbicide, for example, the modified enzyme may not be as effective as the original, inferring a fitness penalty. The significance of this can be that the resistant plants are smaller, less competitive and produce fewer seeds. Where this occurs, swapping to an alternative mode of action herbicide is a very effective strategy, as the proportion of resistant plants in the population will decline over time once the original herbicide is no longer used.

However, there is little or no fitness penalty with many of the more recent examples of resistance, such as glyphosate resistance and in some situations, such as the US, resistant weeds are spreading into areas where the susceptible weed wasn't previously found.

Weed monitoring

The underlying principle of integrated weed management is to continually monitor the presence of weeds and the success or otherwise of the weed management tools used. Where a weed is not successfully controlled by one tool (herbicide, cultivation, chipping etc.), an alternate tool should then be used to manage the weed before it can set seed. This approach of scouting and rotating weed management tools as necessary, will not only result in an effective weed management system, but will also reduce the risk of developing herbicide resistance.

Cotton growers should always check fields after every herbicide application to ensure that the target weeds have been satisfactorily controlled. Where control has not been satisfactory, an alternate management tool should be used. A weed control failure may not be due to herbicide resistance, but could be caused by a variety of other factors such as:

- poor application. Nozzles may have been poorly positioned, such as too high from the target, or too little herbicide hit the target due to inadequate water rates, high temperature, small droplets, strong winds etc.,
- an inappropriate (too low) herbicide rate. Larger weeds generally require higher herbicide rates. Mature weeds may be impossible to control with a given herbicide,
- unsuitable conditions. Weeds may be moisture, heat or cold stressed, or conditions may have been too hot for spraying, humidity too low etc., or
- incorrect weed identification. Similar, closely related weeds may have very different susceptibility to some herbicides.

Where weeds that should have been controlled by a herbicide have survived the application, growers should immediately act to ensure that the surviving weeds do not set seed. Assistance from an agronomist or chemical company representative should then be sought to determine whether the survival of the weeds is due to herbicide resistance. Action to manage the weed must be taken as soon as resistance is confirmed. In most cases a small area of resistant weeds can be readily managed, but a problem that is allowed to become a large area could cause issues for many years.

If resistance is suspected and the plants are likely to set seed before resistance can be confirmed, the area should be treated as if it is resistant and all plants controlled. Where resistance occurs in an out-crossing species or a species with small seed that can be spread by wind, the pollen or seed has the potential to spread for kilometres and it is vital that resistant plants are controlled before they are able to spread.

Suspected herbicide resistance

Many suspected cases of herbicide resistance are due to other factors. Incorrect identification of the weed is a common problem. Similar looking weeds often occur in mixed populations without being individually identified. A good example of this occurs with yellow vine and caltrop. Broad-spectrum herbicides such as trifluralin and glyphosate are equally effective in controlling both weeds, but specific herbicides such as Staple® may only be effective in controlling one species (Staple® only controls yellow vine). An apparent spray failure with Staple® on yellow vine can be caused by Staple® effectively controlling the yellow vine, but leaving a large population of caltrop. An alternative control method is needed for the caltrop.

Another general guide to herbicide resistance is that the problem is most likely to show up in a small area of a field, corresponding to the location of the individual plant that initially had resistance. A resistance problem would be unlikely to first appear on a field-wide basis, unless the problem had been spread by land-levelling in the previous season. A field-wide problem would be a very good indication of an application problem or herbicide rate problem.

If the weed has been correctly identified, and no other problems are apparent, then the simplest method of checking for resistance is to re-apply the herbicide at a range of rates on test-strips, ensuring that no suspect weeds are allowed to set seed. Contact a chemical company representative and a weeds agronomist from NSW Dept. Primary Industries or Queensland Department of Primary Industries immediately if the weeds are still not controlled by the recommended rate.

Managing herbicide resistance

Weeds are relatively immobile and will only move large distances if wind blown or transported by water, animals, people, or machinery. Experience from other cropping systems has shown that resistance can often be confined to a single paddock, or even to an area within a paddock.

Where resistance is identified before it has become widely spread, and appropriate measures are taken, resistance can be relatively easily managed and may eventually be eliminated from an area. The keys to managing resistance are:

- early identification, before the problem becomes widespread,
- treatment, preventing the weeds seeding, and
- isolation, to prevent the weed spreading to new areas.

Glyphosate & the resistance spiral

Simple weed management systems centred around glyphosate have been widely adopted by farmers over the last decade and more, particularly with the use of Roundup Ready cotton, maize and soybean crops.

The glyphosate centred systems have been highly effective for controlling weeds, are relatively inexpensive, can be targeted to growing weeds and can be rapidly applied to large areas. They have been able to replace most other weed management tools, improving timelininess of control and greatly reducing the machinery requirement and labour force needed to manage weeds. The glyphosate system has been an important part of achieving the very high yields that have become the normal in the Australian cotton industry of the new century, valuable both for weed control in-crop, and for managing weeds in fallows, facilitating the development of moisture conservation and stubble retention systems.

Unfortunately, we have been using a glyphosate centred system for many years now, and sufficient time has passed that resistance has developed, and in more than just one species. The system is rapidly falling apart. The system is no longer sustainable in the long-term or even the medium-term and failure to change our approach to weed management now will result in Australia joining a growing list of countries where glyphosate technology has already been effectively lost for many of their most troublesome weeds.

However, it doesn't just stop there. The loss of glyphosate for managing the worst weeds in these countries has been followed by the successive loss of the most useful alternative chemistries, with these herbicides also falling to resistance in rapid succession.

Much of the US cotton industry has gone from being a "magic" industry a decade ago, where all weeds were cheaply controlled by a couple of in-crop applications of glyphosate, back to a "slave' industry, where weeds are king, demanding heavy inputs of expensive herbicides, inter-row cultivation and large amounts of hand-hoeing to manage them. In some instance, requiring levels of inputs that would make the Australian cotton industry economically unviable, with multiple herbicides, cultivation and hand-hoeing bills of over $1000/ha in Australian terms, just to produce a harvestable crop.

That the industry has selected for glyphosate tolerant and resistant weeds over the last decade it not surprising. However, the trap of the glyphosate centred system, is the assumption that problems can be solved by re-introducing single components of the conventional system. A pre-planting application of diuron, for example, is becoming widely used to manage glyphosate-resistant flaxleaf fleabane in Australia. After all, diuron was routinely used for over 30 years without any resistance issues to this herbicide emerging, so it seems like a good option. However, this thinking fails to recognise that diuron was not formally used alone but as one part of a whole system of residual herbicides and other tools, with the system often including diuron, trifluralin, fluometuron, pendimethalin, prometryn, inter-row cultivation and hand hoeing. To now expose glyphosate-resistant fleabane to diuron without any of the other tools is to place very high selection pressure on this weed, and is likely to see resistance emerge to diuron within only a few years.

Similarly, using a double-knock in fallows with glyphosate followed by Spray.Seed is a useful strategy for controlling some of the more difficult weeds. However, it is only effective as long as both glyphosate and Spray.Seed are effective. Relying solely on Spray.Seed to control glyphosate resistant weeds is a recipe for developing Group I resistance. Relying on a Group A or B herbicide to control feathertop Rhodes grass is guaranteed to fail.

The need to develop an approach to weed management that is sustainable in economic terms, in environmental terms, and in functional terms is a far bigger challenge than it may at first appear. The adoption of a glyphosate centred system doesn't cut it, and can't be patched by just adding a 2nd herbicide to manage problem weeds. Persisting with a glyphosate centred system is a sure path to failure, with dire consequences, as the US industry are now proving, with many of the more problematic weeds in the US having multiple resistance often to 4 or 5 modes of herbicidal action.

Control every survivor every time. This single glyphosate resistant awnless barnyard grass plant could be the source of year's of heartache if not controlled before it sets seed.

Summary

Herbicide resistant plants can naturally occur in any plant population. Over-reliance on a single herbicide or herbicide group will cause a species shift to weeds that are tolerant of the herbicide and will eventually result in the emergence of weeds resistant to the herbicide.

The development of herbicide resistance is now a reality for the cotton production system, with glyphosate resistant weeds becoming increasingly common. This has not primarily occurred due to a failure in the cotton system, but due to a widespread failure in the whole farming system, due largely to the long-term use of glyphosate to replace all the other components of an integrated weed management strategy.

Now that resistance has occurred, it is essential that growers change to manage their weeds in a more sustainable fashion, re-implementing an integrated approach to weed management. Growers need to return to using a wider range of herbicides and other weed management tools, ensuring that any survivors of every herbicide application are controlled with an alternative weed management tool before they set seed.

Herbicide resistance can't be solved by just adding a second herbicide to manage the escapes from the first herbicide. This strategy places very strong selection pressure on the second herbicide, which is often an older herbicide that has a history of previous use. The result of the strong selection pressure inevitably is resistance developing to this herbicide, and then the next herbicide and so on.

Herbicide resistance is not unmanageable at the present and it is essential that cotton growers act now to ensure the value of their herbicides into the future. The alternative is returning to cultivation and hand hoeing as the primary methods of weed control, with all the associated issues of this approach.