



UNDERSTANDING POST-EMERGENT HERBICIDE WEED CONTROL IN AUSTRALIAN FARMING SYSTEMS



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Introduction

Since the advent of herbicides for weed control, growers have readily adopted herbicide tactics, as they are generally a very robust and cost-effective way of managing weed populations in cropping situations.

Following many seasons of extensive herbicide use, Australian farming systems have selected for herbicide resistance in key weed species. This has resulted in many situations where a particular herbicide, or mode of action group, is no longer effective on a particular weed population, or other situations where resistance is developing in the paddock and the herbicide may now be only providing a partial level of control.

In the southern and western region production systems of Australia, resistance was first selected in-crop, primarily for annual ryegrass (*Lolium rigidum*) to the herbicide modes of action Groups 1 and 2. In comparison, northern Australian grain production systems have first selected predominantly for grass weeds resistant to glyphosate. This is due to significant use of summer and winter no-till fallow, heavy reliance on glyphosate for weed control, and comparatively less historical use of in-crop grass herbicides compared to the southern and western regions. Resistance to glyphosate in broadleaf weeds has been slower to emerge, largely due to the regular addition of other modes of action (particularly Group 4 herbicides) to fallow applications of glyphosate. However resistance to broadleaf weeds is also on the increase.

It is now common for Australian growers to have one or more weed species on the farm that are resistant to one or more of the post-emergent herbicide groups. Of great concern is the rapid development both in the number of populations and number of weed species that have become resistant to glyphosate.

As post-emergent herbicides generally provide high levels of efficacy and are relatively easy to use, they have been given historical prominence in weed management strategies. This has led to their prolonged and widespread use, often in preference to pre-emergent herbicides. Therefore, selection for resistance to post-emergent herbicides has been greater than for pre-emergent options.

Despite recent increases in resistance, post-emergent herbicides remain an integral component of weed control strategies in many production systems. As resistance to these herbicides evolves and intensifies, it is important for users to fully understand how these herbicides work and how resistance evolves and is expressed, in order to maximise herbicide performance.

This manual has been developed by Independent Consultants Australia Network (ICAN) for the Grains Research and Development Corporation to provide advisers and growers with the background science and information to better understand how post-emergent herbicides work and to better inform their choices when using these products.



Many populations of awnless barnyard grass (*Echinochloa colona*) are now resistant to glyphosate.

Photo: Mark Congreve

1. Understanding the behaviour of key grass weeds

Many Australian paddocks have been farmed using minimum or zero-tillage practices for the past 10 to 30 years, with a very heavy reliance on glyphosate for fallow and pre-sowing weed control. The dominance of no-till systems has also altered the environment for weeds in general and has led to a shift in weed species. Weeds that are resistant to, or difficult to control with glyphosate and weeds that like to germinate at or near the soil surface (surface germinators), have flourished in no- or minimum-till environments.

In tillage-based farming systems, weeds that dominate the landscape are typically larger seeded, many of which can germinate from the depth of cultivation, often 5 to 10 centimetres. For grass weeds, this favours weeds such as wild oats (*Avena* spp.) and annual ryegrass in winter and liverseed grass (*Urochloa panicoides*), and to a lesser extent barnyard grass (*Echinochloa* spp.) in summer, which can all germinate when buried, although these species are also comfortable germinating near the soil surface in no-till systems. In tillage-based systems it is also common to find a range of perennial weed species such as couch grass (*Cynodon dactylon*), Johnson grass (*Sorghum halepense*), and nutgrass (*Cyperus rotundus*).

The move to low disturbance farming systems has seen a significant rise in the number of surface-germinating weeds such as the broadleaf weeds fleabane (*Conyza* spp.) and sow thistle (*Sonchus oleraceus*). For grass weeds, this farming system change has led to increased importance of grass weeds such as feathertop Rhodes (*Chloris virgata*) and windmill grass (*Chloris truncata*), as well as sweet summer grass (*Moorochloa eruciformis*) in Central Queensland, alongside the ever-present barnyard grass and annual ryegrass.

Species dominating in low disturbance farming systems tend to share key traits:

- Typically they are adapted for germination on, or near to, the soil surface. Many surface germinators, especially summer germinating species, do not require a period of extended darkness (that is, burial) to break dormancy.
- For many surface germinators that are problematic in fallows, seed dormancy is typically low, allowing high numbers to germinate after rainfall. They are frequently among the first species to establish after a rainfall event, with many surface germinators also able to germinate following small rainfall events.
- Under conditions of decreasing daylength (for summer weeds) or in a drying soil profile, they typically respond by rapidly moving to a reproductive phase and setting seed.
- Seed left on the soil surface is likely to be exposed to many factors that reduce viability. This includes predation by ants, UV exposure, rapid soil wetting/drying cycles and high daily temperature fluctuations. To overcome these losses, surface germinators tend to produce high numbers of viable seed (often tens of thousands per plant).

- They often have dispersal mechanisms to allow rapid spread to new areas, for example, seed adapted for transport by wind, overland water flow, or on the hides of animals.

These traits, especially high seed production, can lead to these species dominating the paddock in a very short period of time. For example, species like feathertop Rhodes grass have been known to go from individual plants in one spring, to small patches the following summer and full field infestation within 3 to 4 years, where summer rainfall conditions are suitable and control tactics have been inadequate in preventing seed-set.

As many zero and minimum till growers have relied heavily on glyphosate for fallow weed control since the conversion to reduced till farming, many of these surface-germinating species have been selected for resistance to glyphosate.

An understanding of weed ecology allows managers to design strategies to manage key weeds. Table 1A summarises the important characteristics of the primary grass weeds of Australian broadacre cropping.

For surface germinators, management strategies are likely to involve:

- Effective scouting to identify individual plants or patches while still small and removing these before they shed seed.
- Being prepared with a control plan for major germinations, which are likely after suitable climatic events. If traditional fallow herbicides are failing, this is likely to involve a program of both pre and post-emergent herbicides, as well as non-herbicide tactics, possibly including cultivation and burning.
- Competitive crops that can substantially reduce weed establishment and weed seed production.
- Tactics such as interrow cultivation or shielded sprayers may be required to control weeds in summer grass crops (for example, sorghum, maize). There are few selective in-crop herbicide options to control grass weeds in these crops, so any late germinations or survivors from at-planting treatments can replenish the weed seedbank. Growing these crops on a wide row spacing diminishes crop competition, providing an even greater opportunity for seedbank replenishment.
- Monitor for quick maturing, surface-germinating summer grass weeds that can establish late in winter crops after pre-emergent herbicides have 'run out' (if any were used). This may result in weeds already established and past the ideal herbicide application window at fallow commencement when the crop is harvested. At harvest, soil moisture is often low and these weeds are likely to be moisture stressed, making herbicide control even more difficult at the start of the fallow period.
- **Ensure that all plants are controlled before seed-set.** For many of the problem grass weed species, the short-lived seed persistence on the soil surface (often 1 to 3 years) means that where complete control can be achieved, and no new seed is allowed to return to the seedbank, an infested paddock can be 'cleaned up' within a few years.

For species that can germinate from depth and where weed seed has been spread through the soil profile by cultivation, it is likely that burial will have increased seed dormancy for most species. This means it will be more difficult and require more time to reduce the seedbank to manageable levels. Strategies for these situations can include:

- Conversion to a minimal disturbance system may assist in driving down the seedbank over time. Control with pre-emergent herbicides can be particularly challenging during this 'transition' period, with both surface and deep germinating weeds emerging in the paddock, often at staggered times. Further, the use of some pre-emergent herbicides in stubble retained systems can be problematic.

- Switching between summer and winter cropping rotations can be a useful tactic. For example, if a winter cropping program is overrun by wild oats, annual ryegrass or phalaris (*Phalaris paradoxa*), many growers have successfully changed to a summer program for 2 to 4 years and controlled populations in the winter fallow with knockdown herbicides or tillage. A similar strategy can also be used for a range of low dormancy summer grass weeds by rotation to winter crops for several seasons. This tactic can see the seedbank driven down to low levels, allowing a return to a winter or summer cropping phase that is relatively clean of weed seed. Southern and western region growers use a fallow year or brown manure crop in a similar manner to achieve a season with no seed-set.

Table 1A: Characteristics of key weed species including seed production and persistence, germination and establishment and level of outcrossing.

Weed	Seed production and persistence	Germination and establishment	Genetics
Annual ryegrass (<i>Lolium rigidum</i>)	Very high seed production. Up to 45,000/m ² . Little viable seed after four years in zero-till situations. Persistence increases with burial. Short seedbank dormancy (6 months).	Late autumn to early spring emergence following ~20mm rainfall event. Shallow cultivation stimulates germination. Germinates mostly from 0 to 20mm	Obligate out-croser. Diploid
Barley grass (<i>Hordeum glaucum</i> and <i>H. leporinum</i>)	Most seed (99%) will germinate the following autumn. Minimal seedbank dormancy.	Traditionally there has been a rapid germination response to autumn rainfall. However, many populations in southern states have evolved in crop paddocks to now require vernalisation (chilling) before germination. This increased dormancy results in emergence later in the winter crop, therefore escaping many earlier season control tactics. Preferential germination under zero-till and low disturbance systems.	<i>H. glaucum</i> diploid <i>H. leporinum</i> tetraploid
grdc.com.au/resources-and-publications/all-publications/publications/2021/ecology-of-major-emerging-weeds-regional.org.au/au/asa/2010/crop-production/weeds/7047_fleetb.htm			
Barnyard grass (<i>Echinochloa crus-galli</i>)	Very high seed production. One plant can produce more than 40,000 viable seeds. Short dormancy. Most seed will not germinate until the following year.	Predominantly spring to early summer with multiple cohorts following a rainfall event (10 to 20+mm). Most germination occurs from 0 to 10mm.	<i>E. crus-galli</i> hexaploid
Awnless barnyard grass (<i>Echinochloa colona</i>)	Seedbank typically persists for 1 to 3 years from a single year of seed-set. Burial increases seed persistence.		<i>E. colona</i> tetraploid
Brome grass (<i>Bromus diandrus</i> and <i>B. rigidus</i>)	A single plant can produce up to 3000 viable seeds. Short, over-summer dormancy period before germinating. Like barley grass, many populations have developed seedbank dormancy through selection in cropped systems for a single vernalisation gene. Seed can persist for up to 3 years.	Adapted to low rainfall environments. Staggered germinations through late autumn and winter following a significant rainfall event. Late in-crop germinations often set seed after pre-emergent herbicides run out. Prefers shallow soil incorporation with main germination from 10mm, however can establish from up to 150mm.	Octaploid
grdc.com.au/resources-and-publications/all-publications/publications/2021/ecology-of-major-emerging-weeds			
Feathertop Rhodes grass (<i>Chloris virgata</i>)	Each plant can produce up to 6000 seeds. Very short (<10 weeks) seed dormancy. Short seedbank persistence. Most seeds lose viability within 12 months. Persistence does not appear to increase significantly with burial.	Prefers lighter soil types, but will establish on most soils. Mainly germinates in spring and early summer, following a rainfall event of 5 to 10+mm Surface germinator. Practically no germination from below 20mm. Does not like competition from other species, however it is often the first species to emerge and establish in bare soil/zero-till situations.	Diploid
grdc.com.au/GRDC-FS-FeathertopRhodesGrass grdc.com.au/resources-and-publications/all-publications/publications/2021/ecology-of-major-emerging-weeds grdc.com.au/IWMM youtube.com/watch?v=Yk95mS_hvhM&feature=youtu.be&list=PL2PndQdkNRHGq9QqVc9M216m3Ej9zr-6t sciences.adelaide.edu.au/agriculture-food-wine/system/files/docs/2017-ftr-biology.pdf			

Table 1A: Characteristics of key weed species including seed production and persistence, germination and establishment and level of outcrossing (continued).

Weed	Seed production and persistence	Germination and establishment	Genetics
Liverseed grass (<i>Urochloa panicoides</i>)	<p>A single plant can produce up to 3000 seeds under good conditions.</p> <p>Strong dormancy. Seed will not germinate before the following season.</p> <p>Viable seed can persist for up to four years.</p> <p>A single seed burial increases persistence, however continual cultivation may reduce the amount of viable seed by stimulating germination.</p>	<p>Prefers lighter soil types.</p> <p>Typically emerges in spring in a major flush, following 20+mm rainfall. Smaller subsequent emergences may follow, which can replenish the seedbank if not controlled.</p> <p>Prefers to germinate within 50mm of the soil surface, however will emerge from 100mm so can be more problematic in cultivated systems.</p>	
daf.qld.gov.au/_data/assets/pdf_file/0008/55277/Managing-barnyard-and-liverseed-grasses.pdf grdc.com.au/resources-and-publications/all-publications/publications/2021/ecology-of-major-emerging-weeds			
Phalaris (Canary, Paradoxa grass) (<i>Phalaris paradoxa</i>)	<p>Very high seed production. Individual plants can produce >20,000 seeds with >120,000 seeds/m² being recorded.</p> <p>Short seed dormancy prevents germination over the subsequent summer months.</p> <p>Most seed germinates the year after shedding, with little viable seed remaining after two years. Burying seed via cultivation increases seedbank persistence.</p>	<p>Prefers heavy soils with high water holding capacity. Typically emerges from 25 to 50mm with staggered germinations through late autumn and winter. Cultivation stimulates germination. An autumn tickle can stimulate germination before planting, which can then be controlled by knockdown herbicides.</p>	
caws.org.nz/old-site/awc/1999/awc199910711.pdf			
Sweet summer grass (<i>Moorochloa eruciformis</i>)	<p>Up to 4000 viable seeds can be produced per plant. Seed is highly viable the summer following shedding.</p>	<p>Prefers heavy soils, warm to hot conditions with summer dominant rainfall.</p> <p>Multiple cohorts emerge between mid-spring and the end of summer, following good rainfall.</p> <p>Predominantly a surface germinator, preferring to emerge from 0 to 20mm, which makes it adapted to zero-till farming, although can emerge from 50mm.</p>	
Wild oats (<i>Avena fatua</i> and <i>A. sterilis</i>)	<p>Relatively low seed production (50 to >200) per plant, although up to 20,000 seeds/m² have been recorded.</p> <p>Seedbank persistence is relatively short, however will increase with seed burial.</p> <p>Without further recruitment, the seedbank can be largely depleted within ~3 years.</p>	<p>Wild oats typically germinate from up to 50 to 75mm depth, however in zero-till farming systems most seed is retained close to the soil surface.</p> <p>The main cohort (~40%) emerges in autumn or early winter.</p> <p>Staggered germination occurs, and it is often these late germinations (10 to 30% of the seedbank) that replenish the seedbank by escaping pre or early post-emergent herbicides.</p>	<p>Hexaploid (six copies of each gene). 99% self-pollinating.</p>
Windmill grass (<i>Chloris truncata</i>)	<p>Unlike most other annual grass weeds of cropping, windmill grass is a short-lived perennial. Coupled with wind-dispersed seed heads, control can be difficult.</p> <p>Mature plants continue to produce seed heads over summer, which replenishes the seedbank.</p> <p>Minimal seedbank dormancy.</p> <p>Seed loses viability within 18 months, regardless of burial depth.</p>	<p>Prefers lighter soils but will grow on heavier soils. Adapted to low rainfall environments.</p> <p>Predominantly a surface germinator, with little emergence from below 20mm. Suited to zero-till farming systems.</p> <p>Germinates from early summer to autumn following a 20+mm rainfall event.</p>	<p>Tetraploid</p>
qaafi.uq.edu.au/files/5958/Windmill-grass-ecology-and-management.pdf sciences.adelaide.edu.au/agriculture-food-wine/system/files/docs/2017-wmg-biology.pdf grdc.com.au/resources-and-publications/all-publications/publications/2021/ecology-of-major-emerging-weeds			

Information on the ecology and biology of key weeds of cropping can be found at: grdc.com.au/IWMM and grdc.com.au/resources-and-publications/all-publications/publications/2021/ecology-of-major-emerging-weeds.

1.1 Principles of managing grass weeds

Some grass weeds that emerge with or very soon after planting can compete very aggressively. For example, in Figure 1A, a pot trial was established to investigate the competitive effect of wild oats on Spitfire[®] wheat, with wild oats emerging at various times after the wheat.

There was a 20 per cent reduction in wheat yield between the earliest wild oat emergence timing (4 days after wheat emergence) compared to the latest wild oat emergence timing (39 days). This demonstrates the importance of early removal of grass weed competition to maximise yield.

When high weed densities compete with emerging crops, yield loss may have already occurred by the time a post-emergent herbicide can be used (Table 1B). This modelling data shows that substantial gain in yield can be achieved when grass weeds are removed early from the cereal crop (the earlier they are removed, the more yield is protected) and that this effect is magnified with higher weed burden.

The use of a pre-emergent herbicide can significantly reduce these yield losses. However, pre-emergent herbicides when used in isolation from other weed management strategies, often leave sufficient weed survivors to replenish the weed seedbank. It is not until additional tactics are added to reduce weed survivors (that is, a post-emergent herbicide, high levels of crop competition, or harvest weed seed control), that the weed seedbank is put into decline.

Stacking of a pre-emergent herbicide combined with a post-emergent herbicide and harvest weed seed control (Table 1C), helped to reduce the wild oat seedbank over time. Repeated use of a pre-emergent herbicide strategy in isolation was far less effective.

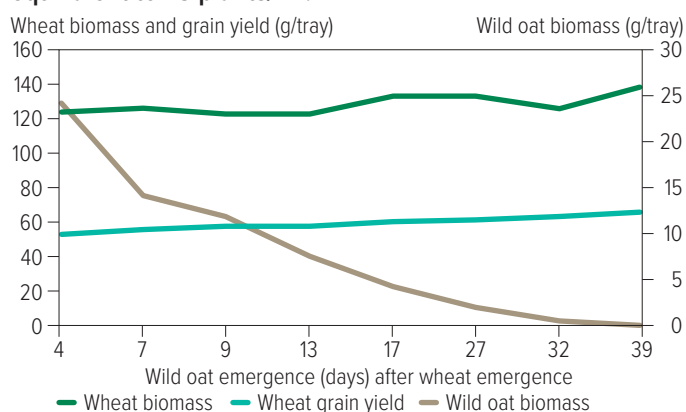
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Chauhan, B., pers. com. (2015). Effect of wild oat emergence time on weed maturity and seed retention in relation to wheat maturity.

Wu, H. & Koetz, E. (2014). *Wild oats seed bank dynamics: a long-term study in southern New South Wales*. Hobart, Tasmania, Tasmanian Weed Society.

Figure 1A: Effect of delayed emergence of wild oats relative to wheat (cv. Spitfire[®]). QAAFI glasshouse trial, 2015. Wheat density equivalent to 115 plants/m², wild oat density equivalent to ~8 plants/m².



Source: Chauhan, B. pers. com. (2015)

Table 1B: Per cent yield gain achieved from removing grass weeds at different application timing.

		Weeds/m ²			
		50	100	200	300
Annual ryegrass	Pre-tillering	12%	20%	30%	35%
	Tillering	10%	18%	26%	30%
	Mid-tillering	9%	13%	19%	22%
Phalaris	Pre-tillering	14%	22%	32%	38%
	Tillering	12%	20%	28%	33%
	Mid-tillering	10%	15%	20%	23%
Wild oats	Pre-tillering	17%	26%	36%	42%
	Tillering	15%	23%	32%	36%
	Mid-tillering	12%	17%	22%	25%
Great brome	Pre-tillering	15%	25%	34%	41%
	Tillering	14%	22%	30%	35%
	Mid-tillering	11%	16%	21%	24%

Note: All data on 2t/ha yields based off the Primary Industries South Australia Weed Decide calculator 1997.

Source: Bayer CropScience

Table 1C: Wild oat seed production and impact on yield by stacking multiple weed control tactics over successive years.

2009 (fallow)	2010 (TT canola)	2011 (wheat)	2012 (wheat)		
				Wild oats/m²	Yield (kg/ha)
Volunteer barley + dense wild oats	Pre-em only	Pre-em only	All had 900gai/ha glyphosate + 3gai/ha metsulfuron-methyl applied in fallow (Feb). 2L/ha Spray.Seed® applied pre-sowing. No further treatments applied.	34.4	2827
		Pre-em + post-em		6.0	3907
		Pre-em + post-em + harvest weed seed control		1.1	4159
Cut for hay and regrowth sprayed out with glyphosate	Pre-em + post-em	Pre-em only		18.5	3219
		Pre-em + post-em		1.4	4203
		Pre-em + post-em + harvest weed seed control		1.4	3932
No seed-set					
LSD (P=0.05)				7.9	287

Pre-emergent treatments: 2010 – 900gai/ha glyphosate + 1000gai/ha atrazine; 2011 – 540gai/ha glyphosate + 2.5L/ha Boxer[®] Gold.

Post-emergent treatments: 2010 – 1000gai/ha atrazine followed by 60gai/ha clethodim; 2011 – 500mL/ha Crusader[®] + oil.

Source: Adapted from Wu, H. & Koetz, E. (2014)

2. Understanding the behaviour of key broadleaf weeds

A vast number of broadleaf weeds infest broadacre crops. Typically, one or two species dominate in a single paddock, with management decisions revolving around these dominant species. However, there are often small numbers of a range of other weeds present in the paddock and frequently these secondary weeds determine the herbicide rate and/or tank-mixing partner.

It is not possible to cover all broadleaf weeds in a reference manual such as this, so only selected weeds are covered.

Broadleaf weeds employ several strategies to prolong their persistence in farming environments. Understanding the germination and persistence patterns (Table 2A) assists in developing the most appropriate management strategies.

Simplistically, broadleaf weeds can be grouped as follows:

SURFACE GERMINATORS

- Key examples include: sow thistle (*Sonchus oleraceus*) and flaxleaf fleabane (*Conyza bonariensis*).
- Commonly, mature plants will produce very large volumes of viable seed. Dormancy is generally low, with a large percentage of the seedbank germinating within 1 or 2 years after seed production, when conditions are favourable. Species survival is based on high seed production.
- Seed is often small and light, typically adapted for rapid dispersal via wind or water movement.
- As the seed is small, it does not contain large seed reserves and typically does not have enough energy to germinate from depth when buried, therefore they are suited to germination from the soil surface and often proliferate under zero-till farming.
- Many species require light to germinate, so frequent cultivation encourages germination of buried seed that is returned to the surface.
- Where not successfully controlled, weed numbers can build-up rapidly over one or two seasons. However, a robust and effective management strategy can generally reduce seedbank numbers just as rapidly, where no further seedbank recruitment is allowed.

SPECIES WITH SEED DORMANCY

- Key examples include: wild radish (*Raphanus raphanistrum*), bladder ketmia (*Hibiscus trionum*) and cow vine (*Ipomoea lonchophylla*).
- Characteristics of these plants usually involve producing a seed that is often (but not always) relatively large, with the seed often protected from the environment by some form of pod or hard seed coat that is impermeable to water.

- Having a pod and/or hard seed coat can result in extended periods of dormancy, as the pod or seed coat needs to break down before water infiltrates to initiate germination. This feature can also stagger germination, with these weeds often germinating from multiple flushes per season and in low numbers for many years, following a single seed-set. Some species also have other mechanisms to enhance dormancy.
- Seed production may be relatively low in comparison to surface germinators, with extended dormancy being the primary tactic for multi-year survival of the species. However, there are also some hard-seeded species that produce large numbers of smaller seeds.
- Having a larger seed may increase the ability to emerge from depth after burial, so these species often tend to dominate over time in cultivated systems.
- Typically, burying seed results in even longer soil persistence, so avoid deep cultivation where there is a desire to drive the seedbank down.

PERENNIALS

- Some broadleaf plants are perennial and survive from year to year.
- Dispersal can occur by seed, with some species dispersing by underground rhizomes or root or shoot fragments.
- Often these species are present in relatively low numbers or in patches that may take years to build-up.
- Perennial weeds have substantively decreased in importance as more glyphosate and other knockdown herbicides have been included in farming systems.

Tactics for management between these different groups may be quite different, but the ultimate strategy in each case is likely to be similar, that is to farm in paddocks with low seedbanks and stop any seed that is produced returning to the seedbank.

By driving down the seedbank to very low levels, strategies that would otherwise be cost-prohibitive (for example, chipping or hand-roguing) may become far more cost-effective in helping to further suppress the weed seedbank.

For weeds occurring in low densities, optical sprayers can be a highly cost-effective and robust tactic to further drive down the seedbank.

Table 2A: Characteristics of some key broadleaf weed species of Australian grain crops.

Weed	Seed production and persistence	Germination and establishment
Brassica weeds		
Indian hedge mustard (<i>Sisymbrium orientale</i>)	Up to 30,000 seeds per plant. Relatively short dormancy.	Mainly germinates in autumn and winter, with some germination into spring. Cultivation will stimulate germination.
	herbiguide.com.au/Descriptions/hg_indian_hedge_mustard.htm grdc.com.au/resources-and-publications/all-publications/publications/2021/ecology-of-major-emerging-weeds	
Turnip weed (<i>Rapistrum rugosum</i>)	Up to 8000 seeds per plant. Dormancy is broken by hot summer temperatures (approximately 35°C). Seed pods extend life in the soil, however once removed from the pod, persistence appears to be short. In a no-till study in southern Queensland, 36% persisted after 2 years, while 7% remained viable after 4 years when buried to 10cm.	Favours heavier soils but will establish on sandy loams. Mostly germinates in winter, however will continue germinating in warmer months with adequate rainfall. Optimal temperature is 10°C to 25°C. Better adapted to hotter/drier conditions than most other brassicas. Very competitive.
	caws.org.nz/old-site/awc/2016/awc201613511.pdf grdc.com.au/resources-and-publications/all-publications/publications/2021/ecology-of-major-emerging-weeds	
Wild radish (<i>Raphanus raphanistrum</i>)	Mature plants can produce approximately 300 seeds per plant. Earlier emerging cohorts produce more viable seed. Seeds are dormant at harvest, with ~70% still dormant the next winter, so the main flush does not occur until year two after seed-set. Dormancy is influenced by many factors (see factsheet link below for details) but significant levels of viable seed may persist for 6 to 10 years. Seed burial below 40mm substantially increases seedbank survival. Outcrossing species – genetically diverse.	Can germinate all year, depending on rainfall. Multiple germinations occur. Optimal temperature is 10°C to 25°C. Can grow on many soil types, but particularly likes slightly acidic, lighter soils. Optimal germination is from 10 to 20mm, with little germination from below 50mm – so ideally suited to cultivated systems. Very competitive, but crop competition from narrow-row competitive cereal crops can be a valuable management tool.
	grdc.com.au/resources-and-publications/all-publications/factsheets/2021/wild-radish-fact-sheet	
Other broadleaf weeds		
Caltrop (<i>Tribulus terrestris</i>)	Up to 20,000 seeds per plant. The seed pod consists of 5 segments, which break apart. Each segment, containing four seeds, has two spines, which aid dispersal by animals. Burying seed increases persistence.	Spring/summer germination.
	grdc.com.au/resources-and-publications/all-publications/publications/2021/ecology-of-major-emerging-weeds	
Capeweed (<i>Arctotheca calendula</i>)	Up to 4300 seeds per plant. 2 to 3 months dormancy, which is broken by hot summer temperatures. Seed persistence is variable – some populations have almost no viable seed after 12 months while others have recorded >20% viable seed after 24 months.	Germinates in autumn after a rainfall event that wets the soil for a few days. May germinate with, or prior to, the winter crop. Large plants are very competitive.
Climbing buckwheat/ Black bindweed (<i>Fallopia convolvulus</i>)	Up to 1000 seeds per plant. High dormancy. Only ~2.5% germinates per year.	Requires vernalisation. Germinates when soil temperature at 50 to 100mm depth reaches 11 to 13°C. Larger germination in wet winters/spring and wide row crops with less competition.
Flaxleaf fleabane (<i>Conyza bonariensis</i>)	Prolific seeder. Up to 110,000 seeds per plant. Small seed, dispersed by wind and water, however most seed falls within 3–5m of the parent plant. No dormancy. When on the soil surface, <5% viable seed remains after 18 months. Burying increases persistence, but seed will not germinate from depth unless brought back to the surface.	Germinates on the surface to 5mm depth. Very low germination if buried >2cm. Will germinate most of the year but prefers milder conditions of spring and autumn. Optimal temperatures 20 to 25°C, especially following a substantial rainfall event (>20mm) that wets the surface for 3 to 4 days. Poor competitor. Establishes in bare patches.
	grdc.com.au/GRDC-FS-Fleabane	
Fumitory (<i>Fumaria</i> spp.)	Seed dormancy is due to an immature embryo, lignified seed wall and phenol-containing seed coat, with dormancy slowly broken by high summer temperature. Seed is extremely persistent, with seed shown to remain viable for up to 20 years.	Autumn to winter germination. Prefers heavier soils, with cultivation increasing germination.

Table 2A: Characteristics of some key broadleaf weed species of Australian grain crops (continued).

Weed	Seed production and persistence	Germination and establishment
Other broadleaf weeds (continued)		
Sow thistle (<i>Sonchus oleraceus</i>)	Prolific seed production – up to 25,000 seeds per plant. No/low seed dormancy. Most seed on the soil surface has germinated or decayed within 12 months, however 30 and 50% of seeds were viable after 18 months when buried at 5 and 10cm respectively. Thought to be mostly self-pollinating.	Seed dispersed by wind. Can germinate all year round, however prefers milder temperatures of spring and autumn. Rainfall is more important than season/temperature. Major germinations after >20mm event. Prefers surface germination but will germinate from shallow burial (0 to 2cm).
	qaafi.uq.edu.au/files/5955/Common-Sowthistle-%28Sonchus-oleraceus-L_%29-ecology-and-management.pdf grdc.com.au/resources-and-publications/all-publications/publications/2021/ecology-of-major-emerging-weeds	
Spiny emex/Doublgee/ Three-corner Jack (<i>Emex australis</i>)	Large seed (~7 to 10mm) with 3 prominent spines. The spines aid in dissemination by animals. Plants can produce up to 1100 seeds per plant. Approximately 15% viable seed after 2 years and <1% after 8 years.	Germinates over a range of temperatures. Rainfall appears to be the primary determinant of germination. Autumn cultivation will stimulate germination, allowing a knockdown herbicide application before planting. Seed on the surface has poor germination, with optimum germination coming from seed buried up to 50mm. Prefers neutral to slightly alkaline soils. Highly competitive.

Note: Only selected broadleaf weeds are covered. The intent is to highlight the diversity in biological characteristics, not to provide a full list of all summer and winter growing broadleaf weeds of grain cropping.

Information on the ecology and biology of key weeds of cropping can be found at: grdc.com.au/IWMM and grdc.com.au/resources-and-publications/all-publications/publications/2021/ecology-of-major-emerging-weeds

2.1 Principles of managing broadleaf weeds

Broadleaf weeds, especially when occurring in high numbers, can be very competitive with grain crops – particularly those with large leaves and tap roots. Table 2B demonstrates the yield advantage that can be achieved by removing a range of key broadleaf weeds from cereal crops.

In addition to competition with the crop, deep tap-rooted broadleaf weeds can be large users of stored soil moisture in the fallow.

2.1.1 Controlling broadleaf weeds that display dormancy, resulting in multiple and staggered germinations

Many broadleaf weeds have staggered germination, with multiple flushes occurring throughout the crop. These weeds typically have pods, hard seed coats or other adaptations that prolong seedbank life and spread germination over many seasons and may also allow for multiple flushes per season.

With multiple cohorts emerging at different times, a decision may need to be made regarding the timing of broadleaf post-emergent herbicide application – applying to small weeds, soon after emergence will maximise control, however a second application may be required for later germinations. Another strategy may be to delay application to allow further germinations to emerge, however that will result in older weeds being larger and more difficult to control.

Knowing the extent of the seedbank and the germination patterns of the broadleaf weeds of concern can help determine the optimum strategy. Where the weeds are very susceptible to the herbicide(s) chosen, there may be more opportunity to delay application and target mixed weed sizes with a single application. However as early-stage herbicide resistance takes hold, it is increasingly important that weeds are targeted when they are very small. This may result in the need for a two-application herbicide strategy, or some other late season control method to be implemented to stop any late germinators from returning seed to the seedbank.

Wild radish – a case study

A good example of changing weed control strategies can be seen with wild radish. Wild radish has multiple germinations through the season.

Without herbicide resistance, wild radish can be effectively controlled in cereals with a single application of a range of low cost herbicides that is, many from Group 2 or Group 4 modes of action. In the absence of resistance, these products often control mixed weed sizes, so have historically been applied towards the end of tillering to target the first two germinations. Crop canopy closure occurs soon after, reducing the establishment and competition from later germinations in competitive crops.

As Group 2 resistant populations are selected, many growers switch to products based on diflufenican or picolinafen (both Group 12) plus a phenoxy (Group 4) or bromoxynil (Group 6), or in some cases a 3-way mix.

Group 4 resistance is now widespread in populations of wild radish in Western Australia and increasing in eastern states, with an increasing number of populations resistant to Group 2, 4, 5, 6 and 12 modes of action, therefore making the above strategies unreliable.

More recently, herbicide control strategies for these multiple-resistant populations are now heavily reliant on herbicides from Group 14 or Group 27 modes of action.

- Cereal selective Group 14 herbicides (herbicides containing carfentrazone or pyraflufen) are contact herbicides, so are limited to early application targeting small weeds for effective control, with no residual control of subsequent germinations at rates able to be applied in-crop.
- Cereal selective Group 27 herbicides (herbicides containing pyrasulfotole, bicyclopyrone or topramezone) provide robust control when mixed with bromoxynil and/or a phenoxy. However, as an industry we need to realise that this mode of action is the main robust herbicide still effective against wild radish and therefore everything possible should be done to extend the life of this mode of action by implementing best management strategies.

Current thinking suggests that the best approach for managing multiple-resistant wild radish involves seven critical steps.

1. Paddock selection – know the seedbank dynamics, the resistance status of the weeds, and the management strategies that can be effective. Crop choice will be important to ensure there are adequate effective management tools available.
2. Effective knockdown pre-planting to remove any existing germinations.
3. Utilise robust crop competition to reduce wild radish establishment. Implement agronomic tactics that favour early canopy closure.
4. Apply the most robust herbicide tactic to the first flush of wild radish. This is best achieved when weeds are small and optimal for good herbicide performance. This is likely to be either a Group 14 or Group 27 based mixture. Controlling the first flush is generally the most important, both in terms of weed numbers and the competitive impact on the crop.
5. Plan for a second herbicide application at mid to late tillering. Usually this will be a phenoxy-based mixture unless phenoxy resistance is high. Always apply the second application, even if subsequent weed numbers are very low, as a few survivors are all that is required to replenish the seedbank.
6. Certain herbicides (for example, 2,4-D, flumetsulam, triasulfuron, Sharpen®) are registered for late-season salvage application in some crop situations. (Always follow label directions as there is a high risk of crop damage from incorrectly timed applications. Some of these herbicides may be ineffective on certain populations due to resistance).
7. At harvest, implement one of the harvest weed seed control techniques. Weeds surviving at this stage are more likely to be resistant to herbicides, so non-herbicide tactics are required. (For more information on harvest weed seed control weedsmart.org.au/big-6/harvest-weed-seed-control/).

Relying on herbicides alone has shown to be ineffective for long-term sustainable control of wild radish. A strategic change in management to a more integrated approach, such as utilising multiple herbicide and non-herbicide tactics is required.

More information on wild radish management strategies:

GRDC factsheet

grdc.com.au/resources-and-publications/all-publications/factsheets/2021/wild-radish-fact-sheet

Spray small radish twice webinar

youtube.com/watch?v=cOMSLvpqxZg

Tactics to manage wild radish

youtube.com/watch?v=XgosN-Cj3UE

2.1.2 Controlling surface-germinating broadleaf weeds

With the adoption of zero-till farming systems, there has been a shift in species in many paddocks, with weeds adapted to surface germination tending to become more dominant in many areas. Surface-germinating weeds such as sow thistle and fleabane have increased in importance, particularly in the more northern growing regions where summer rainfall assists in maintaining populations.

Sow thistle and fleabane produce large numbers of viable seed with relatively short persistence. Their survival strategy is to germinate rapidly when conditions are suitable (usually after a significant rainfall event), so generally there is little or no dormancy. Most seeds fall within a few metres of the parent plant, however the seeds of these species are also adapted for long-distance dispersal by wind and water.

Control of surface-germinating species such as sow thistle or fleabane requires different tactics to those employed against weeds such as wild radish with high levels of dormancy.

Sow thistle and fleabane can germinate for many months of the year with rainfall often a more significant driver than temperature or daylength. A substantial rainfall event that keeps the soil surface wet for 3 to 4 days will usually trigger a major flush of these weeds. The amount of rainfall required to achieve this is typically larger in summer, compared to autumn or spring, so these weeds are often not considered to germinate in summer. However when conditions are suitable, they can, and will, germinate over summer.

These germination patterns can make control difficult. A major rainfall event will trigger a large flush of these weeds, however smaller events may still germinate a few weeds. If these isolated weeds are not controlled then they can effectively replenish the seedbank due to the massive numbers of seed produced.

Table 2B: Per cent yield gain achieved from removing broadleaf weeds at different application timings and weed densities.

		Weeds/m ²			
		50	100	200	300
Wild radish	Pre-tillering	20%	30%	40%	45%
	Tillering	18%	26%	34%	39%
	Mid-tillering	13%	19%	24%	26%
Indian hedge mustard	Pre-tillering	12%	18%	24%	27%
	Tillering	11%	16%	20%	23%
	Mid-tillering	8%	11%	14%	15%
Wild turnip	Pre-tillering	12%	18%	24%	27%
	Tillering	11%	16%	20%	23%
	Mid-tillering	8%	11%	14%	15%
Turnip weed	Pre-tillering	16%	25%	35%	41%
	Tillering	14%	22%	30%	35%
	Mid-tillering	11%	17%	22%	25%
Spiny emex	Pre-tillering	8%	14%	22%	28%
	Tillering	7%	12%	20%	25%
	Mid-tillering	6%	10%	15%	18%
Capeweed	Pre-tillering	9%	15%	24%	30%
	Tillering	8%	14%	22%	27%
	Mid-tillering	7%	11%	16%	19%
Fumitory	Pre-tillering	4%	6%	8%	9%
	Tillering	4%	5%	7%	8%
	Mid-tillering	3%	4%	5%	5%
Wireweed	Pre-tillering	3%	6%	10%	14%
	Tillering	3%	5%	9%	12%
	Mid-tillering	2%	4%	7%	10%

Note: All data on 2t/ha yields based off the Primary Industries South Australia Weed Decide calculator 1997.

Source: Bayer CropScience

Fleabane – a case study

Fleabane has become a major weed of many zero-till systems, particularly in areas with spring/summer rainfall (or summer irrigation systems).

A mature plant can produce more than 110,000 seeds. While most seeds fall within a few metres of the parent, the seed has a pappus, which facilitates long-distance movement via wind or water dispersal.

Fleabane prefers to germinate on the soil surface, with effectively no germination below 20mm. Seed persistence on the surface is short (less than 18 months), however burying the seed will see some seed remain viable for 2 to 3 years (Wu, et al., 2007).

Weed age is important for herbicidal control. Autumn-germinating fleabane will often sit under the winter crop canopy. Leaf growth can be minimal during winter, with these weeds often looking similar to spring germinators when they are still small rosettes. However the weeds present over winter have continued to develop a large tap root. In spring, when the winter crop hays off and the canopy opens to allow light penetration, these weeds can rapidly elongate and control with herbicides can be very difficult on these still small, but quite old and tough weeds.

Weed control strategies need to focus on year-round management, as germination can occur over much of the year. Growers relying only on post-emergent knockdown herbicides may need to spray many times per year. Often a two-pass herbicide application (double knock) will also be required for effective control of each germination.

In fallow, numerous research trials have consistently demonstrated that control with glyphosate alone is highly variable in susceptible populations, especially when weeds pass the small (<5cm) rosette stage. Control with glyphosate alone is consistently poor on resistant populations. Tank-mixing with a Group 4 herbicide (most frequently 2,4-D or picloram + 2,4-D) improves control significantly, however these mixtures generally require a second knock of a contact herbicide (most trials have used a paraquat-based second knock) to achieve high levels (>98%) of control (Amjad & Hashem, 2016; Werth, et al., 2008; Daniel, 2018; Fleet & Gill, 2013).

Grower strategies that have been successful in driving down the fleabane seedbank share several common attributes:

1. Farm hygiene is critical. Keeping the property clean reduces new incursions from outside the paddock. Pay particular attention to roadsides, irrigation channels and waterways. Fleabane is a poor competitor when faced with existing vegetative ground cover. However where these areas are

sprayed with a non-residual knockdown herbicide to remove vegetation, fleabane is often the first weed to establish and dominate these areas.

2. Fleabane that has established in the winter crop will be very difficult to control after harvest, requiring aggressive double knock tactics and/or tillage. Monitor winter crops for germinations and remove weeds when they are small. 2,4-D application in winter cereals is effective against small rosettes, however does not control subsequent germinations. Lontrel® Advanced, FallowBoss® Tordon® or picloram + 2,4-D herbicides will provide knockdown control along with useful residual control. Check labels for re-cropping intervals of these products.
3. Paddocks that are left in fallow during spring/summer require vigilance to ensure fleabane is not allowed to set seed. Monitor for germinations after every rainfall event and spray when weeds are small (<5cm rosettes). A range of herbicides can be effective on these small weeds. Double knock applications will be required on larger weeds.
4. Having access to an optical spray can be highly cost-effective when spraying scattered germinations and can enable higher label rates to be used in some states and situations than can be legally applied through conventional spray equipment. Always check product labels for directions.
5. The inclusion of a residual herbicide (for example, Balance®, FallowBoss® Tordon®, Terbyne® Xtreme®, Valor®) applied early in the fallow can substantially reduce the frequency of spring/summer knockdown applications.
6. For crops where there are limited or no post-emergent options, application of an effective pre-emergent herbicide is strongly recommended (for example, Palmero® TX in chickpeas, Terbyne® Xtreme® in cotton).

As seedbank persistence is short, an aggressive approach to stopping all seed-set can see seed numbers virtually eliminated within a few years. However reinfestation from long-distance wind or water transport means that growers are often constantly battling this species, especially where there are uncontrolled populations in adjacent paddocks, roadways or upstream waterways. Farm hygiene becomes especially important.

More information on fleabane management strategies:

GRDC fleabane factsheet
grdc.com.au/GRDC-FS-Fleabane

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3. Tactics for managing weeds in crop systems

Weeds emerging at planting or early in the crop compete with the crop for light, nutrients and water. This can lead to a substantial reduction in crop yield. Allowing weeds to establish and set seed replenishes the weed seedbank.

There are a range of herbicide and non-herbicide tactics available to manage weed populations. While this manual is primarily focused on the role of post-emergent herbicides, it is important not to overlook the other valuable tools critical to integrated weed management. Growers should build a diverse management approach to their weed management and not rely on a single tool.

3.1 Non-herbicide tactics

3.1.1 Crop competition

The importance of crop competition in reducing weed seed germination and weed growth should not be underestimated. Many studies have demonstrated that crops and/or varieties that provide early season vegetative growth, particularly where row closure can be achieved early in the crop, will result in reduced weed biomass and reduced weed seed production (Figure 3A and Figure 3B).

Where moisture is not a limitation, increasing seeding rate (as can be seen with the varieties La Trobe[®] and Scope[®] in Figure 3B) can result in reduced competition from weeds, which often also results in increased yield.

In addition to selection of crop type and variety, there may be an additional benefit to using an east-west sowing direction

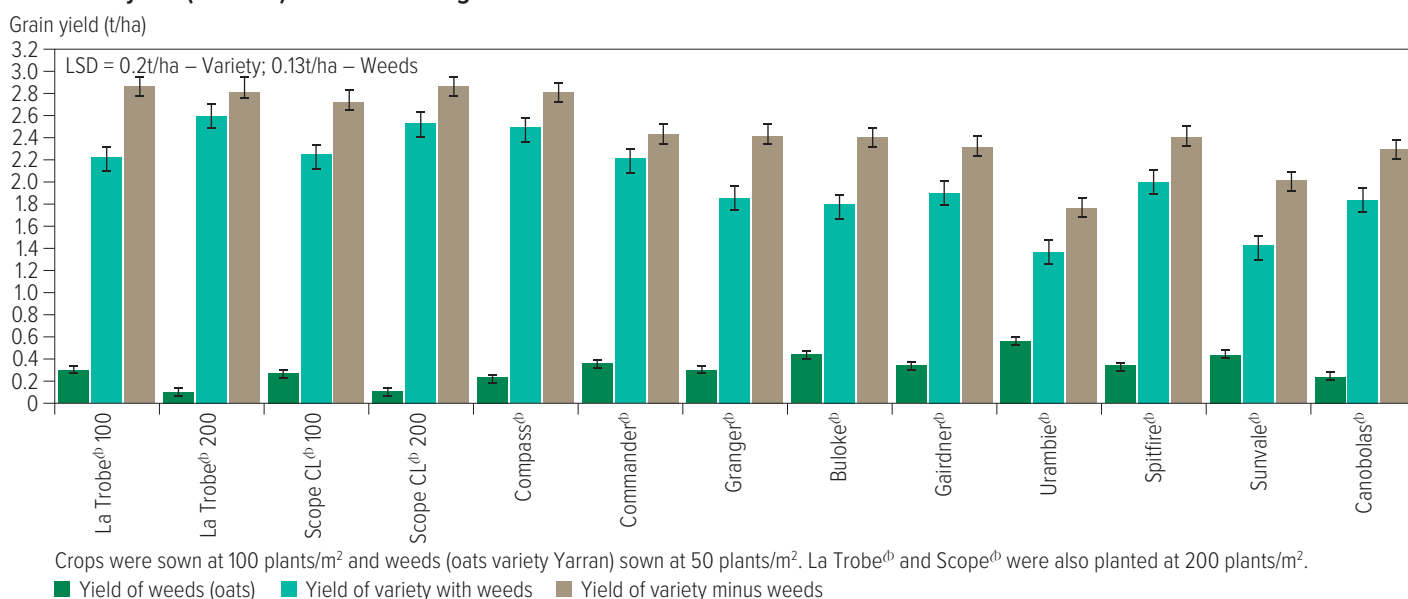
Figure 3A: Canopy development captured 25 days after sowing for Australian barley varieties: La Trobe[®] (low early vigour) and Commander[®] (high early vigour).



Photo: Hickey, et al. (2017)

(Figure 3C) for winter crops the further south they are grown. Light coming from the lower angle of the winter sun is more efficiently intercepted by winter growing crops sown in an east-west direction, compared to those sown in a north-south direction. Crops sown east-west leave less light for interception by weeds growing in the interrow area, resulting in increased crop competition. In northern regions, or in summer in all regions, the sun is more directly overhead and there is adequate light available to the weeds in the interrow, irrespective of row orientation.

Figure 3B: Cereal/weed competition trial showing crop grain yield (t/ha) for different cereal varieties +/- weeds and weed yield (t/ha oat). NSW DPI Trangie 2014.



Source: Brooke (2015)

In this trial at Trangie, NSW (Figure 3C), it was shown that selection of a competitive variety was probably more beneficial than planting direction, although an east-west planting direction provided additional benefit.

Narrowing row spacing, in both winter and summer crops, is highly effective in increasing a crop's ability to out-compete weeds. In a mungbean trial conducted in summer 2014/15 (Figure 3D), the impact of row spacing and timing of weed emergence on crop yield was measured. Three row spacings (25, 50 and 75cm) were evaluated, overlaid with weed-free plots or Rhodes grass; *Chloris gayana* (300 seeds/m²) seed spread across the treatments either at planting or at 3 or 6 weeks after planting. This trial clearly demonstrated the importance of early removal of competition to protect grain yield, while narrowing row spacing provides additional benefit.

Combining crop competition with effective herbicides can lead to higher levels of control. As can be seen in Figure 3E, barley allowed significantly lower wild oat seed production than wheat in the absence of tralkoxydim in this trial. Where the herbicide was used at the full label rate, good control was achieved in both crops. However, where less than the label rate was tested, the less competitive wheat allowed substantially more wild oat seed to be produced, relative to the more competitive barley crop.

More information on the benefits of crop competition can be found in these WeedSmart videos:

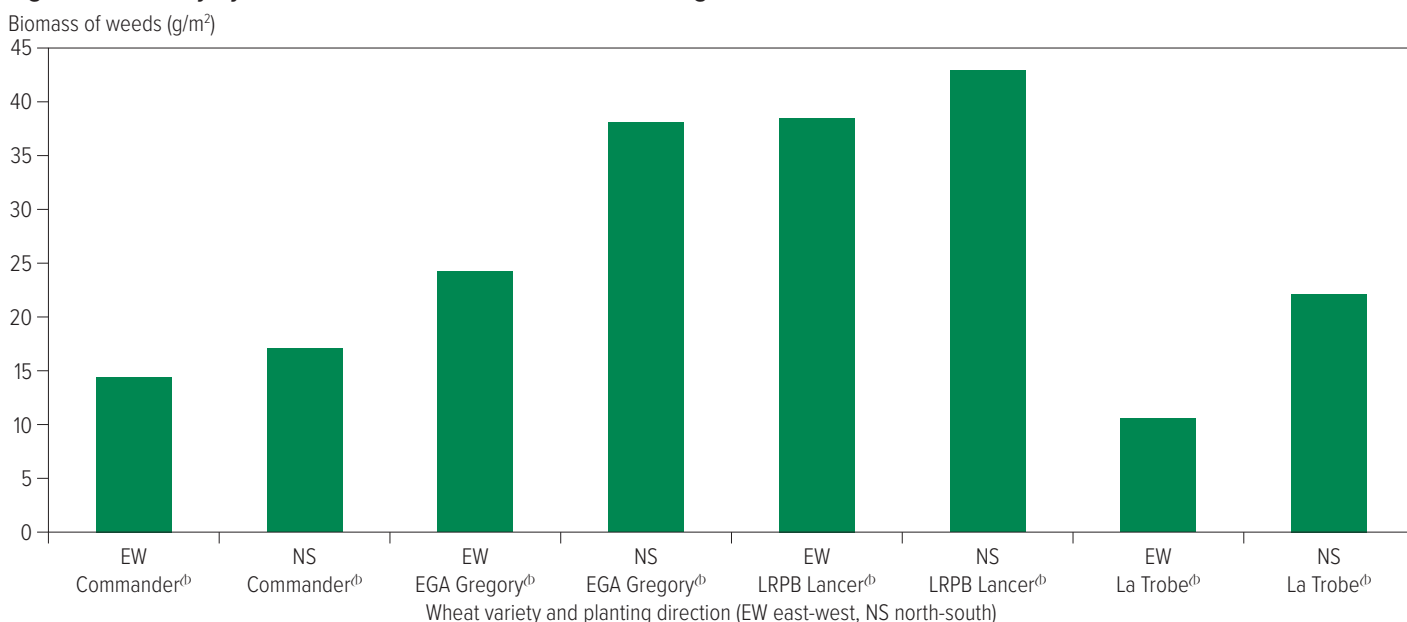
youtube.com/watch?v=sEcjc8uMFaE

youtube.com/watch?v=EhbRpt8SDD0

youtube.com/watch?v=oJOFRQZd3TM

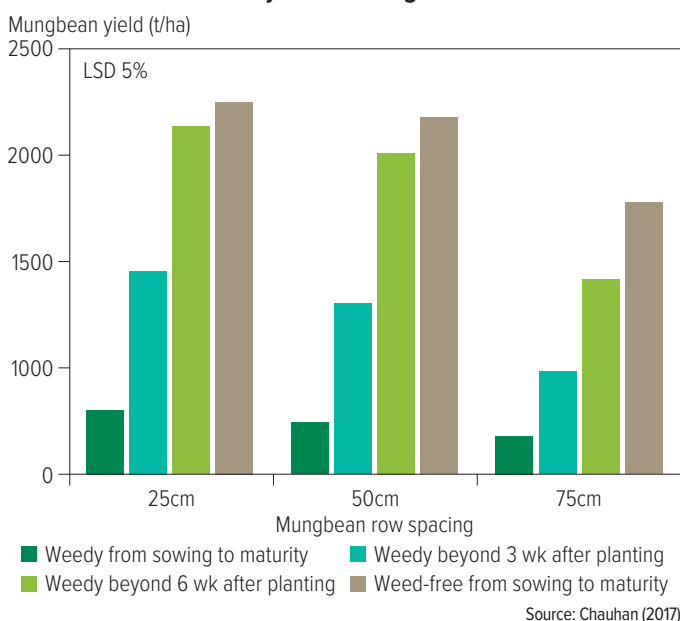
youtube.com/watch?v=IE8ak41f6jA

Figure 3C: Variety by row direction interaction. NSW DPI Trangie 2014.



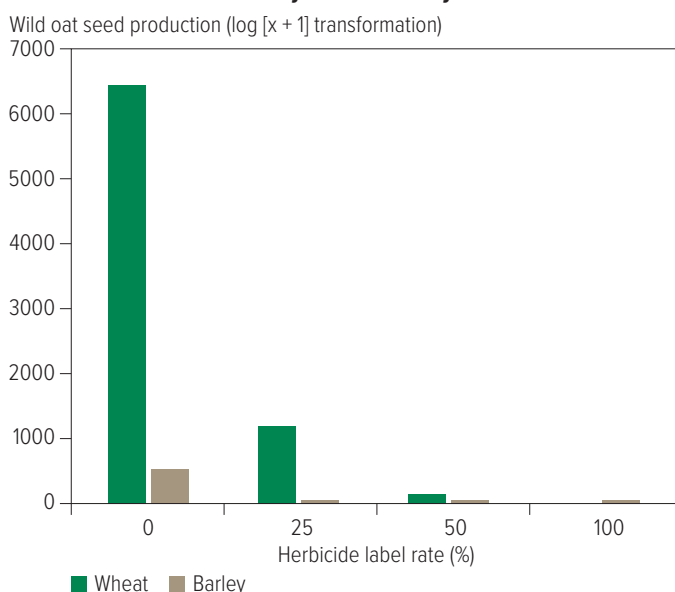
Source: Brooke (2015)

Figure 3D: Impact of row spacing and timing of weed infestation on yield of mungbean.



Source: Chauhan (2017)

Figure 3E: Crop type and competitive ability: effect on wild oat herbicide efficacy with tralkoxydim.



Source: Walker, et. al. (1998)

3.1.2 Keeping weed seedbanks low and the importance of crop rotation/sequencing

Farming, especially when operating in a situation of extensive herbicide resistance, has consistently shown to be most economical where the weed seedbank is very low. This minimises weed competition with the crop and optimises yield. Importantly, low weed numbers also permit the use of diverse weed control tactics and crop options that are possibly uneconomical when the weed seed burden is high.

Keeping weed seed numbers low requires constant vigilance both within the crop and around other sources of weed infestations for example, fence lines, roadsides, watercourses and roadways. Attention to farm biosecurity (farmbiosecurity.com.au/industry/grains/) will be invaluable in the management of weed problems on the farm.

Driving weed seed numbers down requires near zero tolerance to weed seed-set including prevention of any seed returning to the soil. For many of the most economically damaging surface-germinating weeds, seedbank persistence in the soil is relatively short that is, as little as 12 to 36 months. For such weeds, a concerted effort to prevent seed-set over 1 to 3 years can rapidly deplete the weed seedbank and drive down weed numbers.

A key factor associated with increasing weed seed numbers is often an over-reliance on, or dominance of, one particular crop in the rotation. This is especially the case if it is associated with herbicide choice that is either limited, or failing due to herbicide resistance. For example, weeds like annual ryegrass, brome grass and wild oats can rapidly increase in a cereal on cereal rotation, as can barnyard grass and feathertop Rhodes grass in back to back sorghum rotations.

Wherever possible, diversity in crop and herbicide selection is critical. Examples include rotation between summer and winter crops, pasture or fallow phases; and use of options such as manure, hay or silage crops. Diversity in crop sequence alone is not enough – diversity is also needed in the weed control tactics used.

Where weeds have failed to be controlled in the cropping phase, strongly consider weed eradication before the weeds set seed. This may take the form of brown manure (that is, spraying out with a non-selective herbicide), green manure (that is, ploughing in when plants are still green), cutting for hay, slashing or cultivating weed patches or paddock perimeters. The short-term financial cost of lost income by sacrificing the affected portion of the crop is likely to be less than the long-term hit of allowing the weed seedbank to be replenished. Alternatively, a weed seed capture and destruction technique could be used.

Table 3A: Crop sequence and change in annual ryegrass seedbank over 3 years (seeds/m²), Eurongilly, NSW.

Year 1 (2012)	Year 2 (2013)	Year 3 (2014)	Seedbank March 2013	Seedbank March 2014	Seedbank March 2015	
Fallow	RR Canola	Everything had wheat (intermediate herbicide input)	290	NM	56	
Lupin (grain)	RR Canola		748	196	63	
Lupin (BM)	RR Canola		152	NM	110	
Fallow	Wheat (high)		290	NM	118	
RR Canola	Wheat (hay)		208	124	122	
Pea (BM)	RR Canola		464	210	142	
Lupin (grain)	Wheat (high)		748	312	148	
Pea (BM)	Wheat (high)		464	496	162	
RR Canola	Wheat (high)		208	381	219	
TT Canola	Wheat (high)		505	NM	252	
Wheat (high)	RR Canola		777	259	267	
Lupin (BM)	Wheat (high)		152	NM	279	
TT Canola	Wheat (hay)		505	NM	300	
Wheat (low)	RR Canola		5492	797	332	
Wheat (high)	Wheat (high)		777	1379	366	
Wheat (low)	Wheat (high)		5492	3412	523	
Fallow	Wheat (low)		290	NM	970	
Lupin (BM)	Wheat (low)		152	NM	1105	
Lupin (grain)	Wheat (low)		748	6614	1167	
Wheat (high)	Wheat (low)		777	5508	2158	
TT Canola	Wheat (low)		505	NM	2222	
RR Canola	Wheat (low)		208	7770	2387	
Pea (BM)	Wheat (low)		464	7413	3118	
Wheat (low)	Wheat (low)		5492	13148	3140	
P value (2012 treatments)			<0.001	<0.001	<0.001	
P value (2013 treatments)			NA	<0.001	<0.001	
P value (interaction)			NA	0.105	0.699	

NM – not measured BM – brown manure TT – triazine tolerant RR – Roundup® Ready
Crop sequences are ordered from the lowest to highest seedbank in March 2015.

Source: Adapted from Swan, et al. (2015)

To demonstrate the value of crop sequencing, a 3-year trial (Table 3A) was conducted at Eurongilly in southern NSW from 2012 to 2015 (Swan, et al., 2015). This trial commenced in 2012 on a paddock with an average of 1815 annual ryegrass seeds/m² that were resistant to a wide cross section of herbicides. Different crop sequences were applied in the 2012 and 2013 winters. Wheat was planted across all treatments in 2014. The annual ryegrass seedbank was measured throughout the trial, up to autumn 2015. Crop options included:

- **Year 1:** Canola (Roundup® Ready® (RR) or triazine tolerant (TT)), legumes (lupin for grain, or lupin or pea for brown manure (BM)), wheat (high and low input herbicide strategies) or fallow;
- **Year 2:** Canola (RR), wheat (high and low input herbicide strategies) or cereal wheat (hay); and
- **Year 3:** Wheat.

In the above trial, rotation had a large impact on the weed seedbank. The best treatments (fallow/RR canola/ wheat; lupin/ RR canola/wheat; or brown manure lupin/RR canola/wheat) reduced the annual ryegrass seedbank by more than 96 per cent compared to the starting seedbank. The most effective treatments started with an intensive reduction of seed production in the first year, then combined competitive and diverse crop strategies to keep the weed seedbank down. The least effective treatments (low input wheat/low input wheat/wheat; and brown manure peas/ low input wheat/wheat), saw the seedbank increase by more than 170 per cent.

3.1.3 Harvest weed seed control

A key non-herbicide strategy to reduce seedbank replenishment is the capture of weed seeds at harvest and their resultant management.

Research from Western Australia (Table 3B) has shown that harvesting low (15cm above ground) captures a high percentage of weed seeds from species that retain seeds in the seed head at harvest.

Strategies to deal with these weed seeds include:

- **Chaff carts** – trail-behind carts that collect the chaff fraction containing the weed seeds. Chaff is dumped in heaps and either burnt, grazed by livestock or collected and removed from site.
- **Narrow windrow burning** – spreaders on the header are removed and the straw and chaff fractions are concentrated into a narrow windrow (less than 1m) behind the header. This concentrated windrow is then burnt in autumn. By concentrating the windrow, a hot and sustained burn temperature can be achieved. The critical temperature to kill annual ryegrass and wild radish seed has been determined to be more than 400°C for 30 seconds (Walsh & Newman, 2007).
- **Chaff tramlining** – in paddocks using controlled traffic on permanent tramlines, the chaff fraction containing the weed seeds is directed onto permanent wheel tracks. This area is highly compacted and is typically an inhospitable zone where the weeds will have difficulty germinating. They will also be subject to repeated wheel traffic and ‘nearest neighbour’ competition.

Table 3B: Weed seed retention at harvest.

	Weed seeds retained >15cm above ground at start of harvest	Weed seeds retained >15cm above ground at end of harvest (28 days later)
Annual ryegrass	85%	63%
Wild radish	99%	79%
Brome grass	77%	41%
Wild oats	84%	39%

Source: Walsh & Powles (2014)

- **Chaff lining** – involves making a simple chute to divert the chaff fraction (containing weed seeds) into a narrow row in the centre of the harvester. The chaff is then left to rot/mulch, grazed by livestock or controlled with herbicides.
- **Bale direct** – the straw, chaff and weed seeds exiting the header are directed into a square baler trailed behind the header, with the weed seeds leaving the paddock via the straw bale. This can be effective, particularly where there is an off-farm market for the straw.
- **Seed impact mill technology** – located as a trail-behind unit or as an integrated unit contained inside the back of the header. The chaff fraction containing the weed seeds is fed into the mill and pulverised before being ejected back onto the paddock.

More information:

Integrated Harrington Seed Destructor

ihsd.com/

Seed Terminator

seedterminator.com.au/

Redekop

redekopmfg.com/products/harvest-weed-seed-control/

WeedHOG

tecfarm.com.au/tfwp/

With all harvest weed seed control techniques, correct header setup is essential to ensure that the majority of weed seed is fed into the chaff fraction, which is diverted to the destructor unit and the weed seeds are not allowed to exit the header with the straw fraction.

Most harvest weed seed control research has targeted species such as annual ryegrass and wild radish. These species retain a high percentage of weed seeds in the seed head above the 10 to 15cm harvest height (Broster, et al., 2015). Under the right conditions, trials have demonstrated that all these weed seed capture techniques can be effective in reducing the amount of weed seed returning to the seedbank. Research is ongoing to determine the effectiveness on other problematic species encountered (AHRI, 2016).

Each of these techniques has its advantages and disadvantages. Ultimately, it will be a decision for the grower as to which is best suited to their operation. Weedsmart have produced a free on-line learning course ‘Harvest weed seed control 101’ that can be accessed at learninghub.weedsmart.org.au/courses/harvest-weed-seed-control-101.

3.2 Herbicide tactics

3.2.1 Pre-emergent herbicides

Pre-emergent herbicides applied at, or just prior to planting, were a key management tool in grain production prior to the introduction of post-emergent herbicide options in the 1970s and 1980s. In recent years, with the onset of resistance to many post-emergent herbicides, the use of pre-emergent herbicides has made a resurgence. Many growers now rely on pre-emergent herbicides to reduce weed pressure, following with post-emergent herbicides that still have some level of control against resistant populations to 'clean up escapes'.

The role of pre-emergent herbicides and how to use these effectively is a major topic in its own right and is not addressed in this manual. GRDC has produced online and video resources that provide extensive understanding of factors critical to the performance of pre-emergent herbicides used in grain cropping.

grdc.com.au/SoilBehaviourPreEmergentHerbicides

youtube.com/watch?v=s63GYyflzw&t=0s

youtube.com/watch?v=LJNjuMWS57U&t=0s

3.2.2 Post-emergent herbicides

Post-emergent herbicides have been the mainstay of many weed control programs in recent years. Prior to herbicide resistance, many weed control programs relied almost exclusively on these tools, with growers waiting to see what weeds emerge and then selecting the appropriate herbicide or mixture. Post-emergent herbicides have been used extensively in-crop (where crop selectivity permits) and extensively in no-till fallows.

With the rise of herbicide resistance, the role of post-emergent herbicides has changed on many farms.

In many situations, post-emergent herbicides are still used extensively, however they are now seldom the primary tactic for in-crop weed control. The use of post-emergent herbicides has moved to the role of a component part in a broader weed control strategy that contains both pre-emergent herbicides and non-herbicide weed management tactics. Increasingly their role is cleaning up weeds that escape control by pre-emergent herbicides, with at-harvest weed control (harvest weed seed control or late-season herbicides) becoming critical to further prevent survivors from setting seed.

Increased resistance levels are leading to reduced performance of many post-emergent herbicides on one or more key weeds. Chapters 4 and 5 of this manual cover post-emergent herbicides in detail, focusing on how these herbicides work at a cellular level, with strategies to maximise the efficiency of each mode of action.

3.2.3 Late season herbicide use prior to, or at harvest

Often even the best managed weed control programs still leave a few weeds. To prevent seedbank replenishment, these escapes must be controlled.

3.2.3.1 Spray-topping and crop-topping

Spray-topping involves applying a herbicide to reduce viable weed seed production. This may be achieved via a late season selective in-crop herbicide, or via a very late season non-selective herbicide such as paraquat or glyphosate, applied after the formation of an abscission layer on the harvestable crop grain.

Examples of mid/late season sprays to reduce weed seed-set are the Group 1 herbicide pinoxaden (for example, Axial®) and the Group O herbicide flamprop-m-methyl (for example, Oat Master), which target wild oats in wheat.

High frequencies of resistance to both of these herbicides are present in many field populations of wild oats. This may make spray-topping with these herbicides ineffective.

To reduce the level of viable seed, the recommended target wild oat growth stage for selective spray-topping is between jointing (GS31) and mid-booting (GS45). Where all weeds are at a consistent growth stage, applications at the start of the selective spray-topping window, for example, GS30/32 are preferred and will achieve control via high levels of panicle reduction (Cook, et al., 1999). Delaying herbicide application to the later stages of the selective spray-topping window results in lower levels of panicle reduction but may sterilise seed that is produced (Figure 3F, GS39 and GS47–49 timings). Further delaying application past this critical weed development stage (Figure 3F, GS61–65 timing) sees a rapid decline in the ability of the selective grass herbicides to reduce seed viability.

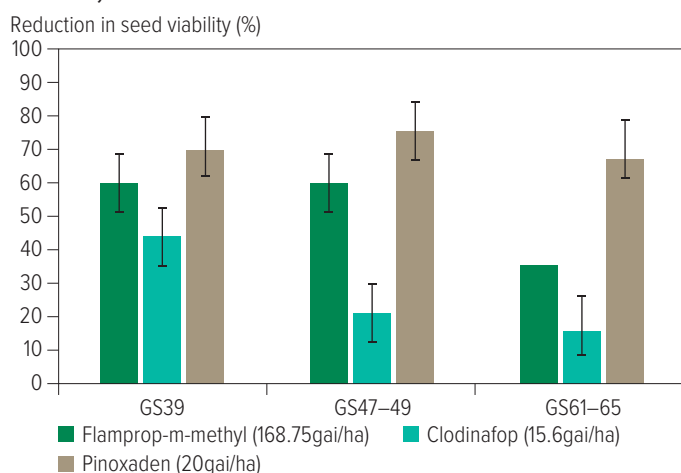
Flamprop-methyl should not be used after the crop flag leaf is fully emerged (GS40) as crop injury may result.

In some winter pulses, the Group 22 herbicide paraquat (for example, Gramoxone®) can be used for spray-topping annual ryegrass (also registered for spray-topping annual ryegrass and barley grass in pastures). If paraquat is applied to pulse crops before physiological maturity, severe damage to the crop can result. Timing is everything and, in some seasons, there may not be a window of opportunity when weeds are still susceptible and the crop is at the right growth stage to avoid damage. Paraquat should be applied to annual ryegrass when the last seed heads have emerged and the majority of seed heads are at, or just past flowering (anthers present or glumes open). Crop losses in excess of 25 per cent may occur if crops still have immature, green pods at application.

Table 3C: Registered growth stage timing (weed and crop) for spray-topping wild oats in cereals.

	Group	Wild oat timing	Crop growth timing
Pinoxaden	1	Stem elongation (GS30), up to and including leaf sheath opening (GS47). Earlier applications reduce panicle numbers. Applications after GS39 reduce viable seed production.	Can be used in wheat and barley. Do not apply after first awns visible (GS49).
Flamprop-m-methyl	O	Stem elongation to booting (GS30 to GS40), preferable when ~20% are at GS31.	Apply before booting of the wheat crop has commenced (GS40). Later application may cause crop injury.

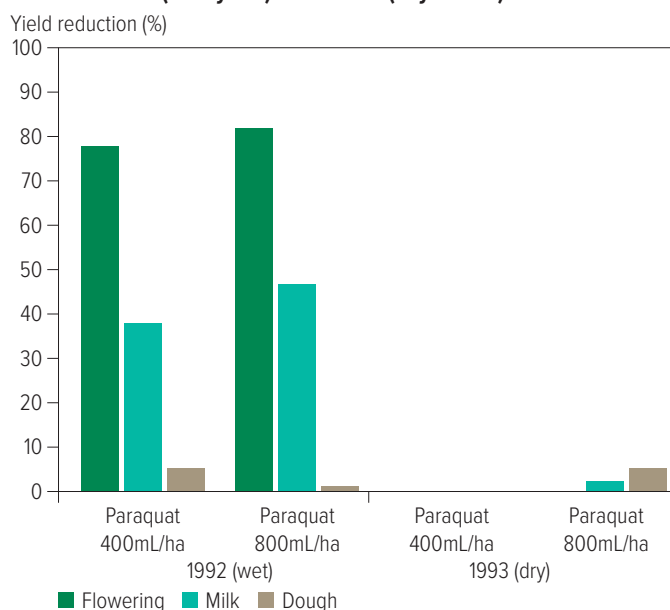
Figure 3F: Selective spray-topping of wild oats with Group 1 herbicides by wild oat growth stage. Average of 15 field trials conducted during 2008 and 2009 across WA, SA, Victoria, NSW and Queensland.



Note: Trials conducted at earlier selective spray-topping growth stages that is, GS31 typically give close to 100% panicle reduction and therefore seed viability cannot be measured. Clodinafop is not registered for selective spray-topping. It was included in these trials to demonstrate that it is not as effective as other registered standards.

Source: Syngenta (2016)

Figure 3G: Yield reduction from crop-topping field peas targeted to the flowering, milk and dough growth stages of annual ryegrass, with two rates of paraquat at Blyth in SA in 1992 (wet year) and 1993 (dry finish).



Source: Mayfield, A. pers. comm.

Being a contact herbicide, paraquat must directly contact all plant parts targeted for control, as translocation is minimal. With crop or pasture topping, the target is the flowering seed head. If the leaf sheath is protecting part of the seed head, the protected component of the seed head will not come into contact with the herbicide and viable seed will still be produced.

As a result, weed species with a short and clearly defined flowering period are best suited to achieving the correct application timing of mid-anthesis. For further information and pictures on correct weed seed growth stages for spray-topping application timing, see the publication 'How do I spray-top to reduce annual weeds in pasture' (Miller, et. al. 2021).

Spray-topping can result in significant crop injury (yield loss) if the herbicide is applied at the incorrect crop or pasture growth stage, which can often be difficult to coordinate with the correct timing for weed seed-set management. Crop injury will be dependent on physiological maturity, so will be influenced by planting date, crop type and maturity of the cultivar. There may also be substantial varietal differences depending on the maturity of individual cultivars (Lines, et al., 2012; Armstrong, et al., 2015).

Trials conducted in South Australia (Figure 3G) showed that in some seasons, it was much more difficult to obtain a window between when the crop had matured sufficiently to enable a safe topping operation and before the weeds had progressed past their optimum time of peak anthesis for spraying. In such seasons, it has usually been possible to delay the spray to the dough stage on the annual ryegrass, and suffer some reduction in weed seed-set control. This work suggested that seasons with dry/early finishes were more likely to provide a window of opportunity as the crop tended to finish faster than the weeds, compared with seasons with longer, cool and wet finishes. Growers still need to be vigilant to achieve correct application timing before the annual ryegrass has hayed off.

Glyphosate may also be used in some situations to spray-top weeds, however the crop must be mature before glyphosate can be applied without causing a yield reduction (refer to product labels for registered uses and application timing). This limits the potential for applications at 'flowering' timing (of the weeds) in most crops. Due to the slower speed of activity, the efficacy of glyphosate declines much faster than for paraquat when applied to weeds post mid-anthesis.

3.2.3.2 Desiccation

Very late season herbicide application (that is, just before harvest) is also often used. This practice is usually focused on drying down the crop (and weeds) to aid harvest operations. Large, established weeds that may be present at this timing may not be killed by the herbicide desiccation. However, they may suffer significant biomass reduction that may 'buy some time' before fallow weed management operations can be implemented. In some circumstances, a level of seed sterilisation may be achieved, however a significant percentage of the weed seed may have already been shed or already be viable by this time.

It is critical to only use approved herbicides and ensure application is in compliance with approved withholding periods (WHPs) for desiccation use patterns. Applications close to harvest are more likely to result in herbicide residues on the grain, so only approved uses that have required Maximum Residue Limits (MRLs) established for local and export markets can be used.

Table 3D: Herbicides registered for crop desiccation (as at June 2023).

Herbicide	Group	Crop	Timing	WHP
2,4-D	4	winter cereals	Apply after firm dough stage. Typically applications will be 14 to 21 days prior to harvest to allow time for weed desiccation.	Not required when used as directed
		Note: 2,4-D is only effective on broadleaf weeds.		
Metsulfuron	2	chickpea	Apply in a mixture with glyphosate when chickpeas are physiologically mature and less than 15% green pods present.	7 days
		Note: Metsulfuron is only effective on broadleaf weeds.		
Sharpen®	14	faba bean	Hilum black in the pods at the top of the canopy (30 to 80% of pods ripe and dark).	7 days Mungbean – Majority of pods are physiologically mature: 90% of pods are yellow or black
		field pea	30% seed moisture or lower, 75% of pods are brown with firm seeds and leathery pods.	
		chickpea	80 to 85 % of pods within crop have turned yellow-brown.	
		lentil	Just after crop starts to yellow (or senesce).	Soybean – Pods are yellow-brown and very late leaf fall (85 to 90%)
		narrow leaf lupin	80% leaf drop. Direct harvesting only.	
		wheat, barley, triticale	Apply between BBCH71 (watery ripe) and BBCH83 (early dough) growth stages.	14 days
	Note: Sharpen® is only effective on broadleaf weeds.			
Diquat	22	winter cereals	Spray as soon as the crop is mature and ready for harvesting.	Not required
		canola	Apply when 70% of the pods are yellow and the seeds are brown or bluish and pliable. Direct harvest four to seven days after spraying.	4 days
		pulses	Apply as soon as the crop has reached full maturity.	0 to 4 days depending on the crop
		Note: diquat is more effective on broadleaf weeds than grasses.		
Paraquat	22	wheat	Not registered. Cannot be used.	
		barley	Not registered. Cannot be used.	
		canola	Not registered. Cannot be used.	
		pulses	Spray-topping use pattern is permitted up to 7 days before harvest.	7 days
Glyphosate	9	wheat	Apply to mature crop from late dough stage (28% moisture) onwards. DO NOT use on crops intended for seed or sprouting.	5 or 7 days depending upon formulation
		barley (except malting barley)	Not all glyphosate formulations are registered for pre-harvest application in barley. Apply to mature crop from late dough stage (28% moisture) onwards. DO NOT use on crops intended for seed or sprouting.	7 days
		canola	Not all glyphosate formulations are registered for pre-harvest application in canola. Refer to labels for specific crop advice. Apply to standing crop from early senescence (20% of seeds on main stem have changed to dark brown/black). Can also be applied at time of windrowing provided application is applied under the windrow. DO NOT apply over the top of the windrow.	Depends on formulation. Refer to individual label for WHP and use pattern information.
		pulses	Not all glyphosate formulations are registered for all pulse uses. Refer to labels for specific crop advice. Application to crops intended for seed production or for sprouting may reduce germination percentage to commercially unacceptable levels.	7 days
		sorghum	Apply when grain moisture is less than 25%. May increase potential for lodging.	7 days

For more information of pre-harvest herbicide use, refer to the GRDC publication *Stewardship for Pre-harvest Application of Herbicides in Winter Crops*.
grdc.com.au/resources-and-publications/all-publications/factsheets/2022/preharvest-herbicide-use-fact-sheet



Problem weeds cannot always be controlled by late herbicide applications. For example, there are no herbicide solutions that can control this feathertop Rhodes grass (*Chloris virgata*) population in sorghum. Photo: Mark Congreve

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4. Understanding and maximising the performance of post-emergent herbicides

Post-emergent herbicides remain a key management tool for many growers, despite increasing levels of resistance. As herbicide resistance increases, these herbicides are not as forgiving as they were when first commercialised. Having a thorough understanding of how each mode of action works within the plant and how resistance is likely to be expressed will arm users with strategies to maximise the performance of these tools.

This chapter provides understanding of the factors that influence herbicide penetration through the leaf, translocation to the site of activity and how the herbicide is metabolised/broken down within the plant.

4.1 Where do post-emergent herbicides work in the plant?

Most herbicides target specific metabolic function(s) in plant cells – typically disrupting a specific enzyme system.

Enzymes work as catalysts by accepting a substrate into a binding site on the enzyme, transforming it into a new substrate, which is then released to be available to the plant to perform another function in the plant.

Herbicides disrupt the function of specific plant enzymes, often by preferentially binding to the target site. This prevents the normal plant substrate from binding and achieving its function, ultimately leading to cell death.

Herbicides are arranged into Modes of Action (MOA) groups. Herbicides are considered to have the same mode of action if they target the same location/enzyme system within the plant. It is possible to have different herbicides, with very diverse chemical structures, that still target the same enzyme. In this case, they are typically classified into different sub-groups within the same mode of action.

For example, Group 1 herbicides are powerful inhibitors of the acetyl-CoA carboxylase (ACCase) enzyme. However, within the Group 1 mode of action, there are three distinct herbicide groups

Table 4A: Group 1 herbicide mode of action sub-groups and active ingredients by sub-group.

Group 1 sub-groups	Grass active herbicides used in grains and broadleaf field crops
Aryloxyphenoxypropionate (Fop)	clodinafop diclofop fenoxaprop fluazifop haloxyfop propaquizafop quizalofop
Cyclohexanedione (Dim)	butroxydim clethodim sethoxydim tralkoxydim
Phenylpyrazole (Den)	pinoxaden

that are structurally and chemically different (Table 4A), however they all target the same plant enzyme system.

Many enzyme systems targeted by herbicides are found within the cell chloroplasts in the leaf (for example, mode of action Groups 5, 6, 10, 12, 14, 22 and 27). To reach a target site located within the chloroplasts, the herbicide may only need to move through the leaf cuticle, across the cell membrane and into the chloroplast of the cells within the leaf.

However, other herbicides such as Group 1, 2 and 9 target enzyme systems that are mainly produced in high levels in the meristematic areas of the plant (that is, the growing points - the root tips, the crown for grass weeds and the apical growing point for broadleaf plants). For effective results, these herbicides need to first penetrate the leaf cuticle, then translocate from the site of plant entry to one, or both, of the meristematic areas of target enzyme activity.

Figure 4A: Glyphosate symptoms 8, 9 and 12 days after application under good translocation conditions that is, hot (January) with a full soil moisture profile and actively growing weeds.

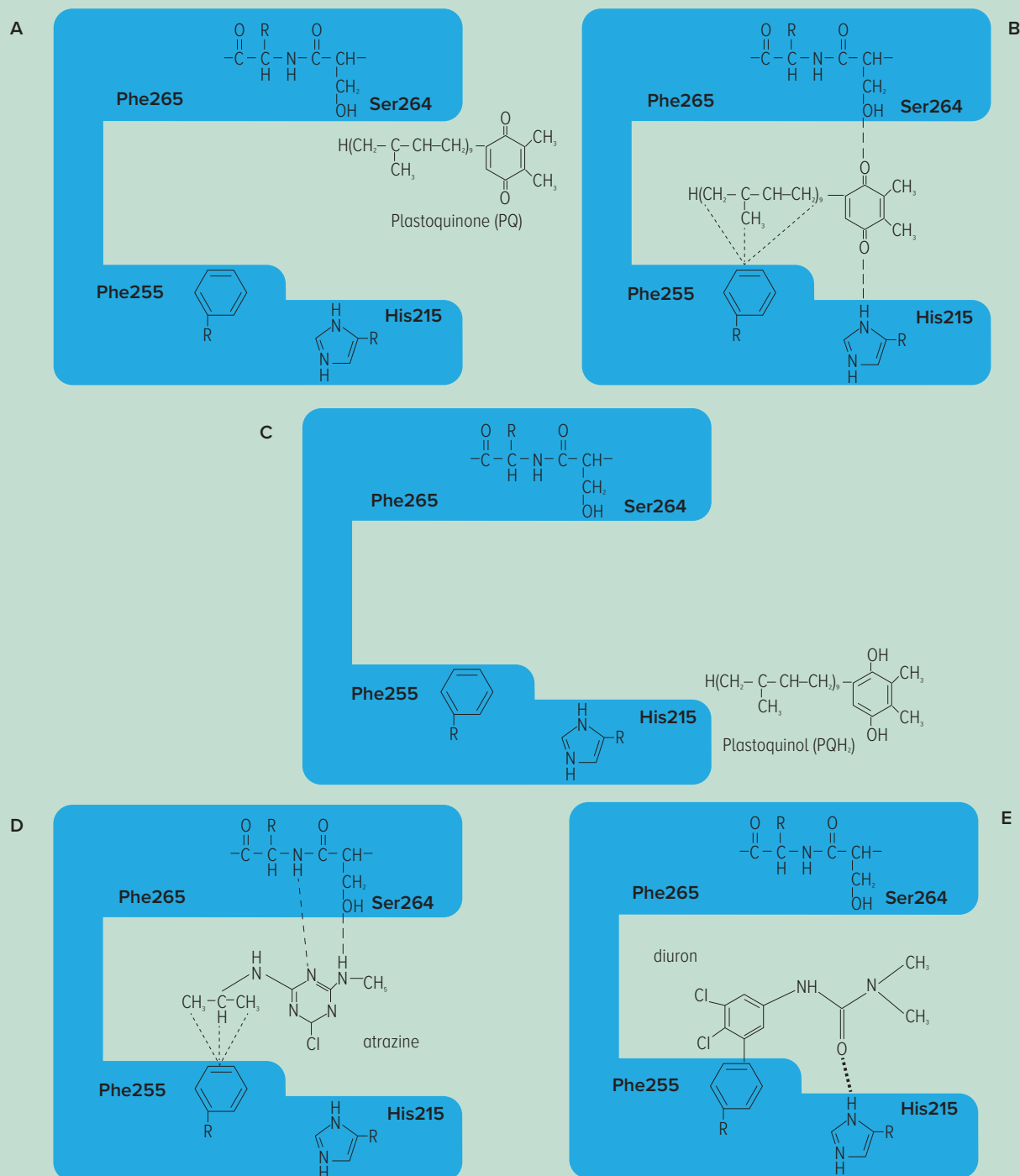


Photos: Mark Congreve

Enzymes, binding sites and herbicides

Understanding the role of enzyme binding sites is important to understand how herbicides work. A binding site is a location where a substrate can bind and then be transformed. The plastoquinone binding site on the D¹ protein is used as an example below to highlight the roles of enzymes, binding sites and substrates.

Figure 4B: The quinone-binding (Q_B) site on the D¹ protein, its role in binding plastoquinone and how Group 5 herbicides bind at the target site.



Source: Adapted from Cobb & Reade (2010) and Preston (2002)

In this example, normally the substrate plastoquinone will move into the binding site (A) and then bind in association with key amino acids at the locations indicated (B). Once bound, plastoquinone accepts two high-energy electrons and two hydrogens and is transformed into plastoquinol. Plastoquinol is then released from the binding site and carries these electrons and hydrogens away from the binding site (C).

Where Group 5 herbicides have been applied to the plant and made their way to the chloroplasts, they will preferentially bind to the target site and are not released. Binding of atrazine (triazine sub-group) and diuron (urea sub-group) are shown (D and E). While these herbicide

examples are from different sub-groups and have different binding to individual amino acids, the presence of either of these herbicides prevents plastoquinone from binding at the target site, and therefore stop it from accepting and removing the high-energy electrons and hydrogen. Without this plastoquinone function, build-up of these reactive electrons causes cell wall leakage and the herbicide effects typically seen following application of Group 5 herbicides.

Note: several additional amino acids (not shown) also influence binding of these herbicides at the binding pocket, however these have been excluded in this example for simplification purposes.

The chemical properties of glyphosate (Group 9) and the ACCase inhibitors (Group 1) dictate their ability to translocate throughout the plant, which is covered in later sections of this manual. Slow or reduced translocation, coupled with the large distance herbicides are required to move from the site of entry to the meristematic regions in grass weeds (particularly for large weeds) is part of the reason why it often takes days/weeks before herbicide symptoms are observed.

4.2 Post-emergent herbicide entry into the plant and translocation to the target site

For post-emergent herbicides to work, they must first enter the leaf and then translocate to the site of activity.

The first key factor affecting herbicide performance is to ensure the maximum quantity of herbicide reaches the leaf surface. Sprayer set-up and application is a complex topic and is not covered in this resource. GRDC has produced a *GrowNotes™ Spray Application Manual for Grain Growers* grdc.com.au/resources-and-publications/grownotes/technical-manuals/spray-application-manual covering spray application in detail.

Spray coverage is critical for all herbicides, however it is especially important for those considered to be contact herbicides (that is, paraquat (Group 22), glufosinate (Group 10) and several Group 14 herbicides). In addition to contact herbicides, Group 1 herbicides are relatively poorly translocated and will also benefit from high levels of droplet coverage. By comparison, products that readily translocate within the plant such as glyphosate (Group 9) and the Group 2 herbicides are typically less sensitive to application coverage.

4.2.1 Weather, herbicide uptake and performance

The weather at the time of herbicide application can have a significant bearing on the performance of a post-emergent herbicide, affecting both how it enters the plant and translocation once inside the plant. As most herbicides block a metabolic plant process, it follows that herbicides are likely to be more effective when the plant is rapidly growing.

- Under dry/low humidity conditions, plants can alter the moisture in the leaf cuticle by moving water out of the cuticle, therefore increasing the density of the waxy layer near the leaf surface. This modification of the cuticle reduces the plant's water loss, however makes penetration of hydrophilic (water-loving) herbicides more difficult. [See Section 4.3.2 Leaf Penetration](#) for more detail.
- Under these same dry/low humidity conditions, plants will close their stomata to reduce moisture losses from transpiration. While penetration via the stomata is not typically a major pathway for herbicide entry into the leaf, the closing of the stomata reduces the plants uptake of carbon dioxide (CO₂) from the air and therefore reduces the rate of photosynthesis.
- Under low light conditions, rates of photosynthesis can reduce by up to 50 per cent. This can therefore reduce the activity of herbicides that directly target the PSI and PSII pathways.
- Where photosynthesis is reduced (either as a result of low light conditions or moisture stress), the production of sugars and activity of other metabolic pathways are also reduced. This often leads to reduced translocation of photosynthetic metabolites within the phloem, which will then affect herbicide

activity of those herbicides that require phloem mobility to get to meristematic regions of the plant where they are active (for example, Group 1 and glyphosate in particular).

- Following a stress event (such as extreme heat, frost or waterlogging), metabolism and associated translocation slows or may completely stop. Plants generally take a few days to fully recover after stress. During this time, the effectiveness of herbicides that require phloem translocation to the active site can be reduced. In addition, several post-emergent herbicides attain their crop selectivity by rapid metabolism in the crop. Without this rapid metabolism, crop damage is likely to increase following a stress event (frost in particular).

Examples include:

- the activity of Group 1 herbicides can be significantly reduced by cold or frost conditions, before or soon after spray application; and
- the crop selectivity of Group 2 herbicides is often reduced when the crop is under stress conditions.

4.2.2. The role of adjuvants

An adjuvant is a product that modifies the performance of another product. In the case of herbicides, this can be in the form of:

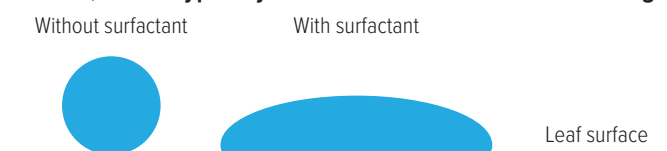
- a surfactant (surface active agent) that modifies the droplet behaviour on the leaf;
- humectants that reduce droplet evaporation;
- products that modify or dissolve the waxy leaf cuticle to assist in cuticle penetration;
- products that modify droplet size;
- pH buffering agents;
- products that assist movement across cell membranes within the leaf; or
- products that enhance compatibility.

A number of commercial adjuvants are blends that perform more than one of these functions.

It is important to use the correct adjuvant(s) for the individual herbicide, specified by the manufacturer on the herbicide label.

A surfactant (sometimes called a wetting agent) reduces the surface tension between the herbicide droplet and the leaf surface, causing the droplet to spread. Not all surfactants/wetting agents provide the same level of droplet spread—some surfactants are designed to give high level of leaf spread, while others may have very limited spread, but may still be useful in increasing droplet adherence or penetration. Leaf surfaces can be waxy or hairy, which may reduce the ability of the herbicide to spread or penetrate the leaf.

Figure 4C: The addition of a surfactant reduces the surface tension on the leaf and reduces the contact angle of the droplet, causing the droplet to spread further on the leaf surface, which typically results in better herbicide coverage.



Depending on the properties of both the leaf surface (that is, different weeds can require different surfactants for best uptake) and the individual herbicide molecule, leaf penetration may benefit from a positive, neutral or negatively charged surfactant to assist leaf penetration. Surfactants can be either:

- anionic: negatively charged;
- cationic: positively charged;
- non-ionic: no charge; and
- amphoteric: cationic in acidic environment or anionic in an alkaline environment.

Organosilicon surfactants are a specific class of surfactants that can create very low surface tensions (as low as 18-20 dynes per centimeter). This not only provides very high levels of spreading, but may also allow the potential for some stomatal entry, but only before the spray deposit has dried (Cobb & Reade, 2010).

Humectants (for example, polyethylene glycol and many others) are often added to the formulations of post-emergent herbicides. Humectants maintain the herbicide in a liquid phase for longer after application, and therefore increase the time available for hydrophilic herbicides to penetrate the leaf cuticle. They also have a role in helping the product concentrate in the drum to remain in a liquid phase.

In some situations, adding a crop oil concentrate (COC) or methylated seed oil (MSO) may reduce the rate of droplet evaporation. COCs and MSOs may also assist in cuticle penetration by modifying the waxy cuticle. Oil-based adjuvants work best with lipophilic herbicides (for example, Group 1 and some Group 5 and 14) however are generally unsuitable for hydrophilic herbicides (for example, Group 22, glyphosate, glufosinate).

It is important to understand the quality (cleanliness, pH, hardness in particular) of water used for spraying so that water conditioning can be undertaken before adding herbicides, should this be required.

pH modifying agents can be built into formulations. For example, pH buffers are often included in sulfonylurea formulations, that would otherwise start to undergo degradation in the spray tank under low pH environments.

Many pH reducing spray adjuvants are also sold as tank-mix additives. In certain situations (that is, extremely alkaline water), there may be an advantage in reducing water pH before adding some herbicides. However this is frequently not required as many post-emergent herbicides are weak acids and will reduce water pH in their own right.

What happens in the spray tank when using hard water and ammonium sulphate with glyphosate?

Hard water contains elevated levels of divalent cations (containing two positive charges for example, Ca^{++} , Mg^{++}) or trivalent cations (containing three positive charges for example, Al^{+++} , Fe^{+++}). Calcium and magnesium are often the most common cations contributing to hard water when it comes to agricultural spraying uses.

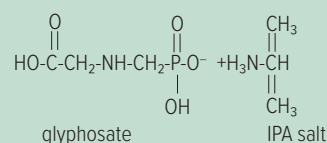
While glyphosate is active in the acid form, the solubility of glyphosate acid is very low (11.6g/L at pH7 25°C; Weed Science Society of America, 2014)). To improve solubility and increase the ability to penetrate the leaf cuticle, glyphosate is formulated as a salt.

If the glyphosate salt formulation is added to the spray tank without preconditioning the water with ammonium sulphate (particularly in alkaline water), some of the glyphosate salt will dissociate (break apart), to the glyphosate acid and the salt (see following example with glyphosate IPA).

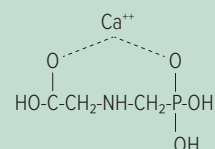
Available cations in hard water (calcium in the example) will preferentially recombine with the dissociated glyphosate, to create a calcium salt of glyphosate. Solubility of a calcium salt of glyphosate is extremely low/poor (~30g/L) so the glyphosate-calcium will precipitate out of solution and will be inefficient in penetrating the leaf surface.

To overcome this effect, ammonium sulphate (AMS) can be added to the spray tank before adding the glyphosate salt. When the AMS is added to hard water, it will dissociate to ammonium and sulphate, with the sulphate reacting with the cation (free calcium in the example below to form calcium sulphate), which will then precipitate out of solution. When the glyphosate salt formulation is then added to the spray solution after the ammonium sulphate, there will be less free calcium available to form the relatively insoluble calcium salt of glyphosate.

Glyphosate is formulated as a salt to increase solubility, cuticle penetration and movement.

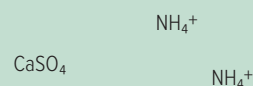


In the presence of calcium and alkaline water, glyphosate dissociates and preferentially binds to calcium.



Glyphosate-calcium has low solubility and is poorly absorbed.

Adding ammonium sulphate ($\text{NH}_4)_2\text{SO}_4$ before adding glyphosate, in the presence of calcium and alkaline water, sees the ammonium sulphate, dissociate and form calcium sulphate which will precipitate out of solution.



Ammonium is also released, which assists glyphosate to cross the plasma membrane surrounding the cell, once inside the leaf.

Adding AMS to the spray tank before the addition of the herbicide is effective where the water contains elevated levels of calcium or magnesium as it can form sulphates of each of these cations.

The following formula calculates the amount of ammonium sulphate required to add to the spray tank:

$$\text{Kg ammonium sulphate/100L} = (0.001 \times \text{Ca (ppm)}) + (0.0006 \times \text{Na}) + (0.0002 \times \text{K}) + (0.0017 \times \text{Mg})$$

For example, a pH-reducing adjuvant is sometimes added to the spray tank to reduce the pH of the solution when using glyphosate, as research has shown that a spray solution pH of 4.5 to 5.6 is generally optimal for glyphosate stability and compatibility in the spray tank, leaf penetration and movement across cell membranes. However, as glyphosate is a weak acid, the addition of glyphosate alone often reduces the pH to these required levels without the need for an additional pH modifying agent. For example, adding the equivalent of 1.2L/ha glyphosate into bore water at water rates equivalent to applying 40L spray volume/ha dropped the pH of the spray solution from 8.4 to 4.9, without any additional buffering or acidifying adjuvants (Dow AgroSciences, 2012).

Ammonium sulphate (AMS) is a useful tank-mix additive in a number of situations, especially to condition hard water for use with soluble herbicides (for example, 2,4-D amine, glyphosate, glufosinate and others). It can also perform as an effective compatibility agent, reducing antagonism between certain herbicides.

Adding AMS can also assist in aiding uptake of weak acid herbicides (in particular this is well documented with glyphosate and the 'dim' herbicides). Weak acid herbicides have a carboxylic acid or other functional group that accepts or loses a hydrogen ion, depending on the pH of the surrounding solution. This means that the herbicide's solubility will change with changes in solution pH.

Free ammonium (NH_4^+) available in the spray tank following the addition of AMS assists negatively charged herbicides to be absorbed more effectively through the cuticle, while the ammonium will also translocate to the cell and cross the cell membrane. Once inside the cell, in a more alkaline environment, it converts to ammonia (NH_3) plus hydrogen (H^+). The hydrogen ions are then required to be pumped back out of the cell into the cell wall to maintain the optimum pH within the cell (7.5 to 8.0). This accumulation of additional hydrogen ions outside the cell further decreases the already acidic pH of the cell wall. When weak acid herbicides are in the presence of this lower pH environment, their lipophilic properties increase, assisting the movement of the herbicide into the cell (University of Nebraska, 2016).

Some growers prefer to add urea ammonium nitrate (UAN) to the spray tank instead of AMS. UAN also provides a source of free ammonium, however UAN does not have the same ability to counteract the effects of hard water that AMS does.

For additional information:

Adjuvants, oils, surfactants and other additives for farm chemicals. Revised 2019 edition (GRDC) grdc.com.au/adjuvants-booklet

Getting chemistry in the spray tank right (Dow AgroSciences, 2015) grdc.com.au/Research-and-Development/GRDC-Update-Papers/2015/02/Getting-the-right-mix

4.3 Herbicide movement within plants

Properties of the herbicide dictate how it moves through the leaf cuticle and translocates to the site of activity.

4.3.1 Herbicide formulation

In some instances, herbicide formulation may be designed to enhance leaf penetration.

One example is the Group 1 aryloxyphenoxypropionates (fops). For these herbicides to control weeds, they need to move into the leaf and then translocate to the meristematic region (predominantly the crown of the plant in grass weeds) where the target ACCase enzyme is present in highest quantities. To provide herbicidal activity, the fop herbicide needs to be in the parent acid form of the herbicide. However, in the acid form, fops have limited ability to penetrate the leaf cuticle. Therefore, to enhance penetration of the leaf surface, they are typically formulated as lipophilic esters, which allow for easier penetration of the waxy cuticle. Once inside the leaf, they rapidly convert to the active acid form by a hydrolysis reaction.

Another example of a herbicide formulation to enhance leaf entry is seen with glyphosate. Glyphosate is required to be in the acid form for herbicidal effect. However, glyphosate acid has poor solubility (~12g/L), which would severely limit formulation concentration, while also having poor leaf penetration properties. Therefore, it is formulated as a salt (for example, isopropylamine, potassium, monoammonium, monoethylamine salts are common commercial formulations). These salt formulations allow for improved leaf penetration and higher formulation solubility (concentrations of 450 to >700g/L are possible depending on the salt used).

Some herbicides, for example the synthetic auxin herbicide 2,4-D, have the ability to be formulated as either an ester or an amine (salt) formulation. In the ester form, leaf penetration will be more rapid as the herbicide is more lipophilic, which may result in lower use rates due to enhanced speed of entry allowing more herbicide to enter the leaf before the spray deposit has dried. 2,4-D in the amine form is more hydrophilic so will take longer to cross the waxy leaf cuticle and will be more subject to drying on the leaf surface or wash-off in the event of rainfall soon after application.

As amine formulations take longer to penetrate the cuticle, they tend to be more responsive to surfactants and other formulation additives that enhance leaf penetration or delay droplet evaporation. Once inside the leaf, both ester and amine forms of 2,4-D rapidly convert to the parent 2,4-D acid, which is the active compound.

4.3.2 Leaf penetration

For any foliar-applied herbicide to be effective, it must be able to move into the plant; commonly through transfer of the spray through the leaf cuticle, which becomes the main barrier to herbicide penetration of the leaf. In certain situations, some herbicide may enter through the stomata (when open), however this pathway for entry is typically minimal as stomata are relatively sparse and mainly located on the underside of the leaf (with most herbicide deposited on the adaxial (upper) surface).

For foliar-applied herbicides, the herbicide properties dictate movement through the cuticle and cell membranes and penetration into the cells. Once a foliar herbicide is sprayed onto a leaf, its movement can be predicted by understanding the octanol/water partition coefficient ($\log K_{ow}$).

The octanol/water partition coefficient ($\log K_{ow}$) is a ratio that suggests how easy it will be for the herbicide to move across the various lipophilic and hydrophilic layers within the leaf cuticle and cell membranes.

The octanol/water partition coefficient is calculated by measuring the concentration of herbicide in an octanol phase compared to in a water phase that is:

$$K_{ow} = C_{\text{octanol}} / C_{\text{water}}$$

where C_{octanol} is the molar concentration of the organic compound in the octanol phase and C_{water} is the molar concentration of the organic compound in water when the system is at equilibrium. This is then converted to a log scale to be reported as a $\log K_{ow}$ value.

The cuticle is comprised of lipophilic waxes and hydrophilic cutin (Figure 4D). After spray is deposited on the leaf surface, herbicides that are more lipophilic (non-polar or higher $\log K_{ow}$) will move faster through the outer cuticular wax. This rapid movement through the external leaf cuticle often results in short rainfast periods (for example, large quantities of herbicide may have moved across the cuticle within an hour). Once through the cuticle wax layer, movement may slow as the levels of the more hydrophilic cutin increase towards the underlying cells. Lipophilic herbicides are generally non-polar and relatively insoluble in water, so have more difficulty moving through the cutin, and the apoplast in between cells, which are both water-based environments.

Most hydrophilic (polar) herbicides have difficulty with the initial penetration of the waxy leaf surface but move faster through the hydrophilic cutin and apoplast once they have penetrated the waxy cuticle. Where the hydrophilic herbicide is negatively charged (for example, many salt formulations), it can take several hours for a hydrophilic herbicide to move through the cuticle. Cuticle penetration rapidly decreases, or stops, after the spray droplet has dried, so adjuvants that reduce droplet evaporation and/or degrade leaf surface waxes can be highly beneficial with these herbicides. Hydrophilic herbicides are prone to wash off should rainfall occur soon after application, as they are highly soluble and require extended periods of time on the leaf surface for penetration.

An exception is the Group 22 herbicides (for example, paraquat, diquat), which are positively charged and will therefore rapidly enter the negatively charged leaf, despite being hydrophilic.

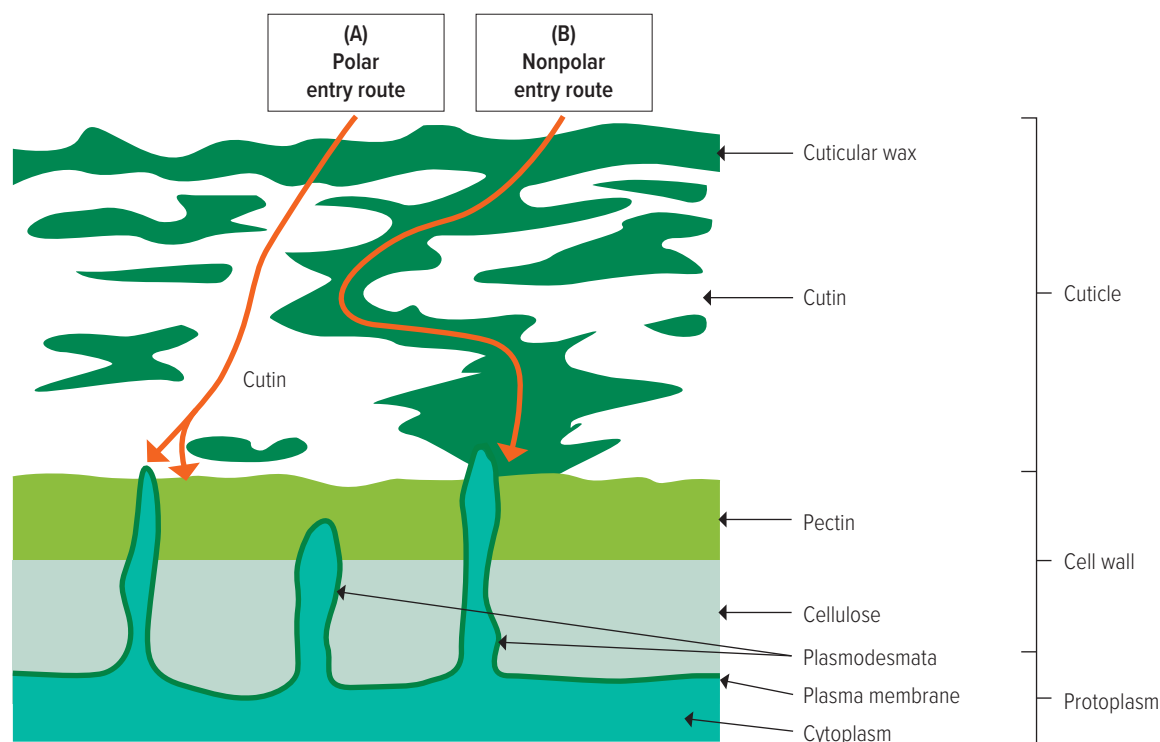
Once through the cuticle, the $\log K_{ow}$ properties also provide an insight into movement across the cell wall and plasma membrane, before entering cell cytoplasm. Herbicides that have difficulty moving across cell membranes are unlikely to move far within the leaf and are typically 'contact' herbicides, while those that can easily move across cell membranes and therefore around the plant, are considered 'systemic' herbicides.

Hydrophilic herbicides ($\log K_{ow} < -1.5$) may have trouble moving across the lipophilic plasma membrane and into the cell by herbicide gradient diffusion. For these herbicides to enter the cell cytoplasm, an active transport mechanism, typically involving enzymes, is often required to move the herbicide across the plasma membrane. For example, auxin influx carriers, phosphate carriers and putrescine carriers assist in moving phenoxies, glyphosate and paraquat respectively across the plasma membrane (Sterling & Namuth-Covert, 2016a).

Herbicides with intermediate lipophilicity appear to be able to move in both directions across the plasma membrane, dependent on the herbicide concentration gradient. Movement across the cell membrane appears to be optimised where the $\log K_{ow}$ is about -1 to 1.5 . Many Group 2 herbicides have these properties and are therefore systemic within the plant.

Lipophilic, uncharged herbicides ($\log K_{ow} > 4$) can easily move across the cell membrane into the cytoplasm. However lipophilic herbicides that are charged (typically weak acids) may be trapped within the cell cytoplasm by a process called ion trapping. Typically, the pH outside the plasma membrane is acidic ($\text{pH} \sim 5.5$), while inside the cell cytoplasm the pH is alkaline ($\text{pH} \sim 7.5$). Weak acid herbicides contain a functional group, often a carboxylic acid, that will gain or lose a hydrogen ion depending on the pH of the surrounding solution. This allows weak acid herbicides to be more hydrophilic or lipophilic, depending on the surrounding pH. Once inside the cell and in an alkaline environment, weak acid

Figure 4D: Absorption route from leaf surface to cytoplasm.



Source: Ashton & Crafts (1981)

herbicides become more ionised (giving up a hydrogen ion) and therefore become more hydrophilic. In the hydrophilic form, the herbicide is trapped in the cell (hence the name 'ion trapping'). The free hydrogen (H^+) is pumped out of the cell by ATPase to maintain the alkaline environment within the cell and the acidic environment outside of the cell wall (Sterling & Namuth-Covert, 2016b). As a result of ion trapping, these lipophilic herbicides are typically less mobile.

For weak acid herbicides, when in the acidic environment outside the cell membrane, the pK_a of the herbicide will influence translocation. pK_a is a measurement of the pH at where 50 per cent of the herbicide molecule has been ionised (dissociated) when in solution. With a pK_a close to the pH of the surrounding environment outside of the cell (that is, pK_a of 3.5 to 5.5), a higher percentage of herbicide molecules will be non-ionised and lipophilic, which will allow greater movement through the plasma membrane.

The combination of pK_a (for weak acids) and $\log K_{ow}$ values will provide an indication of the ability of the herbicide to move in the xylem or phloem (Figure 4E). Herbicides with intermediate lipophilicity are likely to be able to move into the xylem by passive gradient diffusion. Movement in the xylem (often referred to as apoplastic movement) is relatively fast, often 50 to 100 times faster than movement in the phloem, providing the plant is actively transpiring. Once in the xylem, herbicide will move acropetally (that is, from the point of introduction upwards to the leaf tips), where it will accumulate. For xylem-mobile herbicides, damage symptoms will first appear at leaf tips of mature leaves, where most transpiration is happening.

Movement in the phloem, is termed symplastic movement (that is, movement within living cells) and is required for those herbicides that need to move basipetally to reach the meristematic regions of the weed (that is, crown and/or root tips of grasses and the apical meristem of broadleaf weeds) where the target enzyme is being produced in the plant. For a herbicide to be able to move in the phloem it typically requires intermediate lipophilicity and a

pK_a ideally in the range of ~ 1.5 to 5; or a suitable active transport mechanism to move the herbicide through the phloem to the sink. The latter is often seen with many of the weak acids that translocate in the phloem (Cobb & Reade, 2010).

For lipophilic herbicides, phloem transport will generally be poor and these herbicides are generally non-mobile, being trapped within the cell.

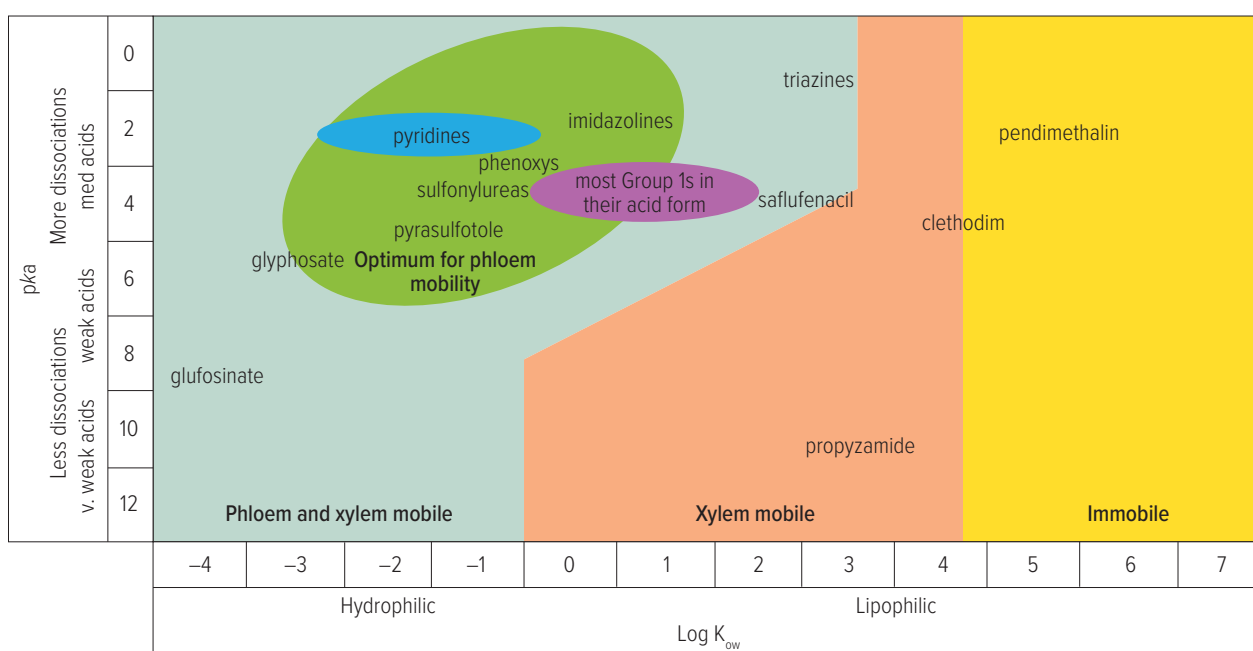
For example, some Group 1 herbicides are relatively lipophilic, however they need to translocate in the phloem to reach the meristematic sinks. Typically with many lipophilic Group 1 herbicides, only a very small percentage of the applied herbicide (often less than 5 per cent for a herbicide such as clethodim) manages to translocate to the site of action. However, these herbicides are extremely potent in inhibiting the ACCase enzyme, so only a very small amount of herbicide needs to reach the target site for the herbicide to be able to control a susceptible weed.

Useful references explaining the biochemistry of herbicide movement within the plant can be found at:

Plant & Soil Sciences eLibrary.
Cellular Absorption of Herbicides
passel2.unl.edu/view/lesson/9e46d907153e/6

Herbicides and Plant Physiology. 2nd Edition.
(Cobb & Reade, 2010)

Figure 4E: The relationship between lipophilicity and acid dissociation can assist in predicting mobility within the plant.



Source: Model adapted from Bromilow, et al. (1986)

Table 4B: Octanol/water partition coefficient ($\log K_{ow}$) values and acid dissociation constant (pKa) values for selected post-emergent herbicides used in Australia.

Herbicide	Class	MOA group	Octanol/water partition coefficient ($\log K_{ow}$) at pH7, 20°C	Acid dissociation constant (pKa) at 25°C
paraquat	bipyridyl	22	-4.5	No dissociation
glufosinate-ammonium	phosphinic acid	10	-4.01	9.15
glyphosate	glycine	9	-3.2	2.34, 5.6 and 10.3 ¹
aminopyralid	pyridine	4	-2.87	2.56
clopyralid	pyridine	4	-2.63	2.01
picloram	pyridine	4	-1.92	1.8
metsulfuron-methyl	sulfonylurea	2	-1.87	3.75
dicamba	benzoic acid	4	-1.88	1.87
thifensulfuron-methyl	sulfonylurea	2	-1.65	4.0
topramezone	pyrazole	27	-1.52	4.06
pyrasulfotole	pyrazole	27	-1.36	4.2
florasulam	triazolopyrimidine	2	-1.22	4.54
bicyclopyrone	triketone	27	-1.2	3.06
pyroxsulam	triazolopyrimidine	2	-1.01	4.67
chlorsulfuron	sulfonylurea	2	-0.99	3.4
amitrole	triazole	34	-0.97	4.14
2,4-D	phenoxy	4	-0.82	3.4
MCPA	phenoxy	4	-0.81	3.73
sulfosulfuron	sulfonylurea	2	-0.77	3.51
iodosulfuron-methyl-sodium	sulfonylurea	2	-0.7	3.22
triasulfuron	sulfonylurea	2	-0.59	4.64
mesosulfuron-methyl	sulfonylurea	2	-0.48	4.35
bentazone	benzothiadiazinone	6	-0.46	3.51
clodinafop	aryloxyphenoxypropionate	1	-0.44	2.911
fluroxypyr	pyridine	4	0.04	2.94
imazapyr	imidazolinone	2	0.11	1.9, 3.6 and 11.0
flumetsulam	triazolopyrimidine	2	0.21	4.6
bromoxynil	nitrile	6	0.27	3.86
haloxyfop-P	aryloxyphenoxypropionate	1	0.27	4.27
triclopyr	pyridine	4	0.36 ¹	2.68 ¹
tribenuron-methyl	sulfonylurea	2	0.38	4.65
imazapic	imidazolinone	2	0.393	2.0, 3.6 and 11.1
imazamox	imidazolinone	2	0.73 ¹	2.3, 3.3 and 10.8 ¹
acifluorfen-sodium	diphenylether	14	1.2	3.86
imazethapyr	imidazolinone	2	1.49	2.1 and 3.9
diclofop	aryloxyphenoxypropionate	1	1.61	3.43
sethoxydim	cyclohexanedione	1	1.65	4.58
metribuzin	triazinone	5	1.7	1.3 and 12.8
butoxydim	cyclohexanedione	1	1.9	4.36

Table 4B: Octanol/water partition coefficient (log K_{ow}) values and acid dissociation constant (pKa) values for selected post-emergent herbicides used in Australia (continued).

Herbicide	Class	MOA group	Octanol/water partition coefficient (log K_{ow}) at pH7, 20°C	Acid dissociation constant (pKa) at 25°C
tralkoxydim	cyclohexanedione	1	2.1	4.3
fluometuron	urea	5	2.28	No dissociation
simazine	triazine	5	2.3	1.62
flumioxazin	n-phenyl-imides	14	2.55	No dissociation
saflufenacil	pyrimidindione	14	2.6	4.41
atrazine	triazine	5	2.7	1.7
diuron	urea	5	2.87	No dissociation
fluazifop-P	aryloxyphenoxypropionate	1	3.18	3.12
butafenacil	N-phenyl-imides	14	3.2	Not available
pinoxaden	phenylpyrazole	1	3.2	No dissociation
trifludimoxazin	N-phenyl-imides	14	3.33	Not available
prometryn	triazine	5	3.34	4.1
terbuthylazine	triazine	5	3.4	1.9
pyraflufen-ethyl	phenylpyrazole	14	3.49	No dissociation
terbutryn	triazine	5	3.66	4.3
carfentrazone-ethyl	triazolinone	14	3.7	No dissociation
halauxifen-methyl	arylpicolinate	4	3.76	2.84
clodinafop-propargyl	aryloxyphenoxypropionate	1	3.9 ²	Note ³
haloxyfop-P-methyl	aryloxyphenoxypropionate	1	4.0 ²	Note ³
clethodim	cyclohexanedione	1	4.14	4.47
diflufenican	pyridinecarboxamide	12	4.2	Not available
fluazifop-P-butyl	aryloxyphenoxypropionate	1	4.5 ²	Note ³
quizalofop-P-ethyl	aryloxyphenoxypropionate	1	4.61 ²	Note ³
triclopyr-butoxyethyl	pyridine	4	4.62 ²	Note ³
diclofop-methyl	aryloxyphenoxypropionate	1	4.8 ²	Note ³
oxyfluorfen	diphenylether	14	4.86	No dissociation
propaquizafop	aryloxyphenoxypropionate	1	4.78	No dissociation
fluroxypyr-meptyl	pyridine	4	5.04 ²	Note ³
picolinafen	pyridinecarboxamide	12	5.43	No dissociation
2,4-D-ethylhexyl	phenoxy	4	5.78 ^{1,2}	Not available
bromoxynil-octanoate	nitrile	6	6.2 ²	Note ³
MCPA-2-ethylhexyl	phenoxy	4	6.8 ^{2,4}	Not available

Lipophilic herbicides easily move across the cell membrane but are mostly trapped within the cell.

Extracted from the University of Herefordshire, Pesticide Properties Database (PPDB) Lewis, et al. (2016) unless otherwise stated.

¹ Herbicide Handbook. 10th Edition (Weed Science Society of America, 2014)

² Some herbicides (particularly aryloxyphenoxypropionates) are formulated as esters, which increases their lipophilicity to assist in cuticle penetration. On entering the leaf, they rapidly convert to the acid form (also consider the properties of the acid form).

³ Does not dissociate in the spray solution when formulated as esters

⁴ fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Evaluation12/MCPA.pdf

4.4 ‘Selective’ herbicides – How does the crop survive?

Herbicides that are active post-emergent (that is, after germination) yet can also be applied over the top of a crop are considered ‘selective’ to the crop, that is, they typically cause little or no crop injury. As ‘selective’ herbicides are sprayed over the crop, they will also enter the cells of the crop.

At registered use rates and timings, crop selectivity (how/why the crop is largely unaffected) to foliar-applied post-emergent herbicides can occur in several ways:

- For some herbicides, the target enzyme is not present in the crop (or in other weeds that are not controlled); or may be present yet insensitive to the herbicide at the rates applied.
- The herbicide may not be translocated to the target site at the same rate within the crop compared to the sensitive weed.
- The crop may be able to rapidly metabolise (breakdown) the herbicide before it can reach the target site and cause a significant level of crop injury.

A good example is seen with Group 1 herbicides. In broadleaf crops, the ACCase enzyme is insensitive to the Group 1 herbicides. As a result, broad-leaved crops are largely unaffected by Group 1 herbicides in most situations.

In cereal crops, selectivity to Group 1 herbicides is dependent on the ability of the crop to rapidly metabolise the individual herbicide. In the case of Group 1 herbicides, this is typically as a result of enzymes in the plant (cytochrome P450s or glutathione S-transferases) that can rapidly detoxify some Group 1 herbicides. The level and functionality of the different enzymes to degrade the herbicide varies between cereal crop and individual herbicide.

Typically with Group 1 herbicides, wheat is the most tolerant cereal crop, barley is somewhat less tolerant, and oats have poor tolerance. Within ACCase herbicides, diclofop and tralkoxydim can be metabolised relatively rapidly in wheat, with registered dose rates generally considered to be ‘safe’ for over-the-top application in wheat.

Other ACCase herbicides such as clodinafop, fenoxaprop and pinoxaden have ‘useful’ levels of metabolism with cereal crops but are not considered ‘fully selective’ when used on their own. The functionality of the metabolising enzymes may be able to be increased, and acceptable levels of crop safety achieved, by addition of a crop safener with these herbicides ([for more information on herbicide metabolism and the role of safeners in relation to herbicide resistance, see Table 6J](#)).

When the crop is under stress (for example, after frost or waterlogging), metabolism typically slows. If tolerance to the herbicide relies on metabolic activity to detoxify the herbicide, stresses on the plant that slow metabolic pathways can result in less crop selectivity and more crop damage. This effect can be seen with several different herbicides but is particularly common with Group 2 herbicides. For example, where plants are exposed to frost or cold/anaerobic growth conditions close to the application of a Group 2 herbicide such as chlorsulfuron, increased herbicide symptoms are often observed where the speed of metabolism, and therefore detoxification is reduced.

4.5 Dose rate and dose response curves

Performance of a herbicide is influenced by the rate applied. Dose response curves highlight how external factors impact on the efficacy of a herbicide.

Dose response curves plot herbicide efficacy (% control) by dose rate (amount of herbicide applied). A hypothetical example of a dose response curve is provided in Figure 4F. Dose response curves can be useful information to have when designing field trials to understand how field performance will vary depending on herbicide rate, adjuvants or conditions. Dose response curves are often used to compare herbicide performance on susceptible and resistant populations.

Four terms commonly associated with dose response curves are:

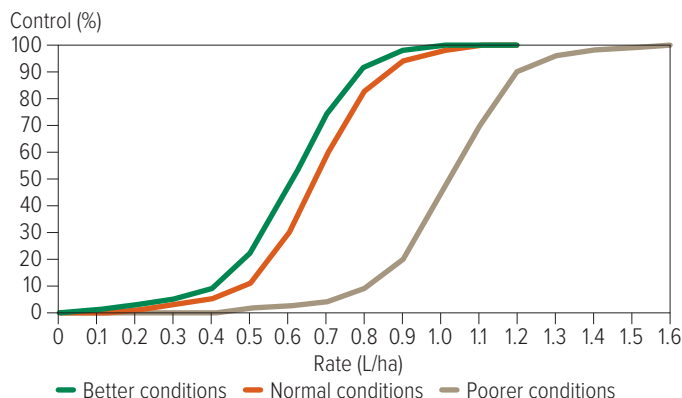
- **Threshold dose rate** – the rate at which ‘most’ weeds are just controlled for prevailing conditions.
- **Commercial dose rate** – the commercial rate selected that includes a margin of error to accommodate minor changes in conditions and still obtain commercially acceptable results.
- **Discriminating dose rate** – a rate that will discriminate between minor changes in product efficacy. Discriminating dose rates are typically 0.5 to 0.7 of the threshold dose rate for a given weed size and condition.
- **LD₅₀ (lethal dose to control 50 per cent of the population)** – calculated rate from the curve that would be predicted to control 50 per cent of the population. Comparative LD₅₀ are often used to calculate the magnitude of differences between tested populations. For example, when comparing a susceptible and a resistant weed population, the dose response curves for both populations are established and difference between the LD₅₀ values is published as the ‘resistance factor’ of the resistance population.

In the example in Figure 4F, under normal growth conditions (orange line), the threshold dose rate would be approximately 1L/ha, the commercial rate selected is likely to be 1.3 to 1.5L/ha and the discriminating dose rate 0.5 to 0.7L/ha.

When conducting herbicide trials, it is often desired to identify small/incremental improvements in product efficacy; such as differences between formulation type, carrier volume or other application settings, adjuvant package, or the impact of tank-mix partners. Selection of a rate close to the threshold dose rate will kill the large majority of weeds in most situations and across different treatments and therefore provide poor resolution around the subtle treatment differences the research seeks to understand.

By comparison, a discriminating dose rate of 0.5 or 0.7 of the threshold dose rate is situated at the steepest part of the dose response curve. At these discriminating dose rates, small changes in the effective dose rate will lead to the largest difference in percentage weed kill. As such, discriminating dose rates of 0.5 to 0.7 of the threshold dose rate for prevailing weed size and conditions for that weed and herbicide combination are commonly used when research is intended to uncover small and subtle differences between treatments.

Figure 4F: Example of herbicide dose response curves showing variation in performance with better, normal and poor conditions.



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5. Key post-emergent modes of action

When new mode of action herbicides are first introduced, manufacturers typically provide robust formulations and use rates. There is often a high level of 'forgiveness' in the label.

As resistant populations are selected over time, there is sometimes a period where the herbicide may still be useful, albeit with reduced performance. In these situations of low-level or emerging resistance, it is critical that users seek to maximise application conditions to ensure everything possible is done to enhance the herbicide performance.

Understanding how each of the key modes of action available for post-emergent weed control work, how they enter and translocate in the plant, and what is required to maximise efficacy is critical knowledge for maximising field performance.

This chapter covers the key modes of action used for post-emergent weed control and discusses:

- how they work as herbicides;
- factors affecting leaf entry and translocation;
- herbicide metabolism;
- resistance; and
- factors affecting field performance.

5.1 Group 1 – Acetyl-CoA carboxylase (ACCase) inhibitors

Table 5.1: Group 1 herbicide mode of action sub-groups and active ingredients by sub-group.

Group 1 sub-groups	Grass active herbicides used in grains and broadleaf field crops
Aryloxyphenoxypropionate (Fop)	clodinafop diclofop fenoxaprop fluazifop haloxyfop propaquizafop quizalofop
Cyclohexanedione (Dim)	butroxydim clethodim sethoxydim tralkoxydim
Phenylpyrazole (Den)	pinoxaden

Plant function targeted

Group 1 herbicides inhibit the acetyl-CoA carboxylase (ACCase) enzyme. One of the outcomes resulting from inhibition of this enzyme is reduction in the production of fatty acids required for construction of cell membranes needed for new cell production.

The ACCase enzyme in most broadleaf plants is insensitive to herbicides from this herbicide mode of action, and therefore there is acceptable crop tolerance in most broadleaf crops and no efficacy on most broadleaf weeds. Some exceptions exist. For example, haloxyfop is able to control the broadleaf weed storksbill or geranium (*Erodium* spp.) while high rates of clethodim can damage canola, particularly when flowering.

The three sub-groups of Group 1 herbicides bind to the target enzyme at slightly different, and overlapping, amino acids. This differential binding can lead to differences in target site herbicide resistance patterns both between and within the sub-groups ([refer to the Acetyl CoA Carboxylase inhibitors section under Section 6.3.1.1 Target Site Substitution for more detail](#)).

Aryloxyphenoxypropionates are typically present as an isomeric mixture of R and S isomers. However only the R isomer is herbicidally active, so the S isomer is often removed to increase the herbicidal activity per gram of active herbicide (Cobb & Reade, 2010).

Herbicide entry

Aryloxyphenoxypropionate herbicides are typically formulated as esters. This increases their lipophilicity, which assists rapid penetration through the leaf cuticle. Once inside the leaf, they are rapidly de-esterified to the acid, which is the active form.

Cyclohexanediones are not formulated as esters. When applied as weak acids, they can be more prone to dissociation in the spray tank when hard water is used. They are particularly sensitive to water with elevated levels of bicarbonate.

The more hydrophilic cyclohexanediones may have increased difficulty in penetrating the waxy cuticle and may be slower to move into the leaf. Where cuticle penetration is slower, some cyclohexanediones can be subject to a level of photodegradation on the leaf surface (Hall, et al., 1999) before entering the leaf.

Free ammonium in the spray tank, as a result of the addition of ammonium sulphate (even in the absence of hard water), may also assist in moving some cyclohexanedione herbicides across the plasma membrane once the herbicide has penetrated the cuticle. This was demonstrated in a series of wild oat efficacy trials conducted in 2014 by the GRDC-supported research group 'Northern Grower Alliance' (Price, et al., 2015) where performance of clethodim was enhanced by the addition of AMS when using soft water.

Herbicide translocation

Many studies have shown that 70 to 98 per cent + of the applied Group 1 herbicide remains in the leaf, close to the site of entry (Cobb & Reade, 2010; Hall, et al., 1999). Being weak acids and often lipophilic, herbicide entering the cell membrane is subject to ion trapping.

Some phloem transport is essential to move the herbicide to the meristematic regions where new cell production is occurring, particularly the meristem region (crown) of grass weeds, as this is the region within the plant of high acetyl-CoA carboxylase production. Group 1 herbicides exhibit some active translocation in the phloem, however this is limited due to low solubility and relatively high K_{ow} in addition to the associated ion trapping.

When they reach the meristem, the activity of these herbicides is extremely effective, so only a low rate of herbicide arriving at the target site is needed to deliver high-level weed control in susceptible weeds.

Within a few days of application, disruption of cell growth at the meristem commences. This causes necrosis allowing the main shoot to be easily pulled out. It is common for agronomists to be seen pulling the main stem from treated weeds a week or two after application and before leaf chlorosis is often evident. The bottom of the stem will be brown and dying. This, and the ease with which the stem can be pulled from the plant, are early indicator signs that the herbicide is doing its job.



Figure 5.1A: Group 1 herbicides cause meristem damage. Their leaves can be easily pulled from the plant.

Photo: Hall, et al. (1999)

Herbicide metabolism

The fop sub-group are typically applied as ester formulations. After entry into the leaf, these esters are rapidly metabolised to the herbicidally active acid form via carboxylesterase activity.

Once inside the leaf, the speed of metabolism of the Group 1 herbicide within the plant influences selectivity to crops (Table 5.1A). Where the herbicide can be rapidly metabolised into inactive substrates before reaching the meristem, there may be adequate selectivity for use in cereal crops.

Levels of metabolic enzymes that can degrade these herbicides are typically higher in wheat than other cereals. Barley has generally less tolerance than wheat, while oats has very little tolerance to these herbicides.

In this example, maize does not have adequate production of metabolic enzymes that can degrade either haloxyfop or tralkoxydim before reaching the target site, with crop death the result of application of these herbicides as they are not able to be metabolised. Whereas in wheat, adequate concentrations of metabolic enzymes that can structurally degrade tralkoxydim are present, which typically allows tralkoxydim to be applied over the top of the wheat crop. Under conditions where metabolism is slowed (for example, stresses such as frost, waterlogging and so on), the speed of metabolism is also slowed and crop effects (yellowing), may be observed with tralkoxydim.

In addition to tralkoxydim, diclofop-methyl can also be applied to wheat with minimal crop injury, as the herbicide is rapidly broken down by these enzymes, before it can reach the target site.

For other Group 1 herbicides, metabolism is slower within cereal crops. This may be due to either low levels of the specific metabolic enzyme required or the enzyme having more difficulty in breaking apart these different chemical structures.

The addition of a crop safener to the formulation may increase the ability to metabolise the herbicide and therefore increase

Table 5.1A: Relative dose (ED_{50} μ M) required to reduce growth by 50%.

Species	Haloxyfop	Tralkoxydim
Maize	19 (susceptible)	18 (susceptible)
Wheat	83 (susceptible)	>760 (tolerant)
Soybean	>6000 (tolerant)	>6000 (tolerant)

Source: Adapted from Cobb & Reade (2010)

crop safety of cereal crops (particularly wheat, where higher levels of enzyme are already present). For example, the safener cloquintocet is often included in formulations of clodinafop or pinoxaden to provide safety to wheat; while mefenpyr has been included in fenoxaprop/sethoxydim formulations used in cereal crops (for more see Table 6J the role of safeners).

Other Group 1 herbicides that are more difficult for the cereal crop to metabolise can be effective in providing control of volunteer cereals in broadleaf crops.

Metabolism of these herbicides in the plant is primarily catalysed by cytochrome P450 enzymes, with an exception being fenoxaprop, which is degraded by glutathione S-transferase enzymes (GSTs). Certain other chemicals can influence the levels of P450 enzymes within the plant. For example, following application of a phenoxy herbicide, a grass plant responds by rapidly increasing the production of P450 enzymes. This increased level of P450 enzymes will increase metabolism of the Group 1 herbicide if it was applied as a tank-mix, or very soon after the phenoxy herbicide was applied (Han, et al., 2013). Mixing phenoxy herbicides such as 2,4-D, and to a lesser extent MCPA, with a Group 1 herbicide can reduce the level of grass weed control by more than 20 per cent in some situations. Where rates are very robust, this may not be noticed in the paddock. However, where rates are more marginal or where metabolic resistance is present, this could easily lead to spray failure.

Where rapid metabolism is the mechanism for selectivity in grass crops, conditions need to be suitable for this to occur. Plants that are under stress (particularly from frost or moisture stress) will have reduced metabolism and therefore herbicide breakdown will be slower. As a result, there is an increased risk of crop injury in these situations.

In the soil, most Group 1 herbicides are readily degraded by microbial activity and have only limited soil persistence. Plant-backs to sensitive grass crops can be as short as a few days/weeks with some of the herbicides where conditions are favourable for microbial breakdown (warm temperature, moist topsoil). However, under conditions less favourable to breakdown, some have plant-back periods of up to 3 to 4 months.

How does resistance occur?

Two primary resistance mechanisms have been identified that confer resistance to ACCase inhibitor herbicides.

Target site substitution can be selected within a few years of use if herbicides are placed under high pressure and survivors are not controlled. This may present as high-level resistance (that is, the resistant plants look like they have not been sprayed at all). Often this is first identified by patches or individual weed survivors that are alive and healthy plants (they look like they have not been sprayed) amongst dead plants, and where there are no signs of a spray miss (such as plants surviving in positions shaded from herbicide application, or a line of survivors indicating a blocked nozzle).

There are many different target site substitutions that can confer resistance to Group 1 herbicides, with these having different resistance profiles across each of the three Group 1 herbicide sub-groups. While high-level target site resistance frequently occurs (that is, control needing >10x the dose rate), the specific mechanism selected may only confer low-level resistance to other ACCase inhibitor herbicides. The only practical method to understand which herbicides still work, is to conduct strip trials or to conduct a resistance test on survivors. It is also highly possible that different target site resistance profiles may exist between patches within the same paddock.

Metabolic resistance has also been identified to Group 1 herbicides, where resistant plants increase their production of cytochrome P450 enzymes which can metabolise these herbicides faster. Typically, this resistance mechanism is not as strong as target site substitution, so it may be possible that these individuals can be controlled by increasing application rate, within label recommendations, particularly in the early stages of metabolic resistance selection. Metabolic resistance mechanisms conferring high levels of resistance have also been identified.

Weeds with metabolic resistance able to degrade ACCase inhibitors are often able to also degrade herbicides from other mode of action groups, even if these other modes of action have never previously been used against that population. An example, commonly seen in the northern grains region involves metabolic cross-resistance between Group 1 herbicides used to control wild oats and the Group O herbicide flamprop-methyl. In many situations, wild oats sent for resistance testing following a Group 1 'failure' are also resistant to flamprop-methyl, regardless of the paddock history of flamprop-methyl use.



Figure 5.1B: High-level target site resistance is often observed as a healthy plant amongst other plants that are dead or dying.

Photo: Mark Congreve

Factors affecting efficacy

The choice of adjuvant is very important to assist cuticle penetration with these herbicides and manufacturers typically recommend a specific combination of spray oil and/or wetter that has been optimised for the particular formulation.

Always use clean water with ACCase herbicides. Diclofop-methyl and fenoxaprop-P-ethyl (in particular) may bind strongly with soil or organic matter suspended in the spray water.

Dim herbicides are applied as weak acids. The addition of ammonium sulphate to the spray tank before adding the herbicide may reduce dissociation in hard water, particularly when using water with high levels of bicarbonate.

ACCase inhibitor herbicides are particularly effective against small, actively growing weeds that are rapidly producing new cells (Hall, et al., 1999) and are therefore producing high levels of ACCase in the meristematic crown. ACCase inhibitor herbicides do not translocate well throughout the plant, often with 70 to >90 per cent of applied herbicide remaining within the treated leaf.

Very small weeds only have a very short distance to translocate to the meristem (Hall, et al., 1999). With small grass weeds, some droplets landing on the leaf may also run down the leaf and be captured in the base of the leaf, closer to the crown (meristematic region).

Any stress that is likely to slow down translocation within the plant is likely to severely affect the performance of Group 1 herbicides. For example, when applying clethodim under cold (frost) temperatures in winter, performance often declines, especially in low-level resistant populations. In particular, frost in the days leading up to herbicide application can significantly impact clethodim performance (Saini, et al., 2016).

Spray coverage is very important that is, treat Group 1 herbicides more like contact herbicides that require excellent coverage. Medium droplets applied at 80 to 100L/ha (or higher) typically provide best results.

As plants mature, or if they are temperature or moisture stressed, Group 1 herbicides are less effective as phloem translocation is further reduced. When spraying large weeds, these poorly translocated herbicides have great difficulty in translocating to the target site. On reaching the meristematic region, there can also be a greater quantity of enzyme requiring inhibition in larger plants.

To control perennial, stoloniferous grass weeds, a herbicide needs to be able to translocate along the stolon and to the meristematic regions at the root tips. This is generally difficult for most Group 1 herbicides, therefore control of established stoloniferous weeds with Group 1 herbicides is often poor.

Differences exist between ACCase inhibitor herbicides in their robustness across the spectrum of grass weeds and control of volunteer cereals. Increasing application rate (within maximum label constraints) can normally overcome differences in tolerance between target grass weeds. However there are situations where growers elect to tank-mix different Group 1 herbicide sub-groups to maximise the strengths of each partner.

At registered label rates, the fop herbicides (such as haloxyfop, quizalofop, fluazifop and propaquizafop) often tend to provide better control of weeds such as wild oats, barley grass and volunteer cereals while typically needing higher application rates to control weeds such as annual ryegrass and phalaris. The relative efficacy of the dim herbicides (sethoxydim, butroxydim and clethodim) is often the reverse, with relatively better efficacy on annual ryegrass and phalaris while higher rates are needed on wild oats, barley grass and volunteer cereals.

Some herbicide labels promote the tank-mixing of a fop and a dim, to maximise the strength of the different mix components on a cross-spectrum of grasses. In practice, this is likely to hide early resistance to either of the individual sub-groups – usually the fops, which typically are selected for resistance faster.

In annual ryegrass, where resistance appeared first and is better studied, the target site substitution that is often first selected may confer resistance to the fop herbicides while the dim herbicides may continue to provide a level of control on some populations.

This has given rise to the term ‘fop till you drop’ as a strategy against annual ryegrass. The theory behind ‘fop till you drop’, is that using fop herbicides only in the first instance is likely to initially select for the more common Trp-1999-Cys, Ile-2041-Asn or Asp-2078-Gly substitutions ([for more information on target site substitution see Section 6.3.1.1](#)), which confers high-level resistance to the fop herbicides. However, these substitutions confer weaker resistance to the dims, which may then allow switching to dims after the fops have begun to fail and may provide several more years of useful paddock life from the Group 1 mode of action group, before resistance is also selected in herbicides such as clethodim or butroxydim. While this strategy is perceived as having been effective in many paddocks, it is possible that the first target site substitution selected could be one that also confers high-level resistance to the dim sub-group (for example, the Ile-1781-Leu substitution). Should the Ile-1781-Leu substitution be the first substitution selected in the paddock, both fops and dims are likely to be lost simultaneously.

Group 1 resistance in other grass weeds has not been as extensively studied. In most other species, it is unknown which target site substitutions will appear first and therefore it is unknown if the ‘fop till you drop’ strategy will work.

Known herbicide interactions

The addition of phenoxy herbicides in particular, and other broadleaf herbicides such as dicamba and some ALS herbicides that induce a metabolic enzyme response in treated plants, can lead to a reduction in grass weed control achieved from the Group 1 herbicide (Li, et al., 2016). The degree of antagonism varies with different broadleaf herbicides depending on their interaction with specific P450s, so there is some difference in compatibility between herbicides. Typically, 2,4-D causes the greatest antagonism.

In some situations, especially where the herbicide performs robustly on the key grass weeds, this antagonism with broadleaf herbicides may not be noticed, and the tank-mix may be acceptable despite some level of antagonism occurring, or the antagonism may be able to be masked by increasing application rate of the Group 1 herbicide. **Note: Most Group 1 herbicide label recommendations for mixtures of broadleaf herbicides were developed in the absence of metabolic resistance. In situations where the weed population is developing metabolic resistance from enhanced P450 activity, antagonism is likely to be further increased when mixing with a broadleaf herbicide.**

Where the antagonism occurs at unacceptable levels and as a result, tank-mixing cannot be permitted, the Group 1 and the broadleaf herbicide application will need to be split. Typically, grass weeds emerge faster and provide more early competition, so typically the Group 1 will be applied prior to the broadleaf herbicide.

As herbicide resistance in grass weeds becomes more prevalent, there is more interest in mixing herbicides with grass weed activity. An increasingly common desire is to tank-mix a Group 1 herbicide with glyphosate. Applying as separate applications is preferred as mixing Group 1 herbicides with glyphosate requires compromise in both the choice of adjuvant and application setup.

- The performance of the Group 1 will be maximised by using a crop oil concentrate (COC). However, the COC may reduce the performance of glyphosate on certain weeds (in particular some summer growing grass weeds for example, barnyard grass). As the main weed drivers for using this mix are generally glyphosate-resistant grass weeds, the choice in fallow situations is usually to use the adjuvant package suited to the Group 1 when tank-mixing, noting that this is not the preferred adjuvant for glyphosate.
- In addition to the choice of adjuvant, there is also a compromise required with application. The performance of glyphosate is optimised by the use of relatively low water volumes and large droplets (coarse or larger spray quality) while the Group 1 will be optimised when applied with water rates in excess of 80L/ha and typically a medium to medium-coarse spray quality.

Always ensure boomsprayers that have been previously used to apply ALS inhibitor herbicides (Group 2) are completely decontaminated before using the boom to apply ACCase inhibitor herbicides (Group 1) over crops sensitive to Group 2 herbicides. The solvents used in Group 1 herbicide formulations are highly effective in stripping any Group 2 residues from spray tanks, lines and filters and these highly active Group 2 herbicides can cause damage to many broadleaf crops at very low concentrations.

Warning: Group 1 herbicides are not completely safe to canola. At high application rates, some canola injury and yield reduction may occur, especially if these herbicides are applied during flowering (therefore labels typically restrict against this application timing). This is most often observed with clethodim, partly because the maximum registered application rate of clethodim (500mL/ha of 240g/L formulation = 120gai/ha) is significantly higher than maximum application rates of other ACCase herbicides used in canola in Australia. Clethodim is not registered for use in canola during flowering.

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5.2 Group 2 – Acetolactate synthase (ALS) inhibitors

This mode of action is the largest class of herbicides used in agriculture and includes several different chemistry sub-groups. Most Group 2 herbicides have broad spectrum activity on a wide range of young broadleaf weeds. Only a few Group 2 herbicides have acceptable tolerance in selected broadleaf crops.

Some Group 2 herbicides (with asterisk, Table 5.2) also have activity on grass weeds, with varying degrees of selectivity in grass crops resulting from different metabolic pathways (see below).

Plant function targeted

Herbicides with the Group 2 mode of action inhibit the acetolactate synthase (ALS) enzyme. In some references, this mode of action is also referred to as 'acetohydroxyacid synthase' (AHAS) inhibitors.

The ALS enzyme occurs throughout the plant, predominantly within the chloroplasts of green plant material. The enzyme is in highest concentration in meristematic regions where new cell growth is most active. Group 2 herbicides are more active on young weeds where cell growth is a primary activity and there are high levels of ALS enzyme activity.

The ALS enzyme is a key enzyme in the biosynthesis pathway that produces the amino acids leucine and valine and other compounds. Following application of a Group 2 herbicide, plant growth is inhibited within hours, despite taking days for symptoms to appear. Necrosis and vein reddening appear first on the young leaves, before spreading throughout the whole plant. Under conditions of rapid growth, plant death may occur within 2 weeks. However, where growth is slow, complete mortality can take many weeks (Cobb & Reade, 2010). Agronomists often refer to plants treated with Group 2 herbicides as 'green skeletons' as susceptible plants often appear severely retarded and sick, but still maintain some green plant material for many weeks.

Herbicide entry

Imidazolinones (IMIs) have intermediate lipophilicity (log K_{ow} 0.1 to 2.5), which means they can penetrate the leaf cuticle relatively easily and are well translocated throughout the plant. With rapid leaf entry, imidazolinones are usually quite rainfast.

Sulfonylureas (SUs) mostly have intermediate lipophilicity, although some are more towards the hydrophilic end of the spectrum; while triazolopyrimidine sulfonamides (TPS) tend to be more hydrophilic.

Tank-mixing with a spray oil enhances foliar uptake through the cuticle. For Group 2 herbicides where crop selectivity is more marginal, the increased speed of leaf entry via the addition of a crop oil may have the potential to increase crop injury.

The main post-emergent herbicides used for grass control in cereals (herbicides containing iodosulfuron, mesosulfuron or pyroxsulam) are usually recommended to be applied with a crop oil concentrate to assist cuticle penetration, however each of these herbicides contain a safener in the formulation to assist the crop to metabolise the herbicide.

Root uptake can also be a significant pathway of entry for many, but not all, Group 2 herbicides.

Table 5.2: Group 2 herbicide mode of action sub-groups and active ingredients by sub-group.

Group 2 sub-groups	Main herbicides used in grain and broadacre field crops
Sulfonylurea (SU)	chlorsulfuron* iodosulfuron* mesosulfuron* metsulfuron sulfosulfuron* thifensulfuron triasulfuron* tribenuron
Imidazolinone (IMI)	imazamox* imazapic* imazapyr* imazethapyr*
Triazolopyrimidine Sulfonamides (TPS)	florasulam flumetsulam pyroxsulam*

* Denotes useful activity on grass weeds.

Soil persistence of ALS inhibitors

Many, but not all, Group 2 herbicides have moderate to long soil persistence, which often provides a level of residual control. The length of residual activity is dependent on the breakdown pathway, solubility of the herbicide and the strength of soil binding for that herbicide.

Once incorporated in the soil following rainfall, many imidazolinones and triazolopyrimidine herbicides are degraded by a relatively slow microbial degradation process.

For sulfonylureas, soil breakdown also occurs via a hydrolysis reaction, however this reaction is pH sensitive. Under acidic or neutral conditions, hydrolysis is the primary breakdown pathway. However as the soil pH increases, the speed of this hydrolysis reaction slows, or stops. Under alkaline conditions, soil breakdown reverts to slow microbial degradation, and therefore soil persistence is longer in higher pH soils.

Most microbial activity occurs in the top 10cm of soil and only when this zone is moist. Microbial populations decline rapidly with depth and as conditions become less suitable for their survival (for example, dry or cold conditions, lack of organic matter as a food source).

As these herbicides are relatively mobile in the soil, they will move down the profile with continued rainfall. Therefore, the worst case for residual carryover occurs where rainfall leaches the herbicide below the top 10 to 15cm soil surface, and out of the zone of greatest microbial activity. At depth, without microbial breakdown, and if combined with a high pH subsoil for sulfonylureas, some of these herbicides can persist for many months (or years). Where there is a change in soil texture, pH or some physical barrier at depth that prevents further leaching, the herbicide may concentrate at this barrier and present symptoms when the roots of the following crop reach this layer.

For more information on soil behaviour of these herbicides, refer to the GRDC publication *Soil Behaviour of Pre-emergent Herbicides in Australian Farming Systems: A Reference Manual for Agronomic Advisers* (Congreve & Cameron, 2023).

Herbicide translocation

Intermediate lipophilicity, activity as polar acid and moderate to high solubility of Group 2 herbicides means these herbicides are ideally suited for translocation in both the xylem and phloem, therefore they are fully systemic within the plant.

Herbicide metabolism

Most sulfonylureas are relatively stable in the spray tank when the water pH level, is neutral or alkaline. However, if the water is moderately to strongly acidic, they undergo hydrolysis and will quickly breakdown. Additionally, solubility typically decreases at lower pH which can lead to higher risk of spray tank compatibility issues when tank-mixing. Therefore, the addition of tank-mix adjuvants that reduce water pH is not recommended when using SUs. Good quality sulfonylurea formulations will contain a pH buffer to prevent the pH of the spray solution lowering too far.

Within the plant, a number of different enzyme-mediated reactions influence metabolism, attacking the herbicide structure at a number of different locations. Depending on the herbicide structure and the plant species, these different reactions result in the considerable difference in selectivity between crop and weed species that is, some Group 2 herbicides control grass

weeds, but also have useful tolerance in cereals; conversely some Group 2 herbicides can be used in broadleaf crops, while most are very damaging.

For example, in wheat, tolerance to chlorsulfuron is due to the crop's ability to detoxify the herbicide via metabolic processes. In warm, unstressed growing conditions, it is rare to see a significant level of damage in a wheat crop treated with chlorsulfuron, as the wheat can rapidly metabolise the chlorsulfuron before the herbicide reaches the target site. By comparison, in cold, wet and/or anaerobic winter conditions often encountered in winter in southern growing regions, crop metabolism slows dramatically, and it is far more likely that an adverse crop response to chlorsulfuron will be seen.

Where these herbicides are not rapidly deactivated within the plant, they are extremely potent herbicides, leading to mortality at extremely low rates.

How does resistance occur?

Target site substitution in the ALS enzyme is one of the most common occurrences of herbicide resistance in Australia. Population sampling indicates that the level of substitution occurring in wild populations (unselected by Group 2 herbicides) is quite common. In a practical sense, as little as four applications with a Group 2 herbicide has been shown to be able to select for high levels of target site resistance in some species. As at 2019, some 26 different amino acid substitutions occurring at eight different locations have been identified across a wide range of weed species, which confer some level of resistance to certain ALS inhibitors ([for further information on ALS target site substitution see Section 6.3.1.1](#)).

Different target site substitutions may result in different performance between the various sub-groups of Group 2 herbicides. For example, it is common to select for a substitution that confers high-level resistance to the sulfonylurea group, but this may not affect an imidazolinone herbicide that may be still able to bind to the target enzyme and provide control. Continued selection pressure is likely to lead to selection of additional amino acid substitutions, which can lead to herbicide failure across multiple sub-groups.

Resistance to Group 2 herbicides can also occur via accelerated rates of metabolic degradation. In the early stages of selection for metabolic resistance, the degree to which herbicides are compromised from metabolic resistance is often substantially less than for target site mechanisms, so may not always be noticed in the paddock.

Factors affecting efficacy

Group 2 herbicides have high water solubility; with most also having log K_{ow} values that permit translocation in both the xylem and phloem. As a result, most Group 2 herbicides are systemic within the plant and will readily move to target sites.

Under conditions of low water pH in the spray solution, sulfonylurea herbicides will typically undergo hydrolysis, which will reduce herbicide efficacy. Many quality formulations contain buffering agents to reduce the effect of low pH (acidic) spray water. DO NOT ADD acidifying spray adjuvants when using sulfonylurea herbicides as this could lead to inactivation via hydrolysis reaction in the spray tank.

Crop selectivity when applying Group 2 herbicides typically results from differential rates of metabolism that is, the crop can

Clearfield® crops – How they work

Imidazolinone (IMI) tolerant crops (traditionally sold under the Clearfield® brand in Australia) have been bred using conventional breeding techniques such as pollen, seed and microspore mutagenesis or in tissue culture in the presence of an imidazolinone herbicide. Plants with the Ser-653-Asn target site substitution have high-level (>100 fold) resistance to the imidazolinone herbicides, yet remain sensitive to sulfonylureas and triazopyrimidines (Cobb & Reade, 2010; Tan, et al., 2005).

Clearfield® crops allow the use of relatively broad-spectrum imidazolinone herbicides, which have been a particularly valuable tool for control of some winter grass weeds where resistance has developed to other post-emergent in-crop modes of action.

Spring (bread) wheat is hexaploid (3 pairs of chromosomes). In the initial commercialisation of Clearfield® wheat, the amino acid substitution conferring resistance occurred on one of the chromosome pairs, but not on the other two. This provided a useful, but not complete, level of tolerance to the imidazolinone herbicides and permitted the use of Midas® herbicide (imazapic + imazapyr) but not Intervix® herbicide (imazamox + imazapyr). More recently, Clearfield® Plus spring wheat varieties were launched in Australia. These varieties contain the amino acid substitution on two of the three chromosome pairs, so therefore have increased tolerance to the imidazolinone herbicides and permit the application of Intervix®.

In recent years, a range of other IMI tolerant crop varieties have been launched that are not branded as Clearfield® crops. Several of these IMI-tolerant varieties achieve tolerance to Group 2 herbicides via different target site substitutions. Do not assume that a Group 2 herbicide that is 'safe' to be used over one IMI-tolerant crop will therefore be safe over all IMI-tolerant crops. Only use the specific Group 2 herbicides that are recommended for the particular variety being grown.

metabolise/break the herbicide down fast enough to prevent damage, while the target weed cannot metabolise the herbicide fast enough to survive. Conditions that reduce the crop's ability to rapidly metabolise the herbicide can often lead to expression of crop injury. Typically this is seen as a slight yellowing of the crop, with internode shortening in some situations. Cold conditions (that is, frost) and/or waterlogged conditions in the days after application of the herbicide often result in expression of damage symptoms in the crop.

Commercial formulations of the cereal-selective herbicides containing pyroxsulam, iodosulfuron and mesosulfuron contain a safener that increases the plant's ability to detoxify the herbicide. Safeners speed up the metabolism of the herbicide and therefore reduce the crop effect. They still require the plant to be actively metabolising, so may not provide full safety when the plant is 'shut down' after a period of significant stress (in particular frost, drought stress or waterlogging).

Known herbicide interactions

Within grass species (cereals and grass weeds), the plant will respond to an application of a phenoxy herbicide by increasing the production of cytochrome P450 enzymes. These same enzymes are also responsible for metabolism of ALS inhibitors, so tank-mixing phenoxy and Group 2 herbicides results in increased rates of metabolic breakdown of the Group 2 herbicide.

In cereals, this is often seen as a 'safening' of the herbicide effect on the crop. However, it may also reduce control of some grass weeds (that is, safening the weeds). Manufacturers may or may not support the tank-mixing of phenoxy herbicides – this will largely depend on the level of robustness of the herbicide on the target weed, that is, if the herbicide rate is robust, a 10 to 20 per cent reduction in herbicide efficacy from tank-mixing with a phenoxy may still be able to be supported. Whereby, in a different situation, the application rate may be more marginal and therefore a reduction in efficacy due to the tank-mixed phenoxy cannot be supported.

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5.3 Group 4 – Synthetic auxins

Table 5.3: Group 4 herbicide mode of action sub-groups and active ingredients by sub-group.

Group 4 sub-groups	Main herbicides used in grain and broadacre field crops
Arylpicolinate	halauxifen florpyrauxifen
Benzoic acids	dicamba
Phenoxy	2,4-D 2,4-DB MCPA MCPB
Pyridines	aminopyralid clopyralid fluroxypyr picloram triclopyr

The synthetic auxins (Group 4) are one of the oldest herbicide groups, with the phenoxy herbicides 2,4-D and MCPA first commercialised internationally in 1945 and 1946 respectively. The pyridine herbicide picloram was first commercialised in 1964, with clopyralid following in 1975.

Herbicides from this mode of action are typically effective on many broadleaf plants, with substantially less activity on grass species. They are often used for selective removal of broadleaf weeds from cereal crops and grass pastures.

A new sub-group of synthetic auxins called arylpicolinates has been commercialised in the mid-2010s with the launch of halauxifen, which prioritises a different target site than both the natural auxin, and other auxin herbicides.

A fifth sub-group (quinolines) contains the herbicide quinclorac. Quinclorac is somewhat unique as it also controls a selected range of grass weeds. Quinclorac is only registered for use in turf grass situations in Australia, so is not covered here in detail.

Plant function targeted

Synthetic auxin herbicides mimic the growth regulator indole-3-acetic acid (IAA), also known as auxin. IAA plays a critical role in managing division, differentiation and elongation at a cellular level while also having a role in controlling seedling morphology, apical dominance, leaf senescence and in many processes at a whole plant level including abscission, flowering and fruit production. At low concentrations, an increase in IAA levels results in significant growth response in roots and particularly shoots. When tissue-specific optimal levels are exceeded, there is an increase in ethylene production, a production of hydroxyl radicals and closing of the stomates which reduces the plant's ability to photosynthesise and ultimately leads to growth inhibition and plant death (Cobb & Reade, 2010) (Goggin, et al., 2016).

IAA binds to auxin-binding proteins located within the cell membrane, the endoplasmic reticulum, cell nucleus and in the cytoplasm. Levels of IAA require careful balancing within the plant, with IAA synthesis and degradation being carefully regulated (Hall, et al., 1999).

Introduced synthetic auxin herbicides disrupt the IAA balance at a cellular level. It is believed that binding of these herbicides to auxin 'repressor' proteins results in unregulated auxin production, inducing a cascade of unregulated growth in susceptible plants within minutes after application (Dow AgroSciences, 2016). A video explaining the mode of action of halauxifen can be viewed at [youtube.com/watch?v=alpVEOB3Wsw](https://www.youtube.com/watch?v=alpVEOB3Wsw)

At very low concentrations, some Group 4 herbicides are used for regulating fruit retention in certain crops for example, citrus. At higher levels, synthetic auxins act as potent broadleaf herbicides, causing unregulated growth and cell division. Initial symptoms appear as twisting, epinasty, stem thickening at the nodes and rapid elongation of new growth, which is often evident within a day or two of application on small, actively growing plants and is consistent with rapidly increasing auxin levels within the plant. Over the following weeks, as the introduced synthetic auxin levels have exceeded critical levels and are not reduced, growth is retarded and growth symptoms in susceptible plants will appear as chlorosis at the growing points followed by wilting and eventual necrosis.

Where sensitive plants are exposed to low levels of synthetic auxins (that is, a herbicide drift event), the symptoms typically appear as cupping of the leaf margins, especially on new growth.

Herbicide entry

Most herbicides within the Group 4 mode of action are active via foliar uptake. Synthetic auxins are active in the parent acid form in the plant, however they are usually formulated as an ester, amine or other salt formulation to either enhance solubility or leaf uptake. After entry into the leaf, these ester or amine formulations rapidly convert to the acid form.

When formulated as a more lipophilic ester, leaf penetration is generally faster than the water-soluble amine formulations. This may result in shorter rainfast periods for ester formulations and, in some situations, lower application rates with ester formulations, as more herbicide can enter the plant before the spray solution has dried.

Short-chain or high volatile (HVE) ester formulations of 2,4-D (for example, 2,4-D ethyl ester, 2,4-D isobutyl ester) can be associated with high volatility and have the potential for off-target vapour movement and are now only registered for use in certain restricted areas in Western Australia. Longer-chain, or low volatile (LVE), ester formations such as the ethylhexyl esters of 2,4-D or MCPA are of significantly lower volatility and can be used with caution in most states, when complying with label use directions and constraints. Industry best practice typically avoids the use of any 2,4-D ester formulations for summer spraying in areas where sensitive crops such as cotton, tomatoes or grapes are grown.

Various amine and salt formulations are available for many of the synthetic auxins. Formulating as an amine increases solubility, however as these formulations are more hydrophilic, leaf penetration may take longer and amine formulations can be subject to rapid droplet drying on the leaf surface before all of the herbicide has had the chance to transition through the cuticle. Amine and salt formulations generally have low volatility, however can still be highly damaging to sensitive broadleaf crops if physically drifted onto the crop. As they are often highly active on crops such as cotton at extremely low rates, a high level of care is required with herbicide application and climatic conditions when spraying.

Soil persistence of Group 4 herbicides varies greatly. Some herbicides only have limited soil activity, due to either strong soil adsorption or rapid breakdown in the soil (for example, dicamba, halauxifen), while others can have substantial activity via root uptake (for example, picloram, clopyralid).

2,4-D in the amine form will be taken up more easily by plant roots than 2,4-D in the ester form (Hall, et al., 1999).

Herbicide translocation

Once through the leaf cuticle, synthetic auxins move across the cell membrane and into the cells via two main processes (Hall, et al., 1999):

- auxin binding proteins located in the cell membrane bind the herbicide and move it across the plasma membrane. Auxin influx carriers move herbicide from the free space outside the cell with auxin efflux carriers then moving herbicide from cell to cell.
- being weak acids, the herbicide will be protonated in the more acidic environment outside the cell (pH ~5.5). This increases lipophilicity and makes it easier for the herbicide to cross the plasma membrane into the cell via diffusion. Once inside the cell (pH ~7.5), the weak acid herbicide will lose a proton, become more hydrophilic and therefore become trapped within the cell. However, it may still move from cell to cell via the auxin efflux carriers.

In addition to binding to auxin carriers for movement across cell walls, an additional binding process occurs whereby the herbicide binds to auxin receptors in the plasma membrane of the cell wall resulting in a cascading series of reactions, ultimately leading to rapid cell elongation within minutes of herbicides reaching the cell.

A graphical representation of auxin herbicide binding and cell entry can be found at passel2.unl.edu/view/lesson/624f3e499e56/4 (Sterling & Namuth, 2004).

Synthetic auxins are mobile in the plant, with properties ideally suited for phloem mobility (see Figure 4E for more information on herbicide properties for translocation).

Herbicide moving in the xylem will reach the leaf margins, however it tends to then enter the phloem, rather than accumulating in the older leaves. Movement in the phloem typically sees rapid accumulation at the growing points, in particular the apical meristem.

Movement in the xylem and subsequently in the phloem can be seen in a study by Reid & Hurtt (Figure 5.3A) whereby picloram taken up by the roots of bean plants increasingly accumulated in the apical tissues and newest trifoliate leaf over 3, 6 and 11 hours, with little herbicide moving out into the lower leaves during this time frame.

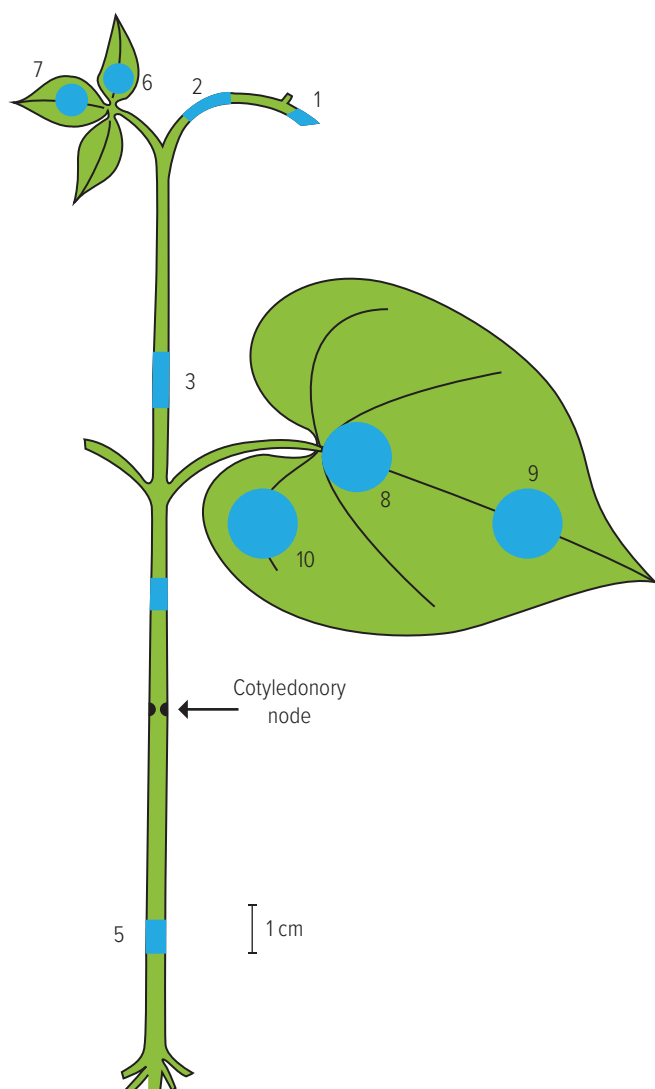


Figure 5.3A: Distribution of radio-labelled C^{14} picloram applied in a hydroponic solution as measured at various times after application.

	ng C^{14} picloram/mg fresh weight at various locations after given times									
	1	2	3	4	5	6	7	8	9	10
3 hours	1.065	0.300	0.112	0.271	0.199	0.484	0.502	0.014	0.007	0.021
	+/- 0.184	+/- 0.048	+/- 0.029	+/- 0.048	+/- 0.042	+/- 0.052	+/- 0.100	+/- 0.004	+/- 0.003	+/- 0.006
6 hours	2.804	0.449	0.231	0.454	0.157	0.885	0.803	0.037	0.036	0.046
	+/- 0.023	+/- 0.076	+/- 0.012	+/- 0.081	+/- 0.011	+/- 0.129	+/- 0.149	+/- 0.010	+/- 0.009	+/- 0.009
11 hours	2.619	0.718	0.367	0.419	0.216	0.958	0.811	0.067	0.040	0.080
	+/- 1.840	+/- 0.615	+/- 0.069	+/- 0.178	+/- 0.043	+/- 0.286	+/- 0.312	+/- 0.021	+/- 0.040	+/- 0.006

Source: Adapted from Reid & Hurtt (1969)

Herbicide metabolism

Group 4 herbicides are generally formulated as esters or salts to assist with leaf uptake or to improve formulation delivery.

Once inside the leaf, Group 4 herbicides are rapidly metabolised to the parent acid form, which is herbicidally active. For example, phenoxy esters are converted to the parent acid by a hydrolysis reaction.

The primary factors driving crop and weed selectivity appear to be differential rates of herbicide reaching the target site and differential selectivity at the target site (Cobb & Reade, 2010).

Phenoxys such as 2,4-D penetrate the leaf cuticle and are rapidly converted to the acid form, with further metabolism being slow in susceptible plants. The majority of the herbicide remains in the parent acid form for days after application (Weed Science Society of America, 2014). In tolerant species, the acid form of the herbicide is quickly transformed to inactive metabolites via a two-stage metabolism process.

For halauxifen, the speed of de-esterification from the applied halauxifen-methyl ester form to the herbicidally active halauxifen-acid varies between species. In tolerant crops such as cereals, the conversion to halauxifen-acid is much slower, with conjugation occurring before halauxifen-acid is formed, giving rise to crop selectivity (Dow AgroSciences, 2013).

Synthetic auxin tolerant crops

Dicamba-tolerant cotton is expected to be the first synthetic auxin tolerant crop to be commercialised in Australia. Dicamba-tolerant and 2,4-D-tolerant crops have been commercialised in the USA in recent years.

Dicamba tolerance results from insertion of a gene coding for DMO (dicamba monooxygenase), which was isolated from *Pseudomonas maltophilia*, Strain DI-6 (ISAAA International Service for the Acquisition of Agri-biotech Applications, 2018). Tolerance is achieved via O-demethylase removing the methyl (CH₃) group from dicamba. To date this has been commercialised in soybeans and cotton, in conjunction with existing glyphosate and glufosinate tolerance traits, and marketed by Monsanto as Roundup Ready® Xtend® crops.

2,4-D tolerance has been incorporated into maize, soybean and cotton varieties and marketed by Dow AgroSciences as Enlist® crops.

In cotton and soybean, 2,4-D tolerance comes from insertion of the aad-12 gene isolated from *Delftia acidovorans*, which codes for production of aryloxyalkanoate di-oxygenase 12 (AAD-12) protein that catalyses the side chain degradation of 2,4-D (ISAAA International Service for the Acquisition of Agri-biotech Applications, 2018). In addition to 2,4-D tolerance, the inserted construct also provides tolerance to glufosinate (via the 'pat' gene).

In maize, 2,4-D tolerance comes from insertion of a synthetic form of the aad-1 gene from *Sphingobium herbicidovorans* which codes for production of aryloxyalkanoate di-oxygenase, 1 (AAD-1) protein that catalyses the side chain degradation of 2,4-D. In addition to 2,4-D tolerance, this gene also provides tolerance to aryloxyphenoxypropionate fop herbicides by degrading the R-enantiomer (ISAAA International Service for the Acquisition of Agri-biotech Applications, 2018).

How does resistance occur?

Despite being in commercial use for more than 70 years, there are relatively few cases of herbicide resistance to the Group 4 mode of action. The first recorded case of resistance to 2,4-D can be traced back to 1957 where resistance was reported in spreading dayflower (*Commelina diffusa*; Hawaii, USA) and separately in the same year in wild carrot (*Daucus carota*; Ontario, Canada) (Heap, 2017).

In Australia, resistance was first reported in wild radish from Western Australia in 1999 followed by South Australia in 2006, Victoria 2009 and New South Wales in 2013. In addition to wild radish, there are also Australian populations of 2,4-D-resistant Indian hedge mustard confirmed from South Australia in 2005 and Victoria in 2016; capeweed from South Australia detected in 2015 and sow thistle from South Australia and Victoria in 2015, and 2022 in northern NSW and Queensland.

The mechanisms of 2,4-D resistance require further study. However, in wild radish populations from Western Australia that had developed field-selected resistance to 2,4-D amine, the mechanism may be due to disruption of an ABCB (ATP-binding cassette sub-family B) transporter(s) that move the herbicide from cell to cell, ultimately reducing the amount of 2,4-D reaching the phloem and then transported to the meristematic points (Goggin, et al., 2016). After treatment with commercial rates of 2,4-D amine, these biotypes developed typical leaf curling, petiole elongation and epinasty after application. These plants however could produce asymptomatic new growth 7 days after application, suggesting limited herbicide translocation to the growing points. This study also tested for differential rates of leaf entry, metabolism, conjugation or vacuole sequestration, however these alternate pathways for herbicide resistance were shown not to occur in these field-resistant populations.

A separate South Australian study in field-collected 2,4-D resistant Indian hedge mustard populations also showed that reduced translocation was likely to be the mechanism of resistance in these populations, conferring 67 and 81-fold resistance levels in these populations. This study suggests that the resistance mechanism is a result of a single dominant gene (Dang, et al., 2017).

In addition, research under controlled glasshouse conditions conducted at the University of Western Australia has also demonstrated the ability of wild radish to develop 2,4-D-resistance conferred by enhanced metabolic breakdown.

Recurrent selection with initially low rates of 2,4-D amine, increasing with each application, shifted the LD₅₀ (lethal dose to kill 50 per cent of the population) required to control wild radish from 16gai/ha to 42gai/ha to 55gai/ha to 62gai/ha and finally 138gai/ha after just 4 generations (Ashworth, et al., 2016). This 8.6-fold increase in tolerance demonstrates the importance to always use robust label rates.

Interestingly in this same study, the 4th generation 2,4-D selected wild radish population had also evolved resistance to the metabolizable Group 2 herbicides metosulam (4-fold) and chlorsulfuron (4.5-fold). The chlorsulfuron population was also tested by pre-treating with the P450 inhibitor malathion before applying the chlorsulfuron, with resistance levels able to be reversed, suggesting that P450 catalysed metabolism is likely to be the mechanism involved. Other herbicides tested: sulfometuron-methyl and imazamox (both non-metabolizable Group 2 herbicides), diflufenican (Group 12), bromoxynil (6), diuron (5), metribuzin (5), atrazine (5), glyphosate (9) and diquat (22) were still able to provide robust control of the 4th generation 2,4-D selected population.

Factors affecting efficacy

Group 4 herbicides are generally able to be used in most cereal crops, however the timing of application needs to be managed. Damage can occur at certain growth stages, typically during periods of rapid growth.

General rules of thumb in relation to cereal crop safety:

- Variation exists between different cereals and within cultivars. Oats are generally more sensitive than wheat or barley.
- Ester formulations will enter the leaf faster than amine formulations and therefore have the potential to cause more cereal crop injury. Application rates (gai/ha) may be lower with some ester formulations due to enhanced rates of leaf entry.
- Adjuvants that enhance speed of uptake have the potential to cause more cereal crop injury.
- Where the crop is under stress following application, the potential for cereal crop injury is higher due to slower herbicide metabolism.
- Cereal safety varies with herbicide (Table 5.3A). 2,4-D generally has the greatest potential to cause damage, especially at earlier timings and particularly at higher use rates.

Known herbicide interactions

The application of synthetic auxins such as 2,4-D causes a range of biochemical responses in the plant. One of these plant responses is an increased production of certain cytochrome P450 enzymes (Hirose, et al., 2007). These same P450 enzymes appear to be important in the metabolism of other herbicides such as the Group 1 herbicide diclofop and the Group 2 herbicide chlorsulfuron (Han, et al., 2013).

Increasing the speed of metabolism of Group 1 and certain Group 2 herbicides following tank-mixing with Group 4 herbicides may present as reduced grass weed control and/or as reduced crop impact of the Group 2 herbicide in certain grass crops.

Group 4 herbicides are often used in tank mixes, either with other Group 4 herbicides or herbicides from different modes of action. This is usually done to broaden the weed spectrum. The phenoxy sub-Group 4s particularly effective on broadleaf weeds from the Brassicaceae family, while the pyridine sub-Group 4s particularly strong on weeds from the Asteraceae and Polygonaceae families.

Both sub-groups are generally effective against the Fabaceae family, although there are differences between individual herbicides. In particular, 2,4-DB and MCPB can be used in certain Fabaceae crops to remove broadleaf weeds. 2,4-DB and MCPB herbicides are not herbicidal in their applied butyric form, however in susceptible plants they are rapidly converted to 2,4-D and MCPA respectively by a beta-oxidation reaction. This reaction does not occur in certain legume crops, with the herbicide remaining in the non-toxic butyric form (Cobb & Reade, 2010).

Table 5.3A: Registered application stages for Group 4 herbicides in winter cereals.

Zadoks growth stage	11	12	13	15	22	23	30	31	37	41	44
	First leaf	2 leaf	3 leaf	5 leaf	2 tiller	3 tiller	Stem elongation	First node	Flag leaf	Early booting	Mid-booting
2,4-D	Refer to label for rate and geography						First node (Z31) to booting (Z43)				
MCPA	5 leaf (Z15) to flag leaf (Z37)										
picloram + MCPA	Early tillering to first node										
picloram + 2,4-D	Z23 to Z31										
fluroxypyr	3 leaf (Z13) to flag leaf (Z39)										
fluroxypyr + aminopyralid	3 leaf (Z13) to first node (Z31)										
fluroxypyr + halauxifen	3 leaf (Z13) to flag leaf (Z39)										
clopyralid	2 leaf to first node										

Note: 2,4-D can also be applied after firm dough stage for salvage control of weeds.

Table 5.3B: Recommended water rate (L/ha) for tank-mixing 2,4-D (Statesman® 720) with glyphosate IPA + AMS.

Glyphosate 450 (IPA) + 2% AMS Rate (L/ha)	Dow AgroSciences Statesman® (720g/L 2,4-D DMEA + DMA) Rate (L/ha)					
	0.8	1.0	1.2	1.5	1.7	2.0
1 to 1.6	50	60	70	90	100	120
1.8	50	60	70	90	110	120
2.0	60	70	80	100	110	120

Source: Dow AgroSciences (2014)

Care needs to be taken when mixing 2,4-D with glyphosate, as physical incompatibility may occur under certain circumstances. Normally 2,4-D ester herbicides have better compatibility with glyphosate.

There are several factors that increase the likelihood of incompatibility between 2,4-D amine (salt) and glyphosate:

- Lower spray volumes where herbicides are applied in a more concentrated form is likely to induce a higher level of physical incompatibility than occurs in a more dilute concentration/higher carrier volume (Table 5.3B). Be especially aware of concentrating herbicides in pre-mixing pots or tanks. The ratio of 2,4-D to glyphosate also has a significant impact.
- Mixing order is important. Pre-condition water first with ammonium sulphate (if required). Ensure at least half the spray tank is full before introducing the 2,4-D amine. Then add glyphosate and finally any other adjuvants.
- Incompatibility problems are more likely to occur in cold water.
- Low pH of spray water. Reducing the spray tank pH below ~5 increases the likelihood of 2,4-D amine 'gelling out' when mixed with glyphosate. Good quality 2,4-D formulations should contain adequate pH buffering to prevent the spray tank pH falling too far.
- Hard water (high levels of cations or bicarbonate) will increase herbicide dissociation and potentially lead to increased incompatibility. Consider pre-treating the water with ammonium sulphate before adding the herbicides if hard water is to be used.
- Mixing different 2,4-D and glyphosate salts can be more problematic than using the same salt formulations (for example, mixing 2,4-D isopropylamine (IPA) and glyphosate IPA formulations will generally have better compatibility than mixing different salts for example, 2,4-D dimethyl-ammonium (DMA) and glyphosate potassium (K) salts). Note: herbicide manufacturers are continually testing different salt and in-built adjuvant systems to enhance leaf uptake and compatibility. While using the same salt formulations for tank mixes generally reduces the risk of incompatibility, certain mixtures of non-alike salts may be supported by manufacturers due to their proprietary surfactant mixtures. Always follow label recommendations.

In addition to physical compatibility, there is a level of biological incompatibility between 2,4-D (amine or ester) and glyphosate. This may only rarely be observed when treating susceptible weeds and can generally be masked by increasing the glyphosate rate. However this biological incompatibility is becoming increasing evident as glyphosate resistance evolves and is often now resulting in spray failures when these products are mixed.

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5.4 Group 5/6 – Photosystem II inhibitors

Table 5.4: Group 5/6 herbicide mode of action sub-groups and active ingredients by sub-group.

Group 5 sub-groups	Main herbicides used in grain and broadacre field crops
Amides	propanil*
Triazines	atrazine* cyanazine prometryn* simazine* terbuthylazine terbutryn
Triazinones	metribuzin*
Ureas	diuron* fluometuron*
Group 6 sub-groups	Main herbicides used in grain and broadacre field crops
Nitriles	bromoxynil
Benzothiadiazinones	bentazone

* Denotes useful activity on grass weeds.

Both Group 5 and Group 6 modes of action block photosystem II by binding to the quinone binding domain, however different sub-groups do this slightly differently and are therefore split into two mode of action groups in the current herbicide mode of action classification. Previously these two Groups were aggregated.

Typically, most Group 5/6 herbicides are effective on a wide range of broadleaf weeds. Some herbicides (with asterisk above) from the amide, triazine, triazinone or urea sub-groups also have some activity on grass weeds.

Plant function targeted

Group 5/6 herbicides disrupt photosynthesis, which occurs in the chloroplasts. Photosynthesis comprises two adjacent pathways (Photosystem I and Photosystem II), with each pathway consisting of several complex reactions that are ultimately responsible for converting light into energy. The two photosystem pathways interact with each other; in that electrons and hydrogen produced by photosystem II are required to progress further reactions in photosystem I. Stopping either the photosystem I or II pathways can lead to plant death. Group 5/6 herbicides are active in disrupting the photosystem II pathway.

Photosystem II extracts light energy to create high-energy electrons that are released when plastoquinone binds to the quinone binding domain (QB), reacting to form plastoquinol. These high-energy electrons are then required for the photosystem I pathway. A replacement electron is extracted from water, resulting in hydrogen ions and oxygen being created as a by-product of this reaction (Cobb & Reade, 2010).

Group 5/6 herbicides compete for the QB site. Once bound, these herbicides do not release from the binding site, preventing plastoquinone from binding and accepting the high-energy electrons. Without plastoquinone accepting these electrons, the build-up of these high-energy electrons causes cell wall leakage and the resultant herbicidal effects.

Herbicide entry

Most Group 5/6 herbicides are mobile in the xylem and are therefore able to be taken up in the soil water via the plant roots and transported to the leaves via acropetal movement in the xylem.

Atrazine and metribuzin have some ability to penetrate the cuticle and therefore have some foliar activity, while foliar uptake of simazine and diuron is typically poor.

The nitrile herbicide bromoxynil is normally applied as an ester, resulting in good foliar uptake. However, as with the other Group 5/6 herbicides, there is little basipetal movement.

Herbicide translocation

Group 5/6 herbicides typically have intermediate lipophilicity. This allows for apical movement via the xylem in transpiring plants. For the majority of Group 5 herbicides, uptake is primarily via the roots with herbicide dissolved in soil water. As solubility is often low, good soil moisture is required for adequate root uptake. As herbicide moves upwards in the xylem, herbicide accumulates at the leaf tips and margins and symptoms are expressed first in these areas.

When Group 5/6 herbicides are applied post-emergent, almost all herbicide that enters via the leaf remains within the treated leaf and does not translocate well throughout the rest of the plant, due to poor phloem movement. Any herbicide that does move out of the treated leaf will move further upwards in the plant via the xylem.

For example, when atrazine is applied as a foliar post-emergent application, herbicide taken up by the leaf behaves more like a contact herbicide that is, activity is primarily limited to where the herbicide enters the plant. A tank-mix with a crop oil concentrate will assist in maximising penetration of the waxy cuticle, which optimises any opportunity for post-emergent activity. In addition, some herbicide is likely to be oversprayed and reach the soil. This herbicide reaching the soil is then available for root uptake, which is important for applications applied post-emergent.

Herbicide metabolism

Group 5/6 herbicides are metabolised in tolerant plants before herbicidal effects can occur via a range of different mechanisms, depending on the herbicide and the crop/weed.

For example, in maize and sorghum, atrazine is primarily inactivated by a conjugation reaction, catalysed by the glutathione S-transferase (GST) super-family of enzymes to form an atrazine-glutathione conjugate (GS-atrazine). This happens before the herbicide reaches the target site in the chloroplasts, preventing damage.

Bromoxynil is usually applied as the octanoate ester when used in wheat. After leaf entry, wheat selectivity results from hydrolysis of the nitrile group, followed by production of a carboxyl group that may be subsequently decarboxylated.

Crop tolerance for different Group 5/6 herbicides applied as a post-emergent application is a combination of application rate, the metabolic pathway for detoxification and the rate of metabolism. With soil applications applied at planting, positional selectivity (that is, keeping the herbicide band away from the germinating seedling) is also important for crop selectivity.

How does resistance occur?

Despite Group 5/6 herbicides being used extensively in some farming systems for more than 30 years, the selection of herbicide-resistant individuals has been relatively low in Australia. Where resistance has occurred in Australia, the mechanism of resistance often appears to be increased metabolic breakdown that is, rapid degradation of the herbicide before reaching the target site. This typically involves the GST family of enzymes.

Target site resistance has been reported internationally. In susceptible plants, plastoquinone binds to proteins on the Q_B site primarily associated with amino acids His215, Phe255 and Ser264. Triazine and urea herbicides (Group 5) appear to bind in association with Phe255 and Ser264. Group 6 nitriles (for example, bromoxynil) appear to bind primarily in association with Phe255 & His215 (Cobb & Reade, 2010). Differential binding between individual herbicides at this site is likely to explain differences in herbicide activity on different weeds.

One of the most common target site substitutions conferring resistance to the triazines, is the substitution of serine with glycine at location 264 (Ser-264-Gly). This substitution prevents binding of triazines and results in high-level tolerance. However, this substitution does not stop ureas, such as diuron, from binding and controlling triazine-resistant weeds.

This same substitution has been commercialised in triazine-tolerant canola and comes with a substantial fitness penalty, meaning that resistant weeds (and crops) containing this substitution, are likely to be less competitive (yield less) than individuals without this substitution. In the case of triazine-tolerant canola, this is reflected in the 10 to 15 per cent yield penalty often seen with these varieties when compared to otherwise similarly adapted genotypes.

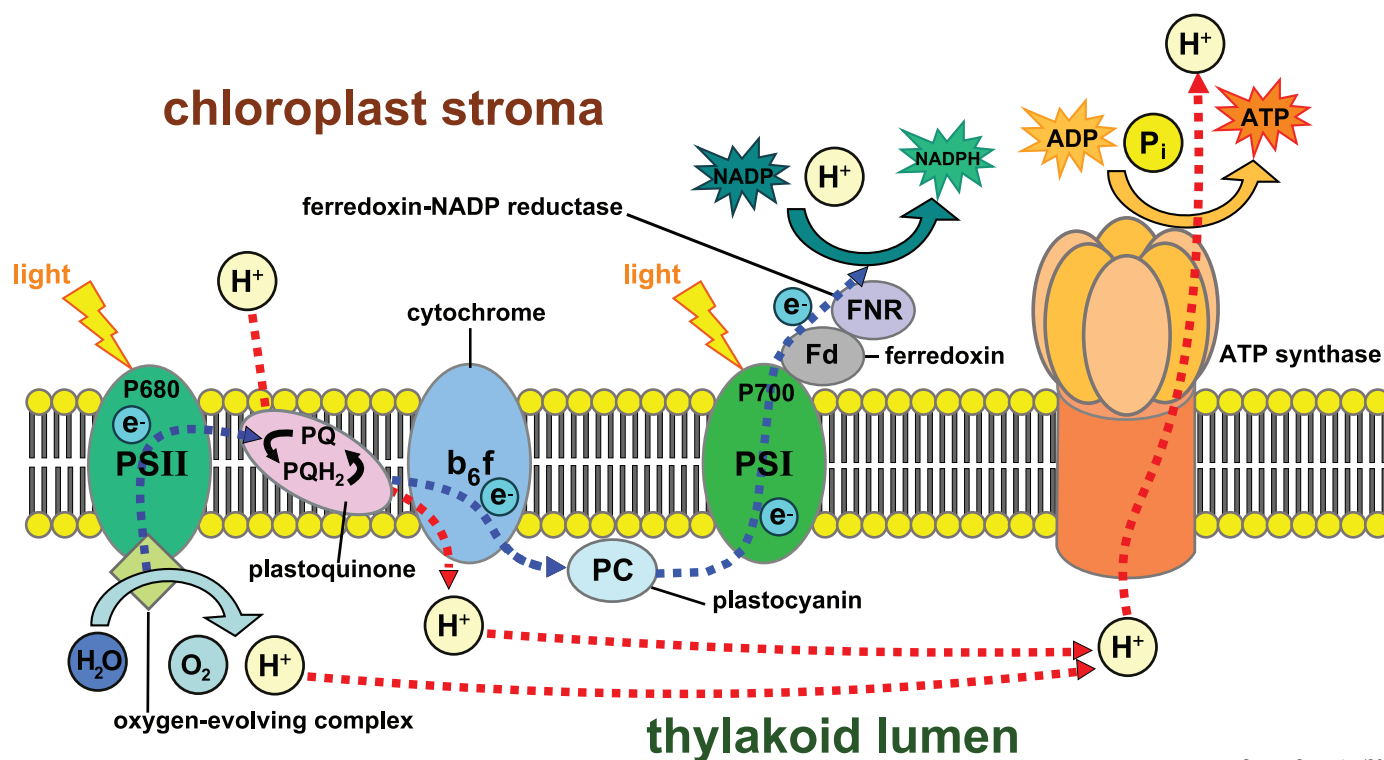
A serine to threonine (Ser-264-Thr) substitution has also been recorded, which confers resistance to both the triazine and urea sub-groups. Other substitutions that have been infrequently identified include Val-219-Ile (resistance to diuron and metribuzin), Asn-266-Thr (bromoxynil), Ala-251-Val (metribuzin) and Phe-255-Ile, which confer resistance across various photosystem II herbicides (Powles & Yu, 2010; Cobb & Reade, 2010).

Factors affecting efficacy

Primary entry into the plant is via root uptake of herbicide dissolved in the soil water, so they are more effective on germinating weeds. Solubility for many of the main Group 5 herbicides is relatively low, so good soil moisture is required for the period of weed control. Soil binding of the triazine herbicides is relatively weak, especially in low organic matter soils. As a result, they can leach below the weed germinating zone with heavy rainfall.

Foliar uptake can be significant for some Group 5/6 herbicides for example, atrazine, bentazone, bromoxynil and metribuzin. These herbicides have better post-emergent activity than many other herbicides in this group. Coverage is important for foliar uptake.

Figure 5.4A: Diagrammatic representation of the key photosystem I and II reactions, showing electron (e⁻) and hydrogen (H⁺) flow.



Source: Somepics (2015)

Known herbicide interactions

Hydroxyl phenylpyruvate dioxygenase (HPPD) inhibitors (Group 27 herbicides) block the production of homogentisic acid, an intermediary required by the plant to synthesise plastoquinone (Cobb & Reade, 2010). As Group 5/6 herbicides compete for the plastoquinone binding site (Q_B) it therefore follows that in an environment of depleted plastoquinone resulting from the activity of a HPPD inhibitor, the Group 5/6 herbicide would have less competition at the Q_B site, leading to increased herbicidal activity.

This synergistic activity has mostly been reported in broadleaf weeds where current HPPD inhibitors demonstrate their greatest activity for weed control. For example, more than 40 per cent improvement in control of both susceptible and triazine-resistant wild radish was demonstrated by a mix of atrazine (Group 5) and low rates of the HPPD herbicide mesotrione (Walsh, et al., 2012). In Australia, this synergist activity has been seen with bromoxynil (Group 6) marketed as a co-formulation with some Group 27 HPPD inhibitors.

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5.5 Group 9 – 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) inhibitors

Table 5.5: Group 9 herbicide mode of action sub-groups and active ingredients by sub-group.

Group 9 sub-groups	Main herbicides used in grain and broadacre field crops
Glycines	glyphosate

Plant function targeted

Glyphosate inhibits the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS). EPSPS is a critical enzyme in the shikimate pathway. The shikimate pathway produces three key aromatic amino acids that are critical to plant life (phenylalanine, tyrosine and tryptophan); plus a range of auxins, phytoalexins, folic acid, lignin, plastoquinones and other secondary products required to fix carbon for plant growth (Shaner, 2006).

Glyphosate binds to and inhibits the EPSPS enzyme, which deregulates the carbon flow within the shikimate pathway. This leads to a reduction in sugar production and a build-up of toxic products such as shikimate (Hall, et al., 1999).

The shikimate pathway is unique to plants, fungi and some microorganisms and does not occur in animals; contributing to the low toxicity to vertebrate and invertebrate animal species.

Herbicide entry

Glyphosate acid is the active form of the herbicide required to provide weed control. However, the solubility of glyphosate acid is relatively low that is, 11.6g/L at pH7, 25°C (Weed Science Society of America, 2014). If glyphosate was to be formulated as a liquid in the acid form, the concentration of glyphosate in the spray drum would be extremely low (<12g/L) and the herbicide would have extreme difficulty penetrating the leaf surface. To increase solubility and improve uptake via foliar application, glyphosate acid is reacted with a base and formulated as a salt (Table 5.5A).

In the salt form, solubility is significantly higher (for example, isopropylamine formulations often contain 450 to 510g/L glyphosate and potassium formulations 540 to 570g/L).

Glyphosate is relatively hydrophilic and is therefore slow to penetrate the waxy leaf cuticle. Adjuvants built into the formulation, or sometimes included as a tank-mix, are critical to optimising leaf coverage and assisting the glyphosate to cross through the cuticle. Some adjuvants may also reduce the speed of droplet evaporation on the leaf surface, allowing increased time for penetration. The type, dose and combination of adjuvant package used can have a significant impact on efficacy and are optimised for the salt used in a particular formulation. Always follow label advice for the recommended choice of adjuvant. Including the wrong adjuvant for glyphosate may decrease performance. In particular oil based adjuvants are not recommended for a hydrophilic herbicide such as glyphosate and will regularly reduce control on some summer grasses.

Glyphosate differs from many other herbicides, in that reduced spray volume (lower water rate L/ha) may improve herbicide control in some situations. For most herbicides, increasing spray volume achieves better leaf coverage and often leads to improved control. However, in the case of glyphosate, a lower carrier volume/more concentrated spray solution will result in droplets with a higher concentration of herbicide and surfactant within the droplet. Applying as a large, concentrated droplet reduces the speed of droplet evaporation while also assisting with penetration through the waxy layer on the leaf surface by maintaining a higher concentration gradient. Note: While the properties of glyphosate are suited to enhancing leaf uptake when applied as a large concentrated droplet, there is still a requirement to ensure enough droplets hit the target. When applying Coarse or larger spray quality, and especially when targeting 1 to 2 leaf erect grass weed seedlings, spray volume of >70L/ha may be required to ensure acceptable coverage.

Dissociation of the glyphosate formulation when added to hard water can be a major problem, reducing the efficiency of herbicide penetration into the leaf. Hard water contains elevated levels of multivalent cations such as calcium or magnesium; or less commonly aluminium or iron. 'Hard' water is most commonly defined as containing >150mg/L of calcium carbonate; CaCO_3 (McDougall, 2012).

Where hard water must be used for glyphosate application, using lower spray volumes achieves a higher concentration of glyphosate per given volume of water. As a result, there will be fewer molecules of calcium, magnesium and other cations present in the spray tank, relative to the chosen rate of glyphosate. Adding ammonium sulphate to the spray tank before adding the glyphosate can also assist in reducing the effect of hard water (see Section 4.2.2 for more information on how ammonium sulphate works). Additionally, water pre-treated with

Table 5.5A: Some common glyphosate salt formulations used in Australia.

Glyphosate salts	Example
Isopropylamine	for example, Roundup® CT, Roundup Biactive®
Dimethylamine	for example, Ripper® 480 Flexi (not currently sold)
Mono-ammonium	for example, Roundup Ready® with Plantshield®
Monoethanolamine	for example, Roundup® Max
Potassium	for example, Roundup Ultra® MAX
Mono-ammonium plus isopropylamine	for example, Weedmaster® Duo®
Mono-ammonium plus potassium	for example, Weedmaster® DST®
Potassium plus isopropylamine	for example, Weedmaster® Argo®
Potassium plus monoethylamine plus mono-ammonium	for example, Crucial®

ammonium sulphate may also assist with physical compatibility of mixtures, along with assisting glyphosate translocation inside the leaf.

Once the glyphosate salt formulation has penetrated the waxy leaf surface, it rapidly converts to the herbicidally active, acid form.

Herbicide translocation

The shikimate pathway occurs mainly in the meristematic areas of the plant, where new growth is happening. For this reason, glyphosate needs to be translocated from the point of entry (leaf) to the roots and growing point. Noting that weeds that are less than 2 true leaf have generally not yet commenced phloem translocation, and this may result in reduced control of newly emerged seedlings.

Glyphosate acid is a negatively charged, hydrophilic (polar) molecule ($\log K_{ow} -3.2$), which makes simple movement across lipophilic cell membranes difficult. To move across the plasma membrane and into the cell cytoplasm, it appears two mechanisms may be involved (Shaner, 2009). Some glyphosate appears to move passively by diffusion across the cell membrane where there is a higher concentration of glyphosate outside the plasma membrane, compared to the concentration in the cytoplasm. In addition, an active transportation process exists that can move glyphosate across the membrane into the cell, against the concentration gradient. In the case of glyphosate, this appears to be a protein carrier that is normally associated with phosphate movement (Sterling & Namuth-Covert, 2016a).

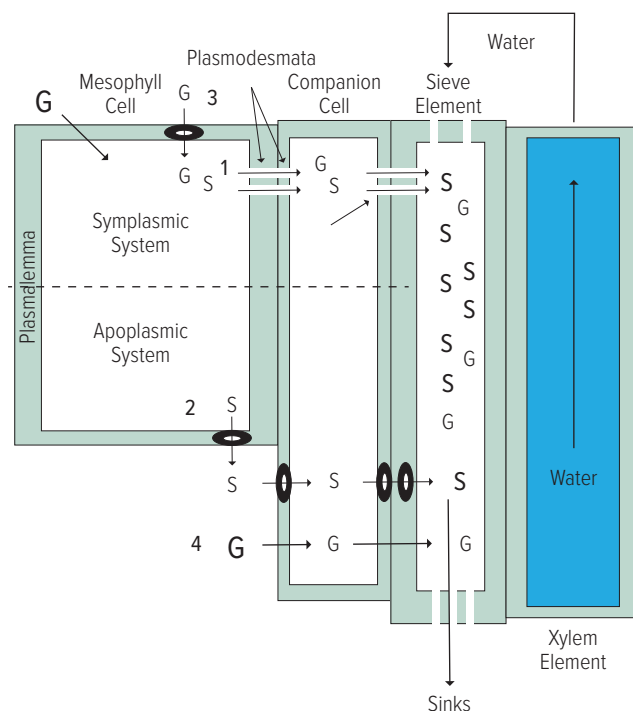
For glyphosate to get to the primary site of activity in the meristematic regions, some herbicide must translocate downwards within the plant via the phloem. Transport mechanisms involved in moving sugars downwards in the phloem are utilised to also move the glyphosate (Shaner, 2009). Once inside the phloem, the glyphosate is trapped by the sieve element, forcing movement downwards towards the sink (Figure 5.5A).

Translocation of glyphosate within the plant appears to be self-limiting. Glyphosate affects photosynthesis in the chloroplasts, ultimately reducing sugar production. Reduction in sugar production that occurs after glyphosate application reduces the sugar flow in the phloem, and therefore the associated movement of glyphosate. Glyphosate translocation over time thereby becomes self-limiting, with the remaining glyphosate trapped in the leaves (Hall, et al., 1999). Up to 70 per cent of absorbed glyphosate has been measured to translocate away from the leaves; however this only occurred for the first 48 to 72 hours after application (Shaner, 2006).

A study of radio labelled [C^{14}] glyphosate in barnyard grass; *Echinochloa crus-galli* (Kirkwood, et al., 2000) showed that translocation peaked 3 days after application (Figure 5.5B). In this study, glyphosate was applied to the fully expanded 4th leaf. There was negligible translocation to the older leaves (1st to 3rd leaf). More than 20 per cent of the applied glyphosate was translocated to the next (5th) emerging leaf in this study, however this was mostly confined to the base of the 5th leaf, highlighting the limited xylem transport of glyphosate. Useful translocation to the meristematic regions in the shoot and roots, resulting from phloem mobility, is primarily responsible for herbicidal performance of glyphosate.

Glyphosate may also translocate passively in the xylem, although this apoplastic movement in the xylem is less significant for weed control. Under conditions of warm/hot temperatures and rapid transpiration, yellowing of apical plant tissue may be seen within a few days of glyphosate application as a result of apoplastic movement in the xylem. While this is visual, it is the symplastic movement down the plant that is primarily responsible for weed mortality.

Figure 5.5A: Movement of sucrose, glyphosate and water into the phloem.



G – glyphosate; S – sucrose/sugar (size of letter indicates the size of pool). Symplasmic system is the cytoplasmic continuous system between cells joined by plasmodesmata. Apoplasmic system is the cell wall and intracellular connections between cells. Grey box is the plasmalemma of the cells. Circles with arrows indicate active transporters, and direction of arrows indicates the direction of the pump (out of cell or into cell across the plasmalemma). Companion cell and sieve element are components of phloem. (1) Symplasmic system: sucrose, produced in mesophyll cell, diffuses to the companion cell via the plasmodesmata. In the companion cell, sucrose is converted to an oligosaccharide that cannot diffuse back through the plasmodesmata but can diffuse into the sieve element. (2) Apoplasmic system: sucrose is actively transported out of the mesophyll cell into the apoplast and is subsequently actively transported into the companion cell via an active influx system. (3) Glyphosate can enter the phloem either via the symplasmic system via an active transporter and then diffuse through the plasmodesmata to the phloem. (4) Glyphosate can enter the companion cell via diffusion.

Source: Shaner (2009)

In the early stages of seed formation, the plant is still moving water up the xylem and it is possible for some glyphosate to translocate to and sterilise the developing seed. This can be useful for sterilisation of weed seeds if glyphosate can be applied during flowering. However, if glyphosate is applied to a grain crop before a seed abscission layer has formed, then yield and germination of the grain/crop seed produced can also be affected. Therefore, it is not recommended to use grain for subsequent planting following a crop-topping application of glyphosate.

Herbicide metabolism

Metabolism of glyphosate within the plant is extremely slow to negligible. Slow metabolism of the glyphosate to the breakdown product amino methylphosphonic acid (AMPA) has been documented in some species (Weed Science Society of America, 2014).

In the soil, glyphosate tends to bind relatively tightly to soil and organic matter and there is practically no available glyphosate for root uptake in most farming soils, with the possible exception of soils with extremely low cation exchange capacity (very sandy soils with almost non-existent organic matter) and where high rates of glyphosate are used.

Biodegradation of any unbound glyphosate in the soil solution occurs relatively rapidly by certain microbes (for example, *Pseudomonas* spp.), using glyphosate as a phosphorous source (Cobb & Reade, 2010).

How does resistance occur?

Several different mechanisms have been identified conferring resistance to glyphosate.

In Australia, target site substitution conferring amino acid substitution has been confirmed as a mechanism present in annual ryegrass, barnyard grass and feathertop Rhodes grass, while a gene amplification mechanism has been identified in brome grass and windmill grass (Han, et al., 2016a; Han, et al., 2016b; Malone, et al., 2015; Hereward, 2016). Initially these mechanisms tend to yield only moderate levels of field resistance, with some level of herbicide control often still being able to be achieved by application of robust rates. However, with further selection pressure, several populations appear to be developing 'strong' resistance, possibly with more than one mechanism involved.

In addition, non-target site resistance is also present. Increased translocation to the leaf tips and reduced herbicide translocation in the phloem have been implicated; with vacuole sequestration also demonstrated in laboratory and field-collected samples of annual ryegrass. Increased vacuole sequestration has also been implicated in fleabane (Hereward, 2016).

Recently it has been proposed that enhanced metabolism by aldo-keto reductase is the mechanism responsible for glyphosate resistance in a population of awnless barnyard grass (*Echinochloa colona*) originally collected from the Ord River in Western Australia (Pan et al. 2019).

The exact mechanism of resistance is often unclear in many situations, with many populations likely to have multiple mechanisms.

Factors affecting efficacy

SPRAY APPLICATION SETUP

The choice of droplet size, adjuvant package and application volume will influence penetration into the leaf. Having a high concentration of glyphosate within each spray droplet has been shown to help maximise entry into the leaf. This benefits uptake in two key ways, firstly by increasing the concentration gradient to assist entry into the plant, and to a lesser extent, by increasing the concentration of surfactant to assist in the breakdown of waxy acids in the leaf surface.

Typically glyphosate will be applied with at least coarse spray quality. Water rates of 50 to 60L/ha may be suitable, however may need to be increased to above 70L/ha when using larger droplets and targeting very small catching surfaces such as, 1 to 2 leaf grass weeds.

WATER QUALITY AND MIXING

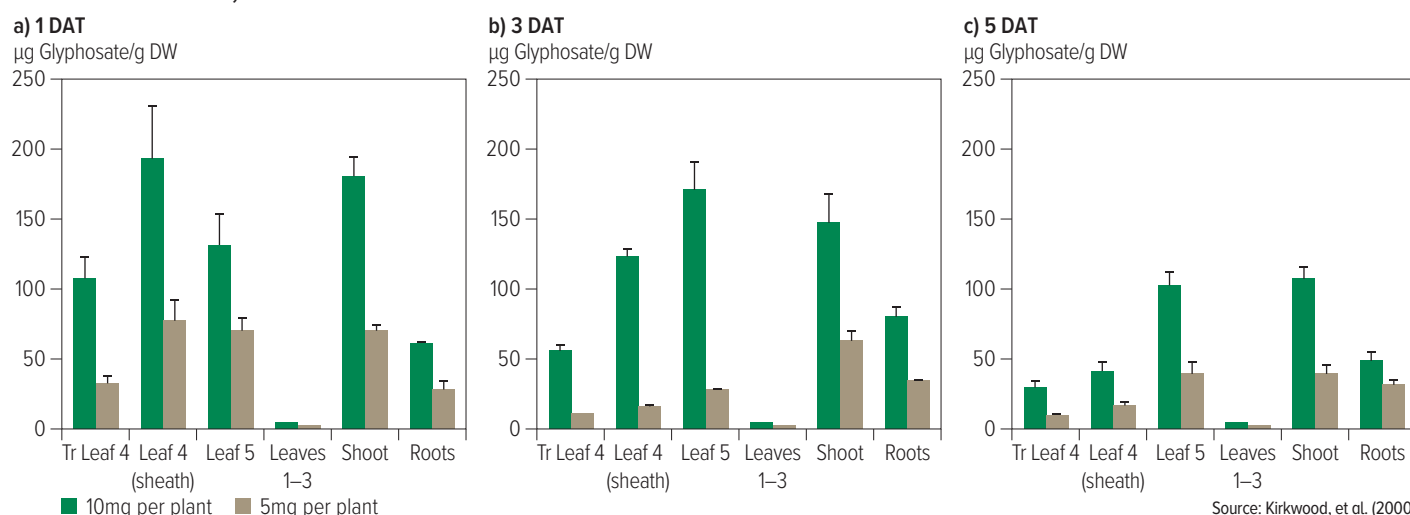
If dirty water containing suspended soil or organic material is used, glyphosate will bind to this and be deactivated in the spray tank.

Spraying under conditions where dust settles on the leaf surface before the spray has dried can also result in herbicide deactivation and poor performance. This is often a problem in lighter soil types prone to dust, and especially around the wheel tracks of sprayers (Figure 5.5C). Lowering sprayer speed and subsequent dust levels, as well as increasing spray nozzle flow rates over wheel tracks are both used to counter weed escapes due to dust inactivation in wheel tracks.

Using hard water containing high levels of cations (calcium, sodium, magnesium, iron or aluminium), or water high in bicarbonates, may result in reduced efficacy.

A recommendation is sometimes given to add an acidifying agent to the spray tank to reduce the pH of the solution, as research has shown that a spray solution pH of 4.5 to 5.6 is generally optimal for glyphosate movement across the plasma membrane surrounding plant cells. However, as glyphosate is a weak acid, the addition of glyphosate alone often reduces the pH to required levels without the need to add a pH reducing agent. For example, adding the equivalent of 1.2L/ha glyphosate into bore water at water rates equivalent to applying 40L spray volume/ha dropped the pH of the spray solution from 8.4 to 4.9, without any additional buffering or acidifying adjuvants (Dow AgroSciences, 2012).

Figure 5.5B: Specific activity of glyphosate in barnyard grass (*Echinochloa crus-galli*) fractions a) 1; b) 3; and c) 5 days after treatment of (green bars) lethal (10mg per plant) and (brown bars) sub-lethal (5mg per plant) glyphosate treatments. Mean values \pm SE, n=3.



Dropping the pH too low can lead to problems with compatibility with other herbicides. Where sulfonylureas are tank-mixed, they will commence breakdown via hydrolysis when the pH of the spray solutions drops to under 4.5 to 5.

Mixing 2,4-D amine products and glyphosate in low pH (acidic) spray water increases the potential for the formation of gel-like precipitates in the spray tank that can block nozzles and filters. Problems are magnified where spray water is also cold and/or hard. Increasing water rate/ha (lower concentration in the sprayer) generally allows more room for separation and less chance of physical compatibility issues. However, if the water is hard and not preconditioned, then increasing water rate/ha can often lead to more compatibility problems as there will be more cations in total in the spray tank.

Applying both herbicides as the same salt also generally improves compatibility that is, applying the IPA salt of glyphosate and the IPA salt of 2,4-D usually results in less compatibility issues than mixing, for example, the potassium salt of glyphosate and a DMA salt of 2,4-D.

It is a good idea to test the water source to be used by measuring the pH after mixing the required amount of glyphosate into the required volume of water, without any pH acidifier or buffer. For example, if doing a jar test and the required glyphosate rate is 1.5L/ha and the spray volume to be used is 50L/ha, then 30mL of glyphosate in 1L of water would need to be added. If the pH after adding the glyphosate is about 5.5 or lower, there is no need to further reduce the water pH by adding additional acidifying agents.

Several commercial adjuvants sold as acidifying agents also modify droplet size and/or reduce the effects of glyphosate disassociation in hard water. It is generally this benefit, rather than the reduction in water pH, that leads to the improvement of glyphosate performance when these products are added.

Often water with high pH is hard (that is, contains high concentrations of divalent and trivalent cations). Where these cations exist in high concentrations, substantial disassociation of glyphosate in the spray tank will often occur. While this may not produce visual particulate precipitation, the recombination of glyphosate with these polyvalent cations will reduce solubility and penetration of the leaf cuticle. A common rule of thumb is that water used for applying glyphosate should be clean, soft and able to easily lather soap. It is important to address the underlying reason for high water pH, typically by pre-treating hard water with ammonium sulphate. Simply modifying spray tank pH alone is unlikely to improve glyphosate efficacy.

ENVIRONMENTAL CONSIDERATIONS

Large droplets that do not spread tend to survive on the leaf for longer. This may help uptake, especially under summer conditions. Adjuvants that spread the droplet on the leaf surface may provide a greater area for leaf uptake, however will result in faster droplet drying. Spreading non-ionic and silicon surfactants typically result in reduced glyphosate leaf uptake, especially under summer conditions.

As glyphosate is usually well translocated in the phloem, excellent coverage is not as important as it is for some other herbicides. Efficacy is generally satisfactory, provided adequate product is on the leaf surfaces and available for uptake for sufficient time.

Glyphosate has shown to be sensitive to high temperatures at application. In a controlled study, glyphosate-susceptible barnyard grass could be controlled at rates of 112gae/ha (and above) under a 25°C/20°C temperature regime, however 100 per cent survived this dose at 35/30°C. Similar trends were observed in a resistant population containing Pro-106-Thr and Pro-106-Leu substitutions. This resistant population could be controlled at rates above 337gae/ha at 25/20°C (so possibly still achieving some level of control at typical field application rates of 450 to 900gae/ha). However, at 35/30°C, 90 per cent of these resistant plants survived the same rate (Han, et al., 2016b).

Under hot conditions (consistent daytime temperature above ~35°C), plants will close their stomata and withdraw moisture from the waxy leaf cuticle, making the cuticle more lipophilic and more difficult for hydrophilic herbicides such as glyphosate, to penetrate and move through the leaf cuticle. This occurs even where soil moisture is good. The plant response to hot temperatures is not instantaneous, taking some time to occur and then also to reverse following the passing of the hot conditions.

Therefore, spraying during a brief window where the temperature drops (for example, early morning) is unlikely to entirely overcome the effects of heat-induced plant stress and subsequent development of a thicker cuticle. Ideally, it is best to have glyphosate applied prior to a run of hot weather, or to wait a few days after daytime temperatures have dropped to below 35°C. Spraying under heat wave conditions will also result in very fast evaporation of the spray droplet. This is likely to reduce the number of smaller droplets reaching the leaf, while also reducing the time available for leaf uptake prior to glyphosate crystallisation on the leaf surface for those droplets making it to the target. Both will reduce the amount of glyphosate entering the leaf.



Figure 5.5C: Poor barnyard grass control in wheel tracks, most likely arising from dust at application probably in association with early onset of moisture stress in compacted wheel tracks.

Where weeds are stressed (in particular moisture and temperature stress), translocation of sugars (and therefore associated glyphosate movement) is reduced, which can lead to poor weed control. Weeds should be actively growing for optimum results. Weeds common in northern Australia that are particularly susceptible to moisture stress, include button grass (*Dactyloctenium radulans*), liverseed grass, awnless barnyard grass and red pigweed (*Portulaca oleracea*). All these weeds have a relatively shallow root system as a common feature.

Some research into the night time application of glyphosate in summer fallow has been undertaken as a means of applying at times of lower heat stress and higher humidity than available during the heat of the day in summer. Any increases in efficacy are generally minor, while the risk of spray drift due to temperature inversions is greatly increased, thereby significantly increasing the risk of off-target drift damage. Temperature inversions occur in the majority of evenings over summer. For more information on temperature inversions grdc.com.au/resources-and-publications/all-publications/factsheets/2022/hazardous-inversion.

Under ideal conditions (warm temperatures, good soil moisture, small plants and high humidity) on sensitive weed species, the first signs of leaf chlorosis may be evident within 4 to 5 days of application. Where weeds are larger/more tolerant, or where plant metabolism is slowed by cold temperatures and/or anaerobic soil conditions, longer periods may be required for symptoms to appear. Older or larger plants have more stored reserves that need to be run down before chlorosis will be evident.

CONTROLLING DIFFICULT WEEDS

The speed of leaf uptake can vary across species (Figure 5.5D). Species that are slower to absorb glyphosate may be subject to more variability in results as less herbicide may enter the plant where herbicide is impacted by rainfall after application or higher rates of droplet evaporation occurs (for example, under summer application conditions).

When using glyphosate as the first application in a double knock strategy, it is important not to apply the second knock too early. It takes 2 to 3 days for the majority of the glyphosate to translocate from the point of application to the meristematic regions in the shoots and roots. To maximise control, delay the second knock for at least 3 days after application of the glyphosate before cultivating or applying a contact herbicide that will destroy the cell structure of the phloem.

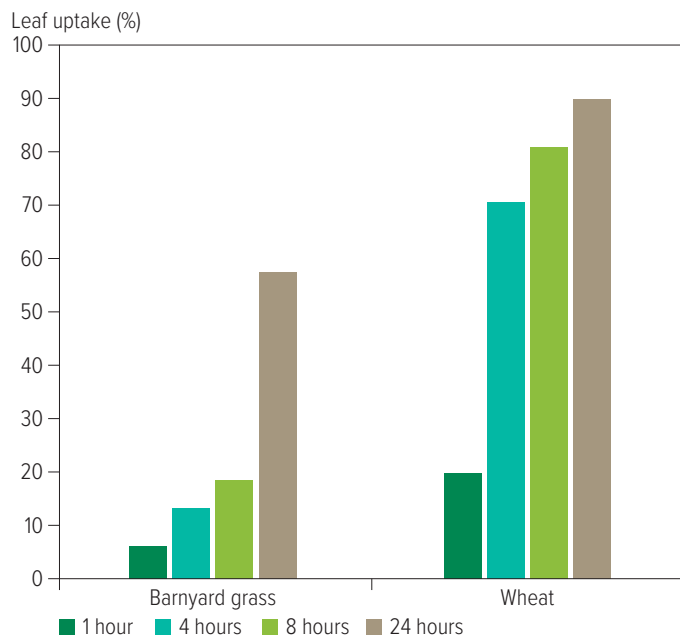
For many years it has been common practice to allow at least 24 hours after applying glyphosate to small annual weeds before significant disturbance by tillage (that is, full-cut disturbance at sowing) or grazing occurs. The labels of some formulations have reduced this to as low as 6 hours where followed by full cut cultivation or planting with tyned seeders. This is the minimum time required to allow some translocation to the roots so that weeds do not keep growing if the roots are cut off from leaves. Where low disturbance seeders are used, the time requirement on labels is generally a minimum of 24 hours.

In perennial species, to enable time for translocation to their larger root systems, labels usually require that soil disturbance or grazing does not occur for at least 7 days post-application.

LATE-SEASON APPLICATION

Application timing is important where glyphosate is to be used in-crop, prior to harvest as either a spray-topping or desiccation application. If application occurs too early, particularly if applied before an abscission layer has formed at the base of the crop seed, then crop yield could be reduced. Even in the absence of symptoms such as plant chlorosis, early application while the crop is still moving sugars into the grain may also result in reduction

Figure 5.5D: Comparison of the speed of radio-labelled leaf uptake of glyphosate (Roundup® 360) between barnyard grass and wheat under glasshouse conditions.



Source: Adapted from Gaskin & Stevens (1993)

of seed viability (Hall, et al., 1999). Refer to glyphosate labels for the earliest application timings for pre-harvest applications. Grain from crops sprayed with glyphosate prior to harvest should not be retained as future planting seed.

As glyphosate breakdown within the plant is negligible, late season application for weed control or crop desiccation applied close to harvest are likely to result in detectable residues on straw and possibly in the grain. Where glyphosate is used close to harvest, it is critical that label recommendations and use patterns are always strictly followed to ensure that Maximum Residue Limits (MRLs) are not breached.

Known herbicide interactions

SPRAYING OILS

The addition of spray oils has been shown to reduce the efficacy of glyphosate on some weeds (particularly difficult to control summer grasses such as barnyard grass and button grass), however this is not always consistent.

Spray oils are added to some spray applications to reduce spray droplet evaporation or modify droplet size, or to assist a partner herbicide that requires the addition of a spray oil to maximise its performance. Potential glyphosate tank mix partners that may benefit from the addition of spray oil include:

- Triclopyr or 2,4-D for control of melons in fallow;
- Group 14 herbicides such as Sharpen® (saflufenacil) and Valor® (flumioxazin) in fallow; and
- Group 1 grass selective herbicides.

In these situations, the addition of spray oil to the glyphosate spray solution may result in reduced control of summer grasses, in particular weeds like barnyard grass. Where the grass weeds are small, susceptible to glyphosate (that is, not selected for glyphosate resistance) and application conditions are excellent; no reduction in performance of the glyphosate may be noticed, especially where the upper end of registered label rates are used.

Strategies to overcome antagonism between glyphosate, a partner herbicide and its recommended spray oil adjuvant include:

- Splitting the application into two distinct applications, usually applying the glyphosate first and then following with the other herbicide plus the spray oil at least 4 to 5 days later. In the case of mixes with Group 1 herbicides in the presence of glyphosate-resistant grass weeds, it may make sense that the Group 1 herbicide be applied first as Group 1 herbicides are generally more sensitive to weed size and growth.
- A double knock strategy where the glyphosate tank mix is followed by cultivation or a contact herbicide, can often mask any antagonism between the glyphosate and oil.
- In some situations, it may be appropriate to replace the spray oil with a non-ionic surfactant to reduce the antagonism of the glyphosate. However, this may also reduce the efficacy of the partner herbicide (contact the manufacturer of the partner herbicide for specific advice).

Usually mixing glyphosate with other broadleaf herbicides provides robust control of broadleaf weeds, without any noticeable impact from the addition of oil-based (lipophilic) surfactants. However, a US study on Canadian fleabane (Table 5.5B) suggests that translocation of glyphosate may be negatively impacted by the addition of a crop oil concentrate (Eubank, et al., 2013).

GLYPHOSATE + GROUP 4

Typically, 2,4-D ester formulations are physically compatible with glyphosate, however tank-mixing 2,4-D salt (amine) formulations with glyphosate requires careful attention.

Glyphosate is commonly available as isopropylamine, monoammonium or potassium salt formulations. 2,4-D amine is available as a range of salt formulations, including isopropylamine (IPA), dimethylamine (DMA), dimethylamine/monomethylamine and dimethylamine/dimethylethanolamine formulations. Tank-mixing similar glyphosate and 2,4-D salt formulations will usually have better compatibility for example, glyphosate IPA and 2,4-D IPA formulations. Dissimilar amine formulations may result in disassociation in the spray tank resulting in incompatibility, especially where the water is hard, cold, low pH and/or spray volume is low and therefore herbicide concentration is higher.

In addition to physical incompatibility, glyphosate can also be biologically incompatible with some Group 4 herbicides on certain broadleaf weeds (for example, sow thistle) and summer grasses. 2,4-D, fluroxypyr and dicamba are particularly problematic.

GLYPHOSATE + TRIAZINES

Mixing a foliar-absorbed triazine herbicide (for example, atrazine) with glyphosate can cause a level of physical antagonism in the spray mix. This antagonism appears to be due to binding of the herbicides within the spray solution and is most likely related to glyphosate binding with the clay carriers used in triazine formulations.

If the triazine is a clay-based formulation (suspension concentrates are an issue, but particularly powder formulations that contain high levels of inert clay material), then the inerts in the triazine formulation may also provide some additional binding of the glyphosate in the tank, which has been shown to result in a loss of 10 to 20 per cent of available glyphosate (Appleby & Somabhi, 1978).

When robust application rates are used, triazine antagonism may not be noticed. However, where marginal rates or unfavourable climatic conditions are experienced and/or low levels of glyphosate resistance has been selected, then the mixture may lead to unacceptable glyphosate performance. Adding an extra 20 per cent of glyphosate (where permitted on the label) and/or preconditioning the water with ammonium sulphate may help mitigate the level of antagonism.

In addition, there is potential for biological incompatibility on some summer grasses. The disruption of photosynthesis resulting from the Group 5 herbicide reduces the performance of glyphosate.

GLYPHOSATE + GROUP 14

Group 14 herbicides can also show a level of antagonism when mixed with glyphosate, particularly when applied under high light intensity. The rapid speed of activity of the Group 14 herbicide causes light-induced necrosis in the cells surrounding the point of droplet entry reducing glyphosate uptake and translocation.

Mixing glyphosate and Group 14 herbicides may be acceptable providing the rate of Group 14 is low, light intensity is low (that is, autumn in southern Australia), weeds are small, the rate of

Table 5.5B: [¹⁴C] Glyphosate translocation and distribution in glyphosate-resistant and glyphosate-susceptible Canadian fleabane, averaged across 24, 48 and 72 hours after treatment.

Trial 1		% of absorbed glyphosate				
		Treated leaf	Crown	Other leaves	Roots	Total translocated
Glyphosate susceptible	Glyphosate	86.8	10.3	2.3	0.6	13.2
	Glyphosate + COC	92.0	6.5	1.2	0.3	8.0
Glyphosate resistant	Glyphosate	89.9	4.8	1.2	4.1	10.1
	Glyphosate + COC	91.4	4.6	0.7	3.3	8.6
LSD (0.05)		NS	2.1	NS	NS	NS
Trial 2						
Glyphosate susceptible	Glyphosate	82.8	14.6	2.0	0.3	17.2
	Glyphosate + COC	89.4	8.6	1.7	0.3	10.6
Glyphosate resistant	Glyphosate	90.6	5.7	1.0	2.7	9.4
	Glyphosate + COC	91.0	5.4	1.3	2.3	9.0
LSD (0.05)		3.0	2.4	NS	NS	3.0

COC = Crop oil concentrates

Source: Eubank, et al. (2013)

glyphosate is increased to compensate for the antagonism and there is no glyphosate resistance. Noting that the choice of adjuvant, preferred spray quality and water rates required for the Group 14 partner are not those recommended for optimum glyphosate uptake.

Applications of glyphosate plus Group 14 herbicides applied in summer (high light intensity) and especially to larger weeds and/or weeds with glyphosate resistance may result in poor control, with excessive regrowth.

A better strategy is typically to apply the Group 14 in mixture with paraquat as the second pass in a double knock strategy.

FOLIAR FERTILISERS

When glyphosate is placed in solution with divalent or trivalent cations (for example, when using hard water), the glyphosate salt formulation may disassociate and reform as a calcium or magnesium salt of glyphosate, which typically has low solubility and extreme difficulty in penetrating the leaf cuticle.

Adding foliar fertilisers to the spray mix that are also available as polyvalent cations (for example, some calcium, copper, iron, magnesium, manganese, molybdenum and zinc foliar fertiliser products) may react in the same way as adding glyphosate to hard water that is, by forming low soluble salts of glyphosate that reduce the ability of the glyphosate to enter the leaf.

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5.6 Group 10 – Glutamine synthetase inhibitors

Table 5.6: Group 10 herbicide mode of action sub-groups and active ingredients by sub-group.

Group 10 sub-groups	Main herbicides used in grain and broadacre field crops
Phosphinic acids	glufosinate

Glufosinate, formulated as glufosinate-ammonium, is the only commercially available herbicide from the Group 10 mode of action. Glufosinate is a broad-spectrum, non-selective herbicide. While having useful grass activity, it is typically stronger against broadleaf weeds.

Plant function targeted

The glutamine synthetase enzyme is essential for nitrogen synthesis. In the plant, it is responsible for converting L-glutamate to an intermediate, which then accepts ammonia to form the amino acid L-glutamine. Glufosinate is an analogue of glutamine and therefore targets glutamine synthetase. It was proposed that the intermediate created from the phosphorylation of glufosinate is not able to bind the ammonia, critical in the process of creating the glutamine amino acid. Inhibition of this process by the introduction of glufosinate may lead to accumulation of ammonia in the plant resulting in cell damage, disruption of photosynthesis and a reduction in the pH gradient across the cell membrane, which can uncouple photophosphorylation (Weed Science Society of America, 2014; Cobb & Reade, 2010).

More recent studies have suggested that the fast speed of activity of glufosinate is a result of accumulation of reactive oxygen species, with ammonia accumulation possibly a secondary (and slower) contributing factor to symptoms (Takano et. al., 2019, 2020).

Table 5.6A: Distribution of ¹⁴C-glufosinate 72 hours after application to 4-leaf canola.

		% of applied dose	
		Glufosinate susceptible variety	Glufosinate tolerant variety
Remaining on leaf surface		12.0%	12.1%
Within the treated leaf	at the leaf tip	15.0%	8.8%
	at the treated area	51.3%	34.3%
	basal leaf	14.2%	4.1%
Shoots above the treated leaf		1.8%	9.1%
Shoots below the treated leaf		3.3%	4.4%
Roots		1.2%	11.4%

Plants were grown under an 18/22°C temperature regime at 70% relative humidity.

Source: Adapted from Beriault, et al. (1999)

Herbicide entry

Despite having high solubility, glufosinate does not have significant soil activity. Glufosinate reaching the soil is highly mobile and rapidly degraded by soil microbes. Any glufosinate that does get absorbed by roots is poorly translocated.

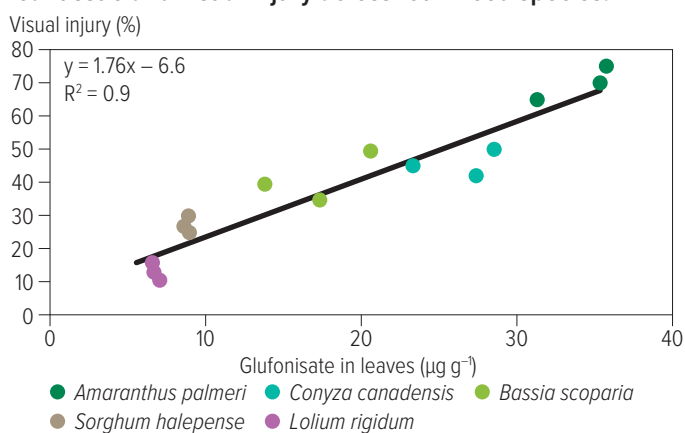
Primary entry into the plant occurs from foliar absorption. Differences in leaf absorption between species have strong correlation to efficacy (Figure 5.6A).

Glufosinate is hydrophilic ($\log K_{ow} -4.01$), which would indicate it requires considerable time in a soluble form on the leaf surface to allow penetration of the waxy leaf cuticle. For this reason, glufosinate is prone to wash off with rainfall should this occur soon after application. In addition, conditions of low humidity can result in alteration of the leaf cuticle and low humidity will increase the rate of droplet evaporation, both reducing glufosinate uptake through the cuticle.

Relative humidity has a significant impact on glufosinate efficacy on wild oats (*Avena fatua*) (Ramsey, et al., 2002). Through a series of trials, the researchers demonstrated control 'failures' where plants were maintained under conditions of 15/25°C temperature and 40 per cent relative humidity, whereas in excess of 80 per cent control was achieved at the same application rate and temperature by exposing the plants to a period of high humidity (99 per cent) for different periods before and after spraying. Across trials, it was concluded that exposure to high humidity for as little as 30 minutes before and after application was enough for increased control, and it was shown that the period of high humidity after spray application was the most critical.

Further, the researchers demonstrated that using standard techniques for applying and measuring leaf penetration of radio-labelled C¹⁴-glufosinate (application of a large single droplet applied to the source leaf) is poorly correlated to herbicide efficacy, as the drying rate of the single large droplet may be significantly different to the drying rate of a typical spray application.

Figure 5.6A: Glufosinate efficacy is proportional to the herbicide concentration in the leaf tissue. Positive correlation between glufosinate concentration within the leaf tissue and visual injury across four weed species.



Source: Takenko et al. (2020c)

The researchers concluded “At present, even with good coverage of glufosinate ammonium on wild oat plants treated at the 3 to 4 leaf stage using a flat fan nozzle, efficacy may be poor if the relative humidity (RH) is low (40 per cent RH). Most likely, the uptake of glufosinate into leaves occurs very rapidly when the droplets are aqueous and little or no uptake occurs once the droplets have dried, a process that will be hastened during conditions of low RH.”

Glufosinate works by creating a rapid increase in reactive oxygen species (ROS) within the leaf. Where ROS production is high, it can overwhelm the capacity of antioxidants within the cells to quench ROS activity, and therefore lipid peroxidation of the cell membrane occurs, leading to rapid cell death.

To achieve greatest control from glufosinate, high ROS production is required. This will be a function of rate applied, weed size, the amount penetrating the leaf (which varies by weed species) and spray coverage (as glufosinate is poorly translocated).

As the production of ROS in the photosynthesis pathway is light dependent, ROS production will be highest when applied under bright, sunny conditions. If applied during the dark, or under low light conditions, no or little ROS is produced and antioxidants may be able to quench the ROS as it is being produced, therefore leading to poor efficacy.

Herbicide translocation

After herbicide entry, the speed of activity of glufosinate is rapid in susceptible species. The resulting rapid cell destruction and inhibition of photosynthesis is likely to be a contributing factor to poor translocation. Glufosinate is considered a contact herbicide, generally with little movement in either the xylem or phloem in susceptible species.

For this reason, high levels of leaf coverage are required with foliar herbicide application. Typically, it is recommended to apply glufosinate using spray volumes in excess of 100L/ha with a medium to medium-coarse droplet spectrum.

In glufosinate-tolerant species, the herbicidally active L-glufosinate is rapidly metabolised to N-acetyl-L-glufosinate, which is not phytotoxic (see following section on herbicide metabolism). Should rapid metabolism of the herbicidally active isomer occur, cell damage reduces and therefore translocation of the inactive isomers and metabolites can occur.

Table 5.6A demonstrates how glufosinate translocation differs in glufosinate-susceptible and glufosinate-tolerant (genetically modified) canola varieties.

As can be seen from the data, there was no difference in uptake into the leaf between each variety, with similar levels of residue being able to be recovered from the leaf surface. However, in the glufosinate-susceptible variety, only approximately 6 per cent of the applied dose had moved outside of the treated leaf by 72 hours after application, indicating that rapid herbicide activity limited further translocation. In the variety genetically modified to tolerate glufosinate, approximately 25 per cent of the applied dose had translocated to other parts of the plant. Further analysis (data not presented here) showed that the recovered ¹⁴C compounds remained as the originally applied D,L-glufosinate in the susceptible species, while approximately 50 per cent of the recovered ¹⁴C from the tolerant variety was present as the non-phytotoxic N-acetyl-glufosinate metabolite. It could therefore be expected that the majority of the remaining glufosinate would be present as the inactive D-glufosinate isomer (see following section on metabolism).

A similar study (Table 5.6B) in glufosinate-tolerant soybean also shows that substantial levels of glufosinate can translocate away from the treated leaf where the herbicide is not damaging the vascular pathways.

In this study, there was a suggestion that downwards translocation may be enhanced at the higher temperature regime.

These data suggests that, where a species can rapidly metabolise glufosinate, translocation of glufosinate can occur. However, where increased translocation does occur, it is likely to mainly be the inactive forms of glufosinate that are translocated.

A further study (Table 5.6C) demonstrated translocation of glufosinate can vary considerably across species. This aligns with the glufosinate susceptibility of these weeds and suggests that some species may be able to partially metabolise the active L-glufosinate. It should be noted that while the measured levels of glufosinate translocating outside of the leaf were quite high in this study, the author stressed that application rates used in this study were sub-lethal and were specifically selected to do minimal damage to the weeds.

Where translocation occurred, it was rapid, with little increase in translocated levels after 12 hours post-application. This is consistent with any available L-glufosinate beginning to damage vascular structures soon after application, and therefore reducing potential for further translocation.

Table 5.6B: Translocation of ¹⁴C-glufosinate in Liberty Link[®] soybean.

Hours after treatment	Temperature (°C)	% of absorbed glufosinate			
		Treated leaf	Leaf above treated leaf	Leaf below treated leaf	Roots
3	15	91 a	1 d	6 c	2 c
	25	86 ab	2 cd	8 c	4 bc
12	15	84 abc	3 cd	7 bc	6 bc
	25	80 c	5 ab	10 abc	6 b
24	15	80 bc	5 ab	12 ab	5 bc
	25	81 bc	4 abc	8 bc	6 b
48	15	78 bc	6 a	12 a	4 bc
	25	71 d	6 a	10 abc	14 a
LSD (0.05)		7	2	4	4

Within each column, treatments followed by same letter are not statistically different (P=0.05, LSD).

Source: Pline (1999)

Table 5.6C: Translocation of ¹⁴C-glufosinate across a range of weed species from a sub-lethal application rate of glufosinate.

Hours after treatment	Species	% of absorbed glufosinate			
		Treated leaf	Leaf above treated leaf	Leaf below treated leaf	Roots
12	<i>Asclepias syriaca</i>	53	15	26	6
	<i>Setaria faberi</i>	20	43	25	12
	<i>Chenopodium album</i>	56	30	13	2
	<i>Senna obtusifolia</i>	71	1	18	10
	<i>Solanum carolinense</i>	22	56	17	5
	LSD (0.05)	16	12	12	4
48	<i>Asclepias syriaca</i>	49	16	30	5
	<i>Setaria faberi</i>	19	49	18	14
	<i>Chenopodium album</i>	63	26	8	3
	<i>Senna obtusifolia</i>	80	2	11	7
	<i>Solanum carolinense</i>	19	59	15	6
	LSD (0.05)	15	11	11	4
72	<i>Asclepias syriaca</i>	53	16	24	7
	<i>Setaria faberi</i>	28	45	14	12
	<i>Chenopodium album</i>	58	28	11	3
	<i>Senna obtusifolia</i>	80	2	11	7
	<i>Solanum carolinense</i>	19	59	15	6
	LSD (0.05)	14	12	10	3

Source: Pline (1999)

Herbicide metabolism

Glufosinate is a racemic mixture of two stereoisomers, D- and L-glufosinate. L-glufosinate is the herbicidally active isomer, however L-glufosinate can be metabolised by adding an acetyl group onto the amino group, forming N-acetyl-L-glufosinate, which is a non-phytotoxic metabolite. D-glufosinate is not phytotoxic to plants and is not acetylated.

While glufosinate is generally considered non-selective in nature, differences in absorption, translocation and metabolism can give rise to differences in the weed spectrum controlled.

Glufosinate-tolerant crops

Globally, certain crops have been genetically engineered to tolerate over-the-top application of glufosinate herbicide. Tolerance to glufosinate is achieved by the introduction of either the 'bar' (bialaphos resistance) gene sourced from the *Streptomyces hygroscopicus* bacterium (often marketed as Liberty Link® varieties) or the 'pat' (phosphinothricin acetyl transferase) gene sourced from *Streptomyces viridochromogenes*. Both the bar and pat genes code for phosphinothricin acetyltransferase (PAT) enzyme production. The insertion of either of these genes allows the plant to increase the speed of conversion of glufosinate to the non-phytotoxic N-acetyl-L-glufosinate metabolite (Carbonari, et al., 2016).

At the time of writing (2023), cotton and canola are the only crops to have been commercially released in Australia with tolerance to glufosinate.

How does resistance occur?

No resistance has been detected to date in Australia.

Internationally, the first reported resistance was detected in 2009 from populations of crowsfoot grass in Malaysia (Jalaludin, et al., 2010).

Further research from Malaysia identified a field-selected population that confers a high level of resistance to glufosinate as well as glyphosate, paraquat and the ACCase herbicides haloxyfop, fluazifop and butoxydim, however was still susceptible to clethodim, sethoxydim and the ALS inhibitor herbicide imazapyr (Jalaludin, et al., 2015). While this study, and a further study (Jalaludin, et al., 2017) on the same population, did not identify the mechanism for glufosinate resistance, the research points to non-target site mechanism(s) being involved. Studies confirmed that the ACCase resistance was a result of a Trp-2027-Cys amino acid substitution, indicating that more than one resistance mechanism is present in this population.

Resistance has also been detected in perennial ryegrass populations in the USA (Avila-Garcia & Mallory-Smith, 2011; Avila-Garcia, et al., 2012) and New Zealand (Ghanizadeh, et al., 2015). In the USA population, an Asn-171-Asp target site substitution was identified.

Glufosinate-resistant Palmer amaranth was first detected in the USA for field collections taken in 2019 and 2020 (Priess, et. al. 2022).

Factors affecting efficacy

The hydrophilic nature of glufosinate makes penetration of the waxy leaf cuticle difficult. To achieve adequate penetration through the cuticle, glufosinate requires time in the liquid phase on the leaf surface. Once the spray has dried, there is limited further uptake through the leaf. Application under high humidity conditions (that keeps the spray deposit liquid for the longest period of time) enhances the uptake of glufosinate.

In a Canadian study, glufosinate leaf uptake and subsequent control of wild oats was significantly increased when plants were exposed to conditions of high humidity (99 per cent) for short periods (as little as 30 minutes) after spray application through a standard spray cabinet. Approximately 80 to 85 per cent reduction in dry weight occurred from the high humidity treatments at application rates of 200 to 400gai/ha, compared to plants maintained at 40 per cent RH throughout the experiment; approximately 20 to 45 per cent reduction in dry weight at application rates of 200 to 400gai/ha (Ramsey, et al., 2002).

Further, in this same study, applying radio-labelled ^{14}C glufosinate using the more common experimental technique of applying product as large discrete droplets (~1500 micron in this experiment) showed minimal impact on leaf uptake when plants were introduced to the high humidity treatment for 1 hour before and after application (approximately 40 to 45 per cent leaf uptake at 1, 2, 4 and 72 hours after application). However, applying the radio-labelled ^{14}C glufosinate as spray (400 to 500 micron droplet size in this study) while maintaining the other parameters, significantly reduced the leaf uptake (approximately 10 per cent uptake at 1 hour after application, rising to approximately 24 per cent at 72 hours after application). The authors propose that this demonstrates differences in drying time of droplets based on droplet volume, with the spray-applied treatment supporting the results of earlier studies and showing that maintaining droplets in the liquid phase for as long as possible after application is critical for glufosinate leaf penetration.

Table 5.6D: Calculated LD₅₀ values (gai/ha) for glufosinate against wild radish and Indian hedge mustard under cool (5/10°C) or warm (20/25°C) temperatures.

	Cool (5/10°C)	Warm (20/25°C)
Wild radish	1160	165
Indian hedge mustard	237	125

Source: Kumaratilake, et al. (2002)

Most Australian glufosinate labels recommend that application occurs under conditions of greater than 50 per cent relative humidity and under warm conditions (up to 33°C) with a minimum of 6-hour rainfast period. Spray volume should be in excess of 100L/ha, using a medium droplet spectrum, so as to achieve maximum leaf coverage. A medium droplet is typically classified as a droplet spectrum of 175–250 volume mean diameter (VMD).

While applying at 100L/ha with a medium droplet spectrum will achieve good leaf coverage (important for a contact herbicide such as glufosinate), a droplet of this size would be expected to undergo very rapid evaporation at the margin of the label conditions (up to 33°C and 50 per relative humidity).

The mode of action of glufosinate, including the resultant disruption of photosynthesis, means that speed of activity is enhanced under warm and sunny conditions.

A study on glyphosate-tolerant soybeans (Figure 5.6B) demonstrated that leaf uptake of ^{14}C glufosinate was enhanced at 25°C, compared to 15°C.

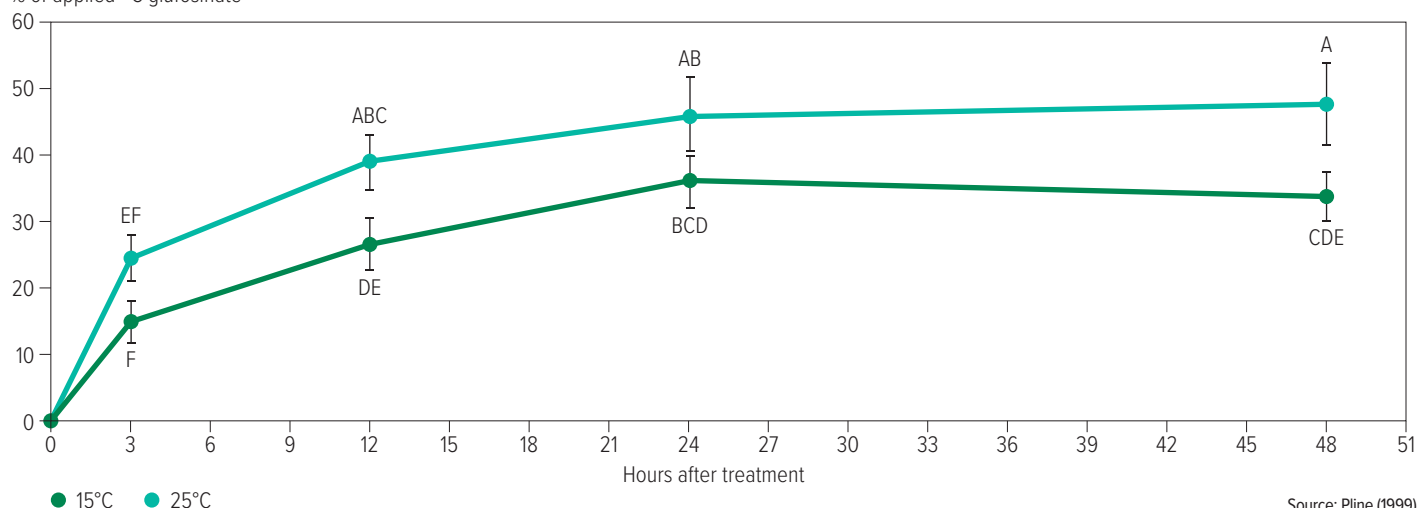
An Australian study (Kumaratilake, et al., 2002) targeted at winter growing brassica weeds demonstrated similar effects of temperature. In this study (Table 5.6D), wild radish and Indian hedge mustard were grown at 5/10°C or 20/25°C regimes before being exposed to application of glufosinate at up to 1200gai/ha. For wild radish, application at the higher temperature resulted in 92 per cent control from an application of 300gai/ha, with rates above 600gai/ha giving 100 per cent control; whereas at the cooler temperature regime the highest rate tested (1200gai/ha) only achieved 47 per cent control. Indian hedge mustard was considerably more susceptible to glufosinate, with 300gai/ha required to achieved 100 per cent control at the warmer regime while 600gai/ha was required to achieve the same level of control under cooler conditions, again showing a considerable advantage to applications in warmer conditions.

Commercial formulations usually contain adequate surfactants, however there may be weed control benefits in certain situations from the addition of a surfactant that increases spreading/coverage and/or reduces the rate of evaporation when targeting weeds with a leaf surface that is difficult to wet. However, when used over the top of glufosinate-tolerant crops, additional surfactant may also increase crop injury.

USA labels often recommend the addition of ammonium sulphate. Tank-mixing ammonium sulphate increased leaf absorption, and resulting efficacy, on barnyard grass, foxtail and velvet leaf (*Abutilon theophrasti*), but not on waterhemp (*Amaranthus rudis*) or fat hen (Maschhoff & Hart, 2000).

Figure 5.6B: Absorption of ^{14}C -glufosinate by Liberty Link® soybeans grown at 15° and 25°C.

% of applied ^{14}C glufosinate



Known herbicide interactions

International research shows that in a study on glyphosate-resistant Palmer amaranth (Botha, et al., 2014), glufosinate was shown to partially antagonise dicamba and tembotrione, but not 2,4-D. In a different study on giant ragweed (Ganie & Jhala, 2017), the addition of glufosinate to 2,4-D or dicamba resulted in additive control.

Due to the speed of activity of glufosinate and resulting destruction of vascular tissue used to translocate systemic herbicides, it is likely that glufosinate may negatively interact with systemic herbicides that require translocation to the target site. A study across a range of grass weeds, Johnson grass, broadleaf signalgrass (*Urochloa platyphylla*), fall panicum (*Panicum dichotomiflorum*), crowsfoot grass (*Eleusine indica*), large crabgrass (*Digitaria sanguinalis*) showed antagonism across a range of Group 1 grass-selective herbicides (clethodim, fluazifop, quizalofop, or sethoxydim), which was not able to be overcome by the addition of ammonium sulphate or increasing the rate of the grass herbicide (Gardner, et al., 2006). In this study, it was shown that the grass herbicide needed to be applied either a minimum of 3 days before or at least 5 days after the glufosinate. For barnyard grass, trials suggest that results with mixing clethodim are likely to be more antagonistic than with quizalofop (Eytcheson & Reynolds, 2015).

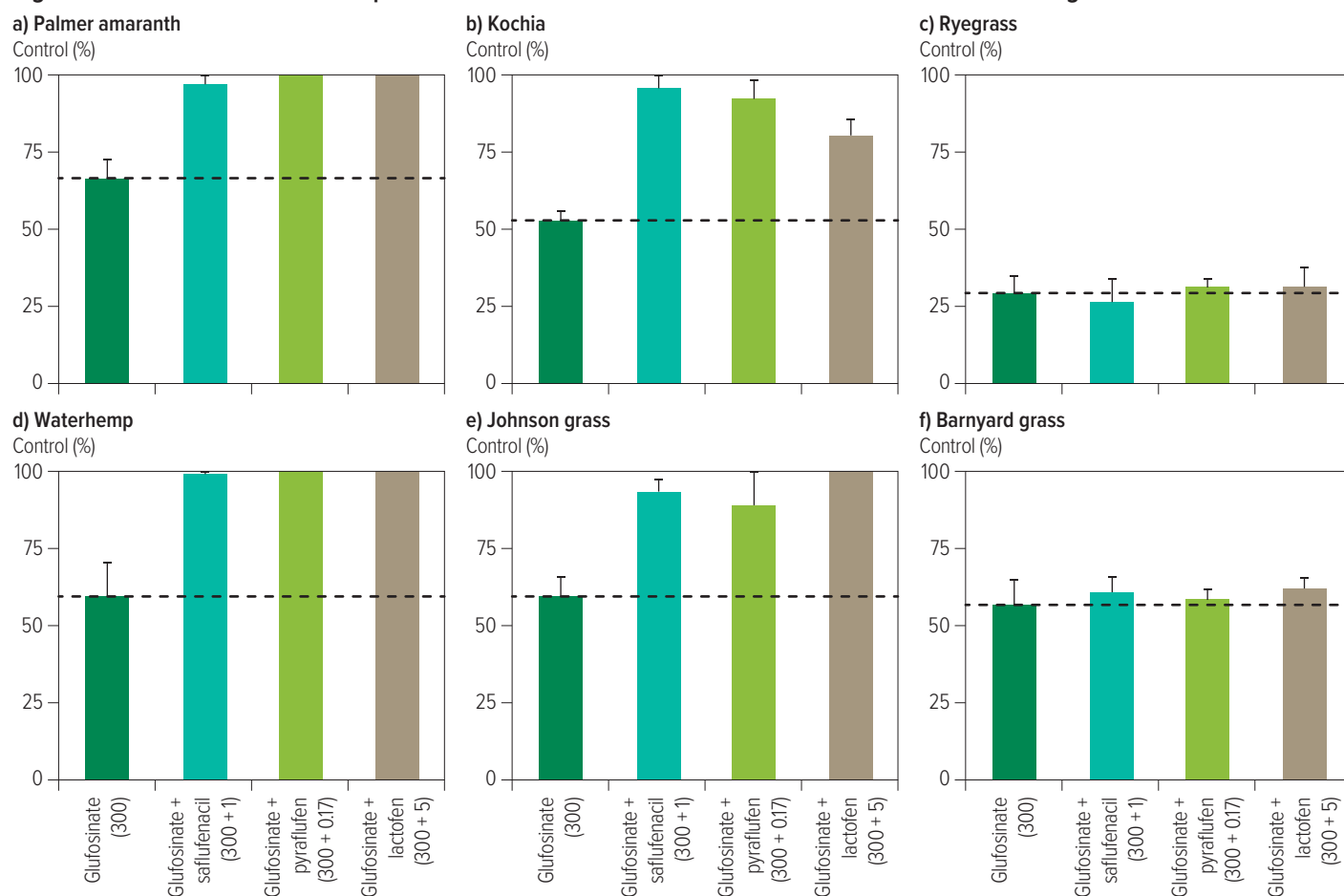
As glyphosate resistance increases in fallow situations, or genetically modified crops are introduced that can tolerate both glyphosate and glufosinate, it is likely that growers may want to consider mixing glyphosate and glufosinate. A study (Chuah, et al., 2008) on crowsfoot grass demonstrates strong antagonism between these two herbicides, so mixing should be avoided.

PPO inhibitors (Group 14) are relatively fast-acting (contact) herbicides that are frequently used in fallow situations. Most Group 14 herbicides recommend tank-mixing with a non-selective knockdown partner in fallow situations. Trials conducted in the USA (Jhala, et al., 2013) suggest that there is no/minimal impact on grass weed control from mixing glufosinate with saflufenacil (saflufenacil is predominantly a broadleaf herbicide with little grass activity), with some additive benefit on broadleaf species, where both herbicides have activity.

However, recent studies have demonstrated synergist activity on some weed species when mixing low rates of PPO herbicides with glufosinate (Figure 5.6C). Further, this study indicated that the mixture may assist glufosinate efficacy under conditions of lower relative humidity, for those species where there is synergistic activity.

Atrazine, a PSII inhibitor, works by disrupting photosynthesis by reducing the flow of electrons from PSII to PSI. As a result, there is a reduction in oxygen produced from photosystem I, and this oxygen is required for optimal glufosinate activity. It has been shown that the presence of a low rate of atrazine resulted in almost full loss of glufosinate activity on Palmer amaranth (Takano, et. al. 2020b).

Figure 5.6C: Control of six weed species where a low rate of PPO inhibitor herbicides are added to glufosinate.



Source: Takano (2020b)

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5.7 Group 12 – Inhibitors of carotenoid biosynthesis at phytoene desaturase (PDS)

Table 5.7: Group 12 herbicide mode of action sub-groups and active ingredients by sub-group.

Group 12 sub-groups	Main herbicides used in grain and broadacre field crops
Pyridazinones	norflurazon
Pyridinecarboxamides	diflufenican picolinafen

A limited number of Group 12 herbicides are used in broadacre situations in Australia. These are often referred to as 'bleachers' as susceptible weeds turn white/yellow/purple soon after application.

The pyridazinone herbicide norflurazon has been registered for long-term pre-emergent weed control in cotton (and some horticultural crops), predominantly targeting nutgrass, however it also has activity on a range of other broadleaf and grass weeds.

Herbicides from the pyridinecarboxamide class are used more commonly in the grains industry. Diflufenican (DFF) has been used extensively against certain broadleaf weeds in cereals and some pulses, with both foliar and soil activity. It is available as a stand-alone formulation (for example, Brodal®) or, more commonly sold for use in cereals as a co-formulation with MCPA ethyl hexyl ester (EHE), bromoxynil octanoate or as a 3-way mixture.

Picolinafen-based herbicides are also effective against some broadleaf weeds via foliar application, however the short soil persistence and low solubility means root uptake is often limited. Similar to diflufenican, picolinafen-based herbicides are available as stand-alone formulations (for example, Sniper®), or as a co-formulation with MCPA EHE or a 3-way mixture of picolinafen plus MCPA EHE plus bromoxynil octanoate.

Plant function targeted

Group 12 herbicides inhibit the production of carotenoids. Carotenoids have multiple roles within the photosynthesis pathway. The primary role is to protect chlorophyll from damage by active oxygen species (by quenching triplet chlorophyll and singlet oxygen and dissipating the excess energy as heat). Carotenoids also have a small role in harvesting light energy, although they typically absorb shorter (that is, higher energy) wavelengths than chlorophyll (Cobb & Reade, 2010).

By disrupting carotenoid production, a build-up of these active oxygen species will result in loss of chlorophyll and damage to cell walls (Weed Science Society of America, 2014). This leads to the typical bleaching symptoms observed following application of herbicides from this mode of action. Production of carotenoids is a multi-step process, so disruption at any step is likely to result in the bleaching effects that result from a loss of carotenoids.

Herbicides from the Group 12 mode of action disrupt the synthesis of carotenoids by targeting the phytoene desaturase (PDS) enzyme, which is responsible for one step in the pathway that converts phytoene to phytofluene.

Herbicide entry

Diflufenican and picolinafen are quite lipophilic, meaning the herbicide will rapidly enter the cuticle, hypocotyl or seed coat but may be absorbed within these lipophilic membranes, preventing further movement.

Haynes and Kirkwood (Haynes & Kirkwood, 1992) studied the uptake of diflufenican via soil, hydroponic, leaf and shoot uptake and showed that susceptible weeds accumulate significantly more diflufenican within the plant at seven days after application, compared to 'tolerant' species such as wheat and barley. When applied to the first leaf, as little as 0.3 to 1 per cent of the applied radioactive diflufenican was recovered from within the treated leaf of the tolerant wheat and barley, with 0.1 to 0.2 per cent of the applied dose being translocated out of the treated leaf. More than 90 per cent of the radioactive diflufenican was recovered from a leaf wash in either water or chloroform, with the chloroform wash responsible for most of the capture. This suggests the lipophilic herbicide was trapped in the waxy cuticle. In the susceptible weed species that were also tested, between 6 to 9 per cent of the applied dose appeared to have entered the leaf by seven days after application. Similar results were seen in the soil applications, with the authors proposing differential entry into the plant as the reason for differences in weed tolerance.

Herbicide translocation

Carotenoid synthesis occurs in the apical meristem (growing points). As translocation of diflufenican throughout the plant is limited, it is important to maximise herbicide disposition in close proximity to the apical meristem. This can be achieved by good spray coverage and targeting small weeds (Bayer CropScience, 2005). It is likely that the more exposed apical meristem region in broadleaf weeds is an easier application target for foliar applications than the meristematic region (crown) of grass weeds.

Where a pyridinecarboxamide has been co-formulated with fast-acting bromoxynil, significant structural cell damage can rapidly occur. This is likely to further retard translocation.

Herbicide metabolism

Metabolism of picolinafen is minimal with differential selectivity between species arising primarily from differences between herbicide uptake and translocation (Weed Science Society of America, 2014).

For diflufenican, differential uptake is also an important factor for selectivity. However, in tolerant crops such as cereals, rapid metabolism also occurs via nicotinamide and nicotinic acid (Weed Science Society of America, 2014).

How does resistance occur?

Despite relatively widespread use internationally, there have been relatively few reports of herbicide resistance to this mode of action.

The first recorded case of resistance was documented from Western Australia in 1998 where diflufenican-resistant wild radish populations were identified. Since then, multiple populations have been confirmed in both Western and South Australia. In 2011, an Indian hedge mustard resistant population was also identified from Victoria (Heap, 2017).

In addition to these Australian examples, a small number of other species have developed resistance to PDS inhibitor herbicides (Table 5.7A).

While target site resistance was confirmed in the aquatic species *Hydrilla*, it is proposed that this mutation is unlikely to occur in terrestrial species and that non-target site mechanisms are most likely the source of tolerance. More recently, a diflufenican-resistant Indian hedge mustard population has been identified with a Leu-526-Val target site substitution as the source of resistance, resulting in 140-fold tolerance (Dang, et al., 2018).

Factors affecting efficacy

Diflufenican and picolinafen are relatively lipophilic, aiding rapid entry through the cuticle when applied as a foliar application. Adding a spray oil will enhance speed of entry into the leaf and may increase crop injury, so most labels recommend not to add crop oils when applied in-crop.

As translocation is poor, excellent spray coverage is essential for foliar applications. Typically, a medium droplet is recommended, with water rates in excess of 50L/ha (70 to 100L/ha preferred). The use of a coarse droplet spectrum may be recommended on some co-formulations that include MCPA.

Crops that are stressed, particularly from frost, will have slower rates of metabolism and therefore will be more prone to diflufenican injury. Frost will generally reduce weed control. Bayer CropScience recommend delaying Tigrex® application for at least two days after cold events (minimum temperatures below 4°C or maximum daily temperatures below 10°C; Bayer CropScience, 2005).

Diflufenican has long persistence in the soil, however is quite insoluble and has moderate to high levels of binding to soil and organic matter. This results in herbicide typically remaining close to the soil surface and also limits root uptake from herbicide dissolved in soil water. As a result, weeds germinating from depth may be poorly controlled.

The lipophilic properties of diflufenican are likely to see diffusion into the seed coat should seeds on the soil surface come into direct contact with the herbicide when diflufenican is applied to the soil. During emergence, the germinating shoots will also need to pass through the herbicide barrier at the soil surface, at which time the lipophilic diflufenican can diffuse into the shoots of susceptible weeds.

Picolinafen soil persistence is relatively short. Coupled with tight binding and low solubility, uptake via the soil is limited and picolinafen will only provide limited, short-term, residual control.

Known herbicide interactions

Differential uptake of herbicide through the leaf is one of the primary vehicles for achieving crop selectivity. Therefore, it would be expected that the addition of crop oils, or other herbicides/surfactant combinations that increase the speed of herbicide penetration through the leaf, are likely to reduce crop safety margins.

Tank-mixing herbicides that are also active in disrupting the photosynthetic or carotenoid biosynthesis pathways in the chloroplasts (for example Groups 5, 6, 13, 27, 34) could also be expected to interact with Group 12 activity, and therefore exacerbate any crop injury.

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Table 5.7A: Known resistance to PDS inhibitor herbicides.

Species	Resistant to	Country	First year	Type
<i>Apera spica-venti</i>	diflufenican	Germany	2012	Non-target site?
<i>Hydrilla verticillata</i>	fluridone	USA	2002	Target site
<i>Poa annua</i>	norflurazon	USA	2000	Non-target site
<i>Raphanus raphanistrum</i>	diflufenican	Australia	1998	Not determined
<i>Sisymbrium orientale</i>	diflufenican	Australia	2001	Non-target site

Source: Adapted from Dayan, et al. (2014)

5.8 Group 14 – Protoporphyrinogen oxidase (PPO) inhibitors

Table 5.8: Group 14 herbicide mode of action sub-groups and active ingredients by sub-group.

Group 14 sub-groups	Main herbicides used in grain and broadacre field crops
Diphenylethers	acifluorfen fomesafen oxyfluorfen
Triazolinones	carfentrazone
Phenylpyrazoles	pyraflufen
N-phenyl-imides	betafenacil flumioxazin saflufenacil tiafenacil trifludimoxazin

A range of different chemical sub-groups of Group 14 are registered for use in Australia. The table above lists the primary herbicides used in various broadacre situations.

Some of these herbicides have been registered at relatively low use rates, with use patterns designed to be mixed with knockdown herbicides, primarily to increase the speed of visual symptoms and/or target specific weeds where this mode of action is particularly effective for example, marshmallow.

In more recent years, registrations and products have come to market for stand-alone use, typically at higher use rates.

Some herbicides within this mode of action can also provide residual control, while others are strongly bound to soil and organic matter and are unavailable for root uptake. Oxyfluorfen is used at substantially higher application rates for residual weed control in selected horticultural situations, while certain products have recently been registered for residual control in non-crop areas at very high application rates and for pre-emergent residual use in some crops (see label for details).

Plant function targeted

Group 14 herbicides inhibit the enzyme protoporphyrinogen oxidase (PPO) at a critical step in the pathway of heme and chlorophyll biosynthesis (Figure 5.8A). The resultant build-up of protoporphyrinogen leaks into the cytoplasm where oxidation to protoporphyrin is unregulated. In the presence of light, triple state protoporphyrin and singlet oxygen are formed, which can extract hydrogen from unsaturated lipids and proteins that are attacked by this chain reaction. Leaky cell membranes, along with loss of chlorophyll and carotenoids cause cells to rapidly dry out and disintegrate (Weed Science Society of America, 2014).

Activity is usually fairly rapid, with symptoms observable within a couple of days of application.

Herbicide entry

Typically, PPO inhibitors are moderately to relatively lipophilic in nature, which aids in relatively fast entry through the cuticle for most herbicides. For some, weed control may be reduced under low humidity conditions, where the spray deposit dries too rapidly, limiting cuticle penetration.

The addition of a lipophilic adjuvant (for example, methylated seed oils or crop oil concentrates) is likely to enhance uptake into the leaf (Table 5.8A). Addition of oil-based adjuvants may increase efficacy on weeds, however may also increase crop injury where these herbicides are applied post-emergent to the crop (for example, acifluorfen use in pulses, carfentrazone in cereals).

Herbicide translocation

Once entering the leaf, translocation is generally poor for most PPO herbicides and they tend to be considered contact herbicides. Targeting small weeds and achieving excellent spray coverage are very important for good weed control.

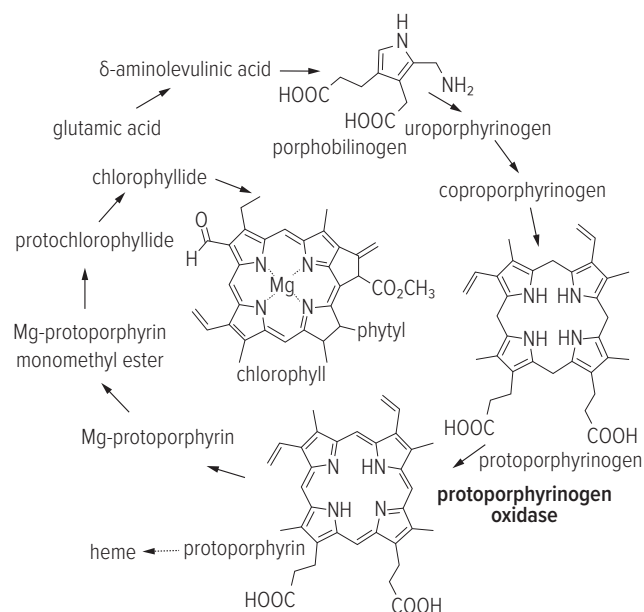
Foliar-applied saflufenacil appears to have more translocation than other PPO inhibitors. Translocation of saflufenacil occurs primarily in the xylem, although some phloem mobility also occurs (Weed Science Society of America, 2014).

Herbicide metabolism

Species tolerance to the PPO herbicides appears to be related to differential speed of metabolism between tolerant and susceptible species. For example, soybeans can detoxify acifluorfen via a reduction of the p-nitro substitution, de-esterification and conjugation. Cytochrome P450 monooxygenases have been shown to be involved in the metabolism of carfentrazone (Dayan, et al., 2014).

Metabolism of oxyfluorfen is limited and therefore selectivity is more limited (Weed Science Society of America, 2014).

Figure 5.8A: Biosynthesis of chlorophyll in the chloroplast, showing the protoporphyrinogen oxidase (PPO) that is inhibited in the presence of Group 14 herbicides.



Source: Dayan, et al. (2014)

How does resistance occur?

Resistance to PPO herbicides has not been recorded to date in Australia.

Internationally, resistance was relatively slow to be identified, however the extent of resistance and the number of species involved and countries impacted is rapidly gathering pace in recent years (Heap, 2017).

Interestingly, where the resistance mechanism has been studied in selected tall waterhemp (*Amaranthus tuberculatus*) populations, the resistance to the diphenylether herbicide lactofen (a PPO inhibitor not used in Australia) appears to arise from a codon/ amino acid deletion of glycine at the 210 location as opposed to the typical substitution of amino acids in most other target site resistance mechanisms (Powles & Yu, 2010).

Target site resistance has also been reported in annual ragweed (*Ambrosia artemisiifolia*) in the USA where a more traditional arginine for leucine substitution Arg-98-Leu was reported (Dayan, et al., 2014).

As crop and weed species selectivity is primarily delivered by enhanced metabolism, non-target site resistance may be possible (Dayan, et al., 2014).

Factors affecting efficacy

As translocation of PPO herbicides is generally restricted, these herbicides tend to be more effective on broadleaf weeds where exposed leaves and the apical meristem are easier to contact with the spray application. The lack of phloem mobility, and resultant inability to translocate herbicide down to the crown of grass weeds, probably plays a large role in the reduced efficacy against grass species when applied as a post-emergent application.

The contact nature of PPO herbicides also results in significantly better efficacy from foliar applications when targeting small seedling and small rosette broadleaf weeds, generally with significant reduction in performance when broadleaf weeds start to elongate. High levels of spray coverage are important, typically involving water rates in excess of 80L/ha when using coarse or larger spray droplets as required by many product labels.

Certain PPO herbicides have soil activity, particularly fomesafen and oxyfluorfen. Trifludimoxazin, flumioxazin and saflufenacil have shorter residual activity, but can provide extended soil activity at higher application rates.

Known herbicide interactions

Often PPO herbicides are mixed with glyphosate to improve the speed of activity over glyphosate alone when used in fallow situations. However, the speed of activity of the PPO herbicide and/or the recommendation to add a lipophilic surfactant have the potential to impact glyphosate uptake and translocation.

An intensive study conducted in the USA looked at various combinations of saflufenacil and glyphosate +/- crop oil concentrate on glyphosate-susceptible and glyphosate resistant Canadian fleabane (*Conyza canadensis*). The study concluded that saflufenacil reduced the translocation of glyphosate by at least 6% on both susceptible and resistant populations, however this may not be noticeable in the paddock (Eubank, et al., 2013). This study also demonstrated that crop oil concentrates can reduce translocation of glyphosate, which may be of concern when tank-mixing PPO herbicides.

Table 5.8A: Control of 10–15cm Canadian fleabane (*Conyza canadensis*) following the application of saflufenacil (25gai/ha).

Treatment	Rate (% v/v)	Control (%)	
		14 DAT	28 DAT
No adjuvant		78 c	71 c
Non-ionic surfactant	0.25%	78 c	72 c
	0.5%	79 c	74 c
Crop oil concentrate	1%	85 b	74 c
	2%	86 b	81 b
Methylated seed oil	1%	91 a	83 ab
	2%	93 a	89 a

Means followed by the same letter within each evaluation period are not significantly different at $P < 0.05$

v/v – volume/volume

DAT – days after treatment

Source: Eubank, et al. (2013)

The tank-mixing of other broadleaf herbicides may provide synergistic or antagonistic effects. A study that looked at mixtures of saflufenacil with the Group 6 herbicide bentazone or the Group 4 herbicide 2,4-D amine +/- non-ionic surfactant showed that generally the addition of 2,4-D increased the level of phytotoxicity, whereas mixing bentazone significantly reduced control compared to saflufenacil alone, across two weed species Flixweed (*Descurainia sophia*) and Dead-nettle (*Lamium amplexicaule*) and winter wheat (Frihauf, 2009). (Note: saflufenacil is highly damaging to wheat and is not registered for early post-emergent application).

When carfentrazone is used in winter cereals, it is typically recommended to be applied with MCPA amine to broaden the spectrum of activity. Mixtures with ester formulations and surfactants should be avoided as they will increase leaf penetration and may increase crop injury. Mixtures with Group 1 selective grass herbicides should be avoided as grass weed control will be reduced and the oil will impact carfentrazone safety. Where grass herbicides are required, apply at least 10 days prior to the application of carfentrazone.

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5.9 Group 22 – Photosystem I inhibitors

Table 5.9: Group 22 herbicide mode of action sub-groups and active ingredients by sub-group.

Group 22 sub-groups	Main herbicides used in grain and broadacre field crops
Bipyridyl	diquat paraquat

Bipyridyl herbicides used in Australia consist of products containing either paraquat or diquat, or a combination of these herbicides. Both herbicides have activity on a wide range of grass and broadleaf weeds, however typically paraquat is stronger on grass species and diquat stronger on some broadleaf species.

Plant function targeted

Bipyridyl herbicides target the photosystem I pathway responsible for energy production within plants. Photosystem I is the second part of the photosynthetic pathway, whereby high-energy electrons created in photosystem II are accepted by iron-containing electron carriers and ultimately converted to energy, after further light activation. See Figure 5.4A for a summary of photosynthesis.

In the presence of the positively charged bipyridyl herbicides, the electrons produced from photosystem II are diverted and combined with an oxygen molecule to form superoxide radicals. These superoxide radicals react in the presence of superoxide dismutase to form hydrogen peroxides and then further react to form highly damaging hydroxyl radicals that quickly disrupt the cell function and cause membrane leakage (Weed Science Society of America, 2014). The bipyridyl herbicide acts as a catalyst. After the bipyridyl herbicide passes on an electron, it reverts to its natural positively charged state and is then free to again accept another electron, so the herbicide is not broken down in the process (Hall, et al., 1999).

For further information on how paraquat works
[youtube.com/watch?v=VKJdRDiBrjg](https://www.youtube.com/watch?v=VKJdRDiBrjg)

Herbicide entry

Bipyridyl herbicides are positively charged, polar, hydrophilic herbicides. As such, they rapidly move across the negatively charged leaf surface and are typically rainfast within minutes of application. After penetration of the cuticle they move quickly through the cutin and then the pectin in the leaf cuticle, to the cell membrane.

To move across the cell membrane, bipyridyls require an active transport mechanism, utilising the carrier that otherwise moves putrescine across the cell membrane.

Once in the cell and in the presence of light, bipyridyl herbicides are quick to start working, with herbicide symptoms often visible within hours of application under high light intensity.

Bipyridyl herbicides bind extremely tightly to soil and organic matter and are therefore not available in the soil water for root uptake.

Herbicide translocation

Generally, once exposed to sunlight, the activity of bipyridyl herbicides results in leaking cell membranes within hours of application. When applied during high light intensity, this rapid action limits further herbicide translocation within the plant by trapping most of the herbicide within the dead cells. Where herbicide movement does occur, this is almost exclusively in the xylem (Hall, et al., 1999). Very little paraquat moves within the phloem. Due to their poor phloem translocation, bipyridyl herbicides are considered contact herbicides.

Spraying in the early evening, or under conditions of low light intensity at and after spraying, delays the onset of cell damage until light activation the following day. Under such conditions, some level of apoplastic translocation is possible before light activation.

Following light activation, the free radicals created by paraquat within the cell cause the cell walls to rupture, releasing water from the cell. This free water, also containing highly soluble paraquat, has a zero water potential which will then flow towards leaf cells above and below the damaged area, which will have negative water potential (Preston, et al., 2005), allowing further translocation to unaffected cells.

Water potential is a measurement of the potential energy of a solution relative to pure water.

Within plants, solutions with zero or positive water potential will move towards areas of negative water potential via osmosis.

Paraquat will then enter these cells and continue the process. Ultimately, if the area of damage was initially large, sufficient quantity of paraquat can reach the meristematic regions of the weed.

While applying paraquat in the early evening can allow more translocation before herbicide activation and create a larger area of initial activity, adequate control of weeds may still be achieved from daytime application where excellent foliar spray coverage of all green plant material is achieved and herbicide rates are robust.

Paraquat is extremely tightly bound to the soil (paraquat K_{oc} 1,000,000; paraquat dichloride K_{oc} 100,000 (Lewis, et al., 2016)). Almost all herbicide reaching the soil is unavailable for plant uptake and microbial degradation. Due to this extremely strong soil binding, paraquat is extremely persistent in the soil (measured in years) but it is not biologically available. Any small amount of unbound paraquat in the soil water is rapidly degraded by soil microorganisms to carbon dioxide, water and ammonia (Syngenta, 2004).

Herbicide metabolism

Bipyridyl herbicides are not metabolised within the plant.

While foliar-applied herbicide quickly enters the leaf, any herbicide that is not absorbed remains on the leaf and will be broken down by UV light (Weed Science Society of America, 2014).

Table 5.9A: Species that have developed resistance to paraquat in Australia.

Species	Common Name	Year confirmed	State	Crop	Resistance to other modes-of-action/ herbicides
<i>Hordeum glaucum</i>	Northern barley grass	1983	Victoria	lucerne	diquat (L)
<i>Arctotheca calendula</i>	Capeweed	1984	Victoria	lucerne	diquat (L)
<i>Hordeum leporinum</i>	Barley grass	1988	Victoria	lucerne	diquat (L)
<i>Vulpia bromoides</i>	Silver grass	1990	Victoria	lucerne	diquat (L)
<i>Mitracarpus hirtus</i>	Small square weed	2007	Queensland	mangoes	diquat (L)
<i>Lolium rigidum</i>	Annual ryegrass	2010	South Australia	pasture seed	A / M – 2 populations
<i>Gamochaeta pensylvanica</i>	Cudweed	2015	Queensland	tomatoes, sugar cane	
<i>Solanum nigrum</i>	Blackberry nightshade	2015	Queensland	tomatoes, sugar cane	
<i>Eleusine indica</i>	Crowsfoot grass	2015	Queensland	tomatoes, sugar cane	
<i>Conyza bonariensis</i>	Flaxleaf fleabane	2016	NSW	grape vines	

Source: Preston (2016)

Table 5.9B: The Spray.Seed® Herbicide label provides advice of recommended water rates.

Winter rainfall areas	Boomspray volumes	Summer rainfall areas
Plant height up to 2cm	50–100L/ha	Small plants (2–5 leaf) and well separated
Plant height 2–5cm	100–150L/ha	5 leaf to early tillering/rosettes; 30–50% ground cover
Plant height 6–10cm	150–200L/ha	Advanced growth; dense and/or tall weed growth
Plant height above 10cm	Use a split application at 150L/ha to remove excessive growth	Very dense and tall weed growth

Applying bipyridyl herbicides at night

Applying bipyridyl herbicides in the early evening (just after darkness) will allow some time for paraquat to translocate throughout the leaf, before light activation the following day and the subsequent rapid cell wall collapse that follows.

While an extended period of darkness following paraquat application may optimise translocation, acceptable results can still be achieved from daytime application where complete spray coverage of the foliage has been achieved and robust application rates applied.

Comparative day versus night trials sometimes show an improvement in control/brownout from night applications. However, applications made at night are typically applied at lower temperatures and/or more favourable Delta T conditions, allowing for better droplet survival. Medium droplets, which are often recommended for bipyridyl herbicides, may be subject to more rapid evaporation under daytime application during summer under poor/marginal Delta T conditions. Therefore, less spray may reach the leaf target under daytime applications, which may lead to poorer performance. Enhanced droplet survival and better spray coverage on the leaf is often the reason for marginal improvement from night applications.

Night applications are more likely to encounter surface temperature inversion conditions with high potential for off-target drift. Do not apply if there are hazardous surface temperature inversion conditions present at the application site during the time of application. When application occurs in an area not covered

by recognised inversion monitoring weather stations, all surface temperature inversion conditions are regarded as hazardous. A surface temperature inversion is likely to be present if:

- mist, fog, dew or frost have occurred;
- smoke or dust hangs in the air and moves sideways, just above the ground surface;
- cumulus clouds that have built up during the day collapse towards evening;
- wind speed is constantly less than 11km/h in the evening and overnight;
- cool off-slope breezes develop during the evening and overnight;
- distant sounds become clearer and easier to hear; and
- aromas become more distinct during the evening than during the day.

Application rate and achieving excellent spray coverage of all green plant material is the most important factor in bipyridyl performance and should be the primary focus of applicators. Increasing water rate, reducing application speed, reducing boom height and applying under climatic conditions that favour excellent leaf coverage will be more important for maximising herbicide efficacy than simply “applying at night”.

How does resistance occur?

Paraquat resistance in Australia has been relatively slow to develop in field populations and the mechanism(s) of resistance have been relatively less studied than for other modes of action.

The primary resistance mechanism conferring paraquat resistance is believed to be sequestration of the herbicide into the vacuole within the cell, where it is inactive (Yu, et al., 2010; Brunharo & Hanson, 2017).

Field-selected paraquat resistance was first confirmed in Australia in 1983 from a barley grass population subjected to continual 'winter pasture cleaning' in lucerne. Since then, a further nine species have been identified in a range of crop situations (Table 5.9A).

Factors affecting efficacy

Bipyridyl herbicides tightly bind to soil colloids. Dirty water should not be used for application of these herbicides. Saline water, hard water, or water with high or low pH does not affect the performance of bipyridyl herbicides.

As bipyridyl herbicides have limited phloem translocation outside of the treated leaf, excellent herbicide coverage is essential. Poor spray coverage will result in poor performance. To optimise coverage while also managing herbicide drift, a medium or medium-coarse droplet quality is typically recommended. To ensure full coverage, application with these size droplets will require higher water rates.

Without translocation in the phloem to the roots, bipyridyl herbicides are often considered to 'burn down' above-ground green plant material, with larger weeds subsequently reshooting from root or crown reserves. For this reason, these herbicides are most effective on small seedlings that have little below-ground reserves and cannot survive after the above-ground material has been desiccated. For grass weed, thorough coverage of the crown will lead to improved mortality. This can be very hard to achieve with a single application on large grasses, as the crown is often well protected by foliage.

In addition to spray volume and weed size, an adjuvant that assists the spray deposit spread across the leaf is important. Most paraquat formulations contain an in-built non-ionic surfactant that is typically in concentrations suitable for most broadacre spraying situations. However, if herbicide rates are low and carrier volumes high, then an additional non-ionic surfactant may be required. Some high load formulations recently released into the market do not contain a non-ionic surfactant in their formulation. Follow label advice for adjuvant recommendations.

Higher humidity spraying conditions reduce the rate of droplet evaporation and increase the percentage of herbicide entering the leaf.

Known herbicide interactions

Group 5/6 herbicides (particularly diuron and atrazine) are often tank-mixed with paraquat. Group 5/6 herbicides work within the photosystem II pathway, blocking the movement of high-energy electrons from photosystem II to photosystem I. Paraquat requires these high-energy electrons for its biological activity. It would be expected that if the supply of high-energy electrons are substantially reduced from the activity of the Group 5/6 herbicide, then the activity of paraquat would be reduced. This is because there are insufficient free electrons available to fully facilitate the paraquat reaction. Reducing the speed of activity of paraquat, by starving it of electrons coming from photosystem II, has been suggested to allow for some additional paraquat translocation within the leaf. Herbicides from Group 5, 6 and 27 are likely to reduce electron flow from photosystem II and may sometimes improve control from paraquat applications. A study investigating mixtures of paraquat plus isoxaflutole suggests synergistic activity on feathertop Rhodes grass (*Chloris virgata*) (Chauhan et. al. 2021).

In practice, if these herbicides are tank-mixed, the rapid speed of activity of the paraquat may have already caused cell rupture before the partner herbicide has been able to reduce supply of electrons – especially under conditions of high light intensity at herbicide application.

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5.10 Group 27 – 4-hydroxyphenyl-pyruvate dioxygenase (HPPD) inhibitors

Table 5.10: Group 27 herbicide mode of action sub-groups and active ingredients by sub-group.

Group 27 sub-groups	Main herbicides used in grain and broadacre field crops
Isoxazoles	isoxaflutole (primarily soil uptake)
Pyrazoles	benzofenap pyrasulfotole topramezone
Triketones	bicyclopyrone mesotrione (only sold as a pre-emergent in Australia)

Some HPPD inhibitors have substantial soil activity. In Australia, isoxaflutole and mesotrione are only used as pre-emergent herbicides.

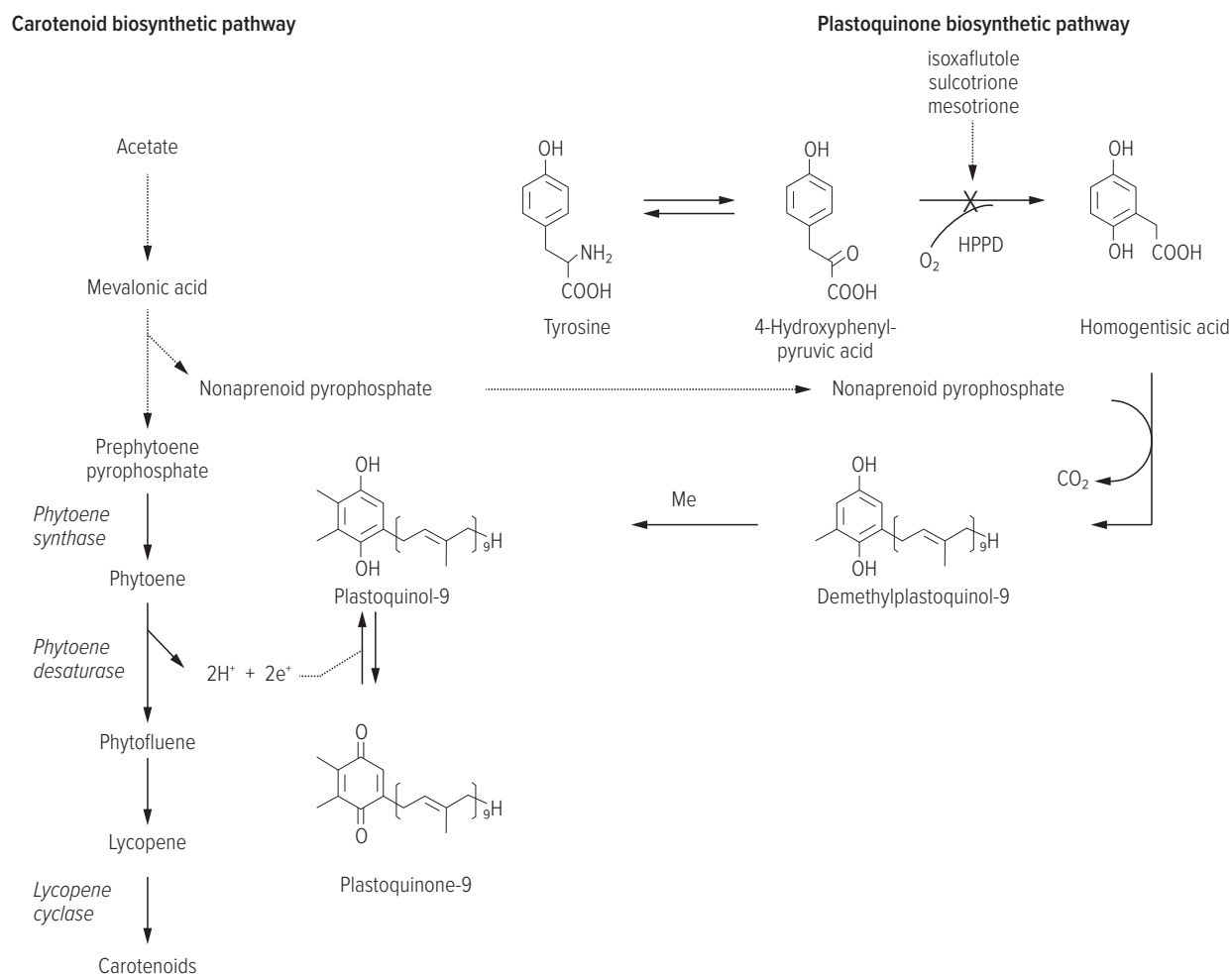
Most HPPD inhibitors are typically more efficacious on broadleaf weeds, where metabolism is slower. Where used post-emergent in cereals, it is typical to apply Group 27 herbicides in combination with either bromoxynil (Group 6) or MCPA (Group 4). This may be either as an in-can formulated product, or as a tank-mix.

Synergistic activity exists on broadleaf weeds when mixed with Group 5/6 herbicides. This may allow for lower rates of the Group 27 herbicide to be used post-emergent in cereals, which may provide additional crop tolerance.

Plant function targeted

Group 27 herbicides inhibit the enzyme 4-hydroxyphenyl-pyruvate dioxygenase (HPPD inhibitors), which is a critical enzyme in plastoquinone synthesis (Figure 5.10A). Plastoquinones are vital cofactors for phytoene desaturase (PDS), leading to the production of carotenoids (Jablonkai, 2011). Without carotenoids to quench triplet chlorophyll and prevent the formation of destructive singlet oxygen, plants are not protected from photo-oxidation and therefore the typical bleaching symptoms are expressed following application of Group 27 herbicides.

Figure 5.10A: Carotenoid and plastoquinone synthesis pathway.



Source: Jablonkai (2011)

Herbicide entry

HPPD inhibitors can enter the plant via foliar application or from the soil via the roots. Entry of individual HPPD inhibitors is often stronger by one pathway based on individual herbicide properties.

Isoxaflutole is primarily taken up through the soil, however it does have some foliar uptake. Isoxaflutole applied to the soil is relatively insoluble, so stays near the soil surface, however it is somewhat lipophilic so will enter seeds through the seed coat where there is direct contact (Armel, 2002). Following rainfall, some isoxaflutole is rapidly converted to diketonitrile, which is more soluble and mobile and therefore will move further down the soil profile and can be taken up by plant roots.

Foliar-applied HPPD inhibitors (products containing topramezone, pyrasulfotole or bicyclopyrone) primarily enter the plant through the leaf cuticle. They are somewhat hydrophilic and are likely to respond to a high quality surfactant/oil type adjuvant that will enhance uptake through the leaf. When co-formulated with a lipophilic ester partner (that is, bromoxynil or MCPA ester), it is likely that the ester co-partner will penetrate the leaf much faster than the HPPD inhibitor.

Data available for mesotrione shows high levels of leaf uptake within 24 hours of application (Figure 5.10B). Note: mesotrione is not registered for post-emergent weed control in Australia as it is too damaging to be applied post-emergent in cereals.

Herbicide translocation

Once inside the plant, the diketonitrile derivative of isoxaflutole is mobile via symplastic and apoplastic movement to the leaves and meristematic tissue. Any movement from young to mature leaves appears to be symplastically driven (Armel, 2002).

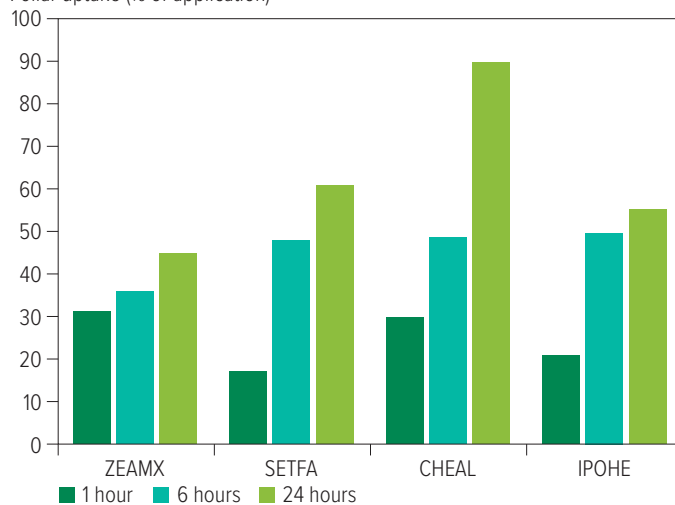
Limited data on the translocation of foliar-applied HPPD inhibitors exists. One study that measured radio-labelled mesotrione showed both acropetal and basipetal movement (Wichert, et al., 1999). In tolerant maize, only 14% of radioactive material moved outside the treated leaf 7 days after application and none of this was as the parent compound, indicating extensive metabolism had occurred. In the susceptible species fat hen, 48% of the radioactivity was measured outside of the treated leaf 7 days after application, and 42% of this material was still present as mesotrione, suggesting a much slower rate of metabolism.

In another study, application of radio-labelled mesotrione to susceptible and HPPD-resistant populations of Palmer amaranth (*Amaranthus palmeri*) showed approximately 30 to 40% of the applied dose moving outside of the treated leaf by 72 hours after application (Nakka, et al., 2017).

It appears that, in tolerant species, translocation will be significant although the herbicide is rapidly metabolised to non-toxic breakdown compounds. For sensitive species, there is theoretically the potential for translocation, however the rapid speed of herbicide injury is likely to reduce the physical opportunity to translocate. Where HPPD inhibitors are mixed with a contact herbicide such as bromoxynil, the speed of herbicide damage will be further enhanced.

Figure 5.10B: Uptake of radio-labelled mesotrione into a single leaf of maize (*Zea mays*) ZEAMX, foxtail (*Setaria faberi*) SETFA, fat hen (*Chenopodium album*) CHEAL and morning glory (*Ipomea hederacea*) IPOHE.

Foliar uptake (% of application)



Source: Adapted from Mitchell, et al. (2001)

Herbicide metabolism

Isoxaflutole is a pro-herbicide and requires metabolism by opening of the isoxazole ring, to form diketonitrile, which is herbicidally active. The herbicide may have entered the plant as isoxaflutole or the herbicidally active diketonitrile derivative. Once inside the plant, conversion to diketonitrile is rapid. For isoxaflutole, differential weed control is a result of the speed of subsequent metabolism to a benzoic acid derivative, with tolerant species being able to rapidly degrade diketonitrile (Pallett, et al., 1998).

For foliar-applied HPPD inhibitors, selectivity arises from a combination of reduced leaf uptake, increased speed of metabolism and a less sensitive target site in more tolerant species (Hausman, 2012). Tolerance differs between species and HPPD inhibitor herbicide.

Pyrasulfotole is metabolised in wheat by one of 2 processes. Pyrasulfotole can undergo demethylation to form pyrasulfotole-desmethyl. This is then glucosylated or conjugated with glutathione leading to pyrasulfotole-sulfinyl-lactate. The second pathway for metabolism results in cleavage of the pyrazole moiety, leaving the pyrasulfotole-benzoic acid and multiple polar constituents (APVMA, 2007).

Where crop selectivity is marginal, herbicide safeners may increase the crop tolerance of post-emergent applications. In the USA, the use of the safener cyprosulfamide is included in some isoxaflutole formulations applied post-emergent to maize (Hausman, 2012). In Australia, the pyrasulfotole-based herbicides Velocity® (pyrasulfotole + bromoxynil) and Precept® (pyrasulfotole + MCPA) contain the safener mefenpyr-diethyl, while Talinor® (bicyclopyrone + bromoxynil) and Frequency® (topramezone) are formulated with the safener cloquintocet-mexyl when these herbicides are used for post-emergent application in cereals.

How does resistance occur?

In North America, with a longer history of use, certain *Amaranthus* populations have developed resistance (first confirmed in 2009). While resistance studies are limited, it appears that enhanced metabolism by cytochrome P450s may be the source of increased speed of degradation in resistant *Amaranthus* populations collected from maize crops (Ma, et al., 2013; Kaundun, et al., 2017).

The first Australian field-selected populations of wild radish (*Raphanus raphanistrum*) resistant to Group 27 herbicides were identified in 2020. These populations were shown to be 4 to 11-fold resistant to pyrasulfotole + bromoxynil, topramezone and mesotrione applied post-emergent, however were still susceptible to mesotrione when applied as a pre-emergent application (Busi, et al., 2022).

Factors affecting efficacy

The recommended adjuvant for foliar applications is generally a methylated seed oil (MSO) or oil concentrate (COC), which will enhance leaf entry.

In addition:

- The Precept® label supports the use of ammonium sulphate instead of a COC if desired.
- The Velocity® label does not support the use of a non-ionic surfactant (except in limited situations) or ammonium sulphate.
- The Talinor® label recommends against using non-ionic surfactants or soya-lipid based adjuvants due to reduced efficacy on the weeds and not to use ammonium sulphate (AMS) or liquid urea ammonium nitrate (UAN) as increased crop damage is likely.

When mixed with bromoxynil, speed of herbicidal damage will be fast, limiting any significant translocation of the HPPD inhibitor. For this reason, herbicides such as Velocity® and Talinor® should be treated as contact herbicides. To maximise weed control:

- Do not apply by air.
- Target small weeds, in low densities, where excellent coverage can be achieved.
- Apply early in the crop to reduce shading from the crop canopy.
- Ensure application set-up maximises coverage. A medium droplet spectrum is generally recommended (consult product labels), utilising water rates of 50 to 150L/ha (Velocity®) or 75 to 150L/ha (Talinor®) or 80 to 150L/ha (Frequency®).

- Consider temperature, light and environmental stress.

- Bayer CropScience recommends applying Velocity® under warm temperatures with at least 1 hour of sunlight left in the day to maximise speed of activity. This will maximise uptake and movement to the chloroplasts. Velocity® should not be applied to weeds that have recently been affected by frost as this will decrease herbicide uptake.
- Syngenta recommends avoiding applying Talinor® under high light intensities in northern grain regions, as increased leaf uptake has led to higher levels of phytotoxicity to wheat in some situations. Syngenta recommends that applications should occur in the morning, under good soil moisture conditions, cool temperatures leading up to application (but noting that frost may reduce weed control) and to avoid warm conditions favouring rapid growth for the 7 days following application.
- BASF advise that when using Frequency®, transient bleaching of the crop may occur, particularly under cold or frosty conditions and can be exacerbated in northern areas where frost/cold starts are followed by warm bright sunlight days.

Known herbicide interactions

Synergistic activity has been widely reported between HPPD inhibitors and PSII inhibitors (Walsh, et al., 2012; Abendroth, et al., 2006; Armel, et al., 2005; Bollman, et al., 2006; Hugie, et al., 2008). It is believed that the modes of action of these two herbicides are complementary. HPPD inhibitors block the production of tocopherols and plastoquinone while PSII inhibitors prevent plastoquinone from binding on the D1 protein.

In Australia, some HPPD herbicides are formulated in a mixture with bromoxynil (PSII inhibitor).

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6. Herbicide resistance

The advent of post-emergent, in-crop, selective herbicides in the 1970s provided farm managers with highly effective tools to remove most of the competitive weed species of cropping. Prior to the availability of these herbicides, weed control was typically achieved by pre-plant cultivation, pre-emergent herbicides, crop competition and rotation. With the arrival of post-emergent herbicides, the use of cultivation and pre-emergent herbicides declined significantly.

By 1982, the first resistance to post-emergent grass-selective herbicides in Australia was confirmed in annual ryegrass. Since then, herbicide resistance has continued to increase rapidly.

6.1 Extent of herbicide resistance

Herbicide resistance in Australian farming systems is causing a rapid and major rethink in the way herbicides and non-herbicide weed management tactics are used. There are now tens of thousands of field populations of grass and broadleaf weeds with confirmed resistance to one or more modes of action.

For grass weeds, resistance is known to occur to most post-emergent grass herbicide modes of action available in Australia.

Table 6A: Confirmed presence of herbicide resistance in key grass weeds of Australian cropping (as at February 2018).

Weed	Mode of action groups											
	1		2			5	3	23	15	22	9	34
	fop	dim	SU	IMI	TPS	Triazine	DNA					
Annual ryegrass												
Wild oats												
Brome grass												
Phalaris												
Barley grass												
Barnyard grass												
Liverseed grass												
Feathertop Rhodes grass												
Windmill grass												
Sweet summer grass												
Crowsfoot grass												
Summer grass												
Silver grass												

Note: Orange indicates resistance has been confirmed by laboratory testing.

Other combinations of resistance are likely to be present, however have not been formally confirmed by resistance testing.

Source: Heap (2018)

Table 6B: Confirmed presence of herbicide resistance in key broadleaf weeds of Australian cropping (as at February 2018).

Weed	Mode of action groups											
	2			5	3	12	14	4		15	22	9
	SU	IMI	TPS	Triazine	DNA			2,4-D	MCPA			
Wild radish												
Indian hedge mustard												
Wild mustard												
Turnip weed												
African turnip weed												
Wild turnip												
Prickly lettuce												
Willow leaf lettuce												
Capeweed												
Broadleaf fumitory												
Paterson's curse												
Lincoln weed												
Calomba daisy												
Tridax daisy												
Common ice plant												
Three-horned bedstraw												
Stinging nettle												
Climbing buckwheat												
Flaxleaf fleabane												
Sow thistle												
White eye												
Blackberry nightshade												
Cudweed												

Note: Orange indicates resistance has been confirmed by laboratory testing.

Other combinations of resistance are likely to be present, however have not been formally confirmed by resistance testing.

Source: Heap (2018)

Further information

An *International Survey of Herbicide Resistant Weeds* database collects information on the first occurrence of a new combination of mode of action x species x location (Heap, 2018): weedscience.org/Home.aspx

Note: This database uses internationally recognised common names for weeds which are often different to Australian naming conventions.

Plant Science Consulting

plantscienceconsulting.com.au/

and Charles Sturt University

csu.edu.au/plantinteractionsgroup/herbicide-resistance

offer commercial resistance testing and periodically report summarised results from their testing programs. These reports give an indication of the frequency of herbicides resistance to key weeds.

6.2 How to test for herbicide resistance

Two different herbicide tests are commercially available in Australia.

When conducting resistance testing, it is important to test to confirm if resistance is present to the suspected mode of action in question. There may also be value in testing multiple application rates to determine if the population may be susceptible to higher application rates (label permitting).

In addition to testing the suspected resistant herbicide mode of action, it is also recommended to test other herbicides that may be considered being used in the future. This will provide valuable information as to the likely levels of performance before these herbicides are applied in following seasons.

6.3 What do we know about resistance mechanisms for key weed species?

Herbicide resistance was first detected in Australia in 1982. Since then, considerable research has been undertaken to identify the types of herbicide resistance present in field populations. However, it is clear that we still do not fully understand all the mechanisms that can evolve within plants to allow them to overcome different herbicide modes of action.

Many different mechanisms conferring resistance have been identified in Australia.

6.3.1 Target site alteration

6.3.1.1 Target site substitution

One of the most common, and frequently studied group of resistance mechanisms are target site substitutions. A target site substitution results in a change (substitution) to the amino acid sequence on the chromosome that alters the binding site on the target enzyme within the plant. This change precludes one or more herbicides from the mode of action group targeting that enzyme, to effectively bind to the target site. As a result, the herbicide may not be able to effectively disrupt the critical enzyme process and the weed survives.

Target site substitution often, but not always, results in high order (very strong) levels of resistance. Increasing application rate is not effective when addressing resistance as a result of high order target site resistance.

Target site substitutions are commonly the cause of resistance in many weed populations to Group 1 (ACCase enzyme inhibitors) and Group 2 (ALS enzyme inhibitors) herbicides. However, target site resistance also occurs for other modes of action.

ACETYL COA CARBOXYLASE INHIBITORS

One of the most studied resistance mechanisms in Australia has been target site resistance conferred by changes to the Acetyl CoA Carboxylase (ACCase) enzyme in annual ryegrass. In a South Australian study (Malone, et al., 2014) that covered collections of annual ryegrass from 653 paddocks sampled between 1998 and 2008, genomic sequencing identified 12 different amino acid substitutions in annual ryegrass that conferred resistance to ACCase inhibitor herbicides. Of the 2008 samples, 23% contained more than one target site alteration.

Identified amino acid substitutions were recorded at seven

SEED TEST

Viable seed is collected from mature plants prior to harvest.

- Log onto the website of the selected testing facility and register the sample.
- Post seeds and associated paddock/location data to the testing facility.
- Seed is received at the testing facility.
- Seed dormancy is broken (where required).
- Plants are grown and treated with herbicides requested by the client.
- Results are returned to the client within 3 to 4 months.

An example of Plant Science Consulting instructions for sampling procedure can be found at plantscienceconsulting.com.au/weed-resistance-seed-test/

Organisations providing a commercial seed testing service

Plant Science Consulting

plantscienceconsulting.com.au/

Charles Sturt University

csu.edu.au/plantinteractionsgroup/herbicide-resistance

QUICK TEST

Live plants are collected from the field and prepared for express postage.

- Log onto the website of the testing facility and register the sample.
- Collect plants and associated paddock/location data and send to the testing facility via express post.
- Plants are received at the testing facility, trimmed and separated, potted up and allowed to regrow.
- Plants are treated with herbicides requested by the client.
- Results are returned to the client within 4 to 6 weeks.
- The Quick Test is only suitable for screening post-emergent herbicides and is limited to grass weeds and certain broadleaf weeds only.

An example of Plant Science Consulting instructions for sampling procedure can be found at:

plantscienceconsulting.com.au/weed-resistance-quick-test/

YouTube videos

youtube.com/watch?v=ukkpmpQUElpQ

youtube.com/watch?v=qjGGmZJpDw

Organisations providing a commercial testing service

Plant Science Consulting

plantscienceconsulting.com.au/

different locations on the chromosome, with multiple different substitutions at some locations. The substitutions Ile-1781-Leu/Val; Trp-1999-Cys/Leu; Trp-2027-Cys; Ile-2041-Asn/Asp/Thr/Val; Asp-2078-Gly; Cys-2088-Arg/Phe; and Gly-2096-Ala were identified. In this study, the Ile-2041-Asp substitution was the most frequent

occurrence (34 to 46% of the populations tested), followed by Asp-2078-Gly (17 to 27%), Ile-1781-Leu (9 to 17%) and then the Cys-2088-Arg substitution (11 to 14%). Other substitutions listed above were found infrequently, typically in less than 5% of individuals tested.

These different target site alterations can result in differences in field performance between different Group 1 herbicides. A combination of partially overlapping binding sites of the three classes of Group 1 herbicides (aryloxyphenoxypropionate (fop), cyclohexanedione (dim) and phenylpyrazole (pinoxaden)) and the structure of the variable molecules of these herbicides explains cross-resistance among and between the three classes of Group 1 herbicides, as well as differences in their specificity (Jang, et al., 2013).

Different regions of the ACCase enzyme binding site are altered by the substitution. Depending on where the substitution occurs, different Group 1 herbicides may be affected (Figure 6A).

As shown above, fop, dim and den herbicides have overlapping binding locations on the enzyme, so it is possible that a target site alteration resulting from one amino acid substitution may render one group of herbicides less effective. However, another group of herbicides may still work, as their binding site has been less affected by the amino acid substitution.

Additional detail on ACCase binding, the effect of the Group 1 sub-groups and how the chemical structure of specific ACCase herbicides can affect binding site can be found in a paper by Jang, et al., (2013).

Typically, for the more common substitutions identified in the South Australian study above (Malone, et al., 2014); that is, 2041 and 2078 substitutions, a higher level of weed tolerance to the fops is usually conferred (Table 6C). In annual ryegrass, it is common for dim herbicides to still have activity, after the fops have failed. A strategy based on this has been coined ‘fop till

Table 6C: Selected amino acid substitution in ACCase endowing resistance to herbicides selected in weeds.

Amino acid substitution	Relative resistance of ACCase to herbicides	
	Aryloxyphenoxypropionates (Fops)	Cyclohexanediones (Dims)
Ile-1781-Leu	XX	XXX
Trp-1999-Cys	XXX	o
Trp-2027-Cys	XX	X
Ile-2041-Asn	XXX	X
Asp-2078-Gly	XXX	XX
Cys-2088-Arg	XX	XX
Gly-2096-Ala	XX	X

Relative resistance compared to susceptible enzyme: o = <2 fold, X = 2–10 fold, XX = 11–100 fold, XXX = 101–1000 fold

Source: Adapted from Preston (2009)

you drop’. This strategy has seen growers rely on fop herbicides for annual ryegrass control until the fop herbicides fail, before switching to dim herbicides.

However, some substitutions may confer strong resistance to the dim chemistry. For example, the Ile-1781-Leu substitution, which was the third most frequent substitution identified in the South Australian study above, expresses high level resistance to both fops and dims (Table 6C). Should one of the substitutions that confer dim resistance be selected early in the use of Group 1 herbicides, then the ‘fop till you drop’ strategy will not be successful in that paddock.

Why does annual ryegrass develop resistance faster than some other grass weeds?

Annual ryegrass in Australia has developed resistance to at least eleven different modes of action – considerably more than any other species. The number of different mechanisms conferring resistance is also higher than other grass weeds, despite many other grass weeds also being in the same paddocks at the same time and therefore being exposed to an identical herbicide application history. Selection for resistance is being detected in these other species, however it often appears to take much longer to express as herbicide failures.

The Australian Herbicide Research Initiative has produced an excellent article to explain how annual ryegrass and wild oats differ, and why it is harder and takes longer to select for resistance in wild oats ahri.uwa.edu.au/wild-oat-always-the-bridesmaid/ (Busi, et al., 2016).

Simplistically, the likely difference is due to the fact that wild oats is largely a self-pollinator, while annual ryegrass is an obligate out-croser (with pollen-mediated gene flow being reported to occur over distances of up to 3km; Busi, et al., 2008). This results in much greater genetic diversity within annual ryegrass, leading to higher frequency of substitution occurrence.

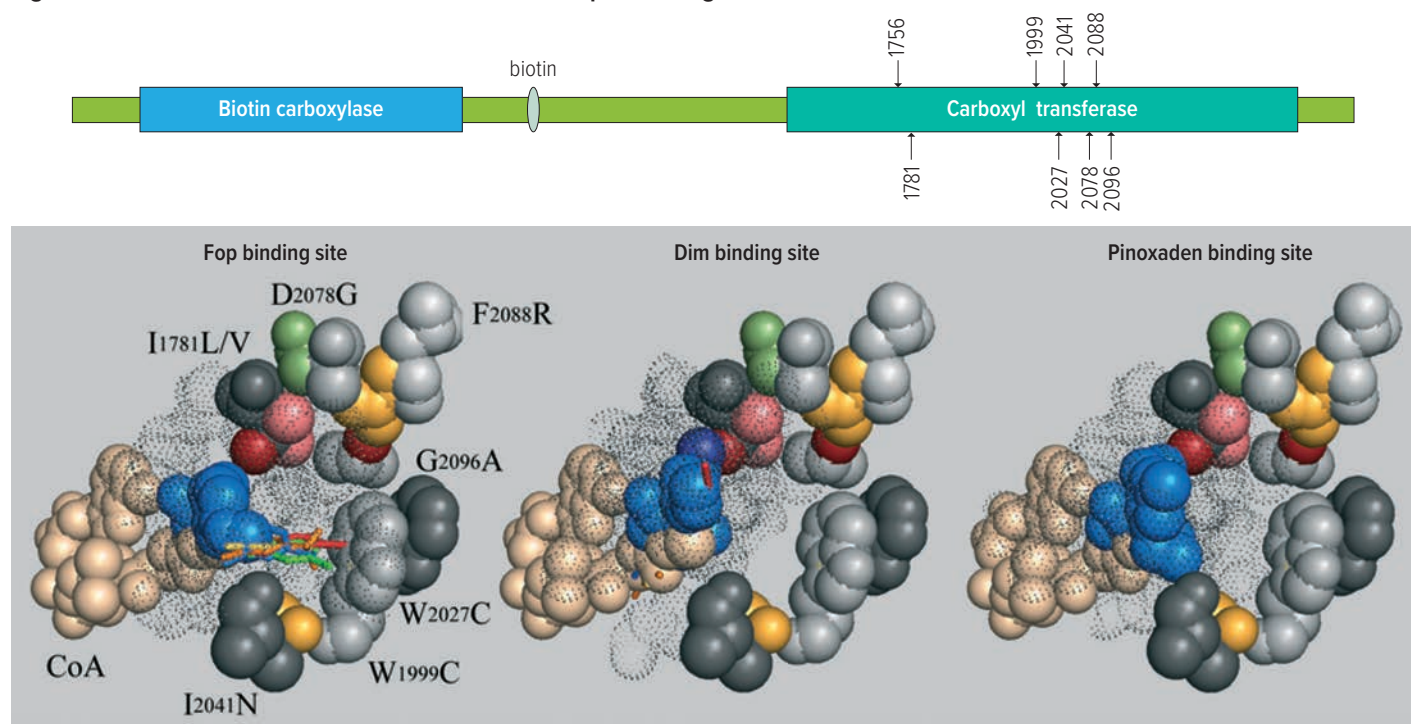
In addition, annual ryegrass is a diploid species (a set of two paired chromosomes with a copy of each gene on both chromosomes), whereas wild oats are hexaploid (six chromosomes with a gene replicated on three homologous copies). Therefore, in a hexaploid species like wild oats,

the impact of a single substitution at one location on one chromosome, is diluted by the other unaffected genes. If this substitution confers herbicide resistance, it is more likely to be only low order (due to the chromosome dilution) and is less likely to become homozygous due to the self-pollinating nature of the wild oat biology.

The difference in herbicide resistance development between species can be seen in a study (Busi, et al., 2016) where a population of wild oats was exposed to a low-dose of diclofop-methyl for 3 consecutive generations, developing 2-fold resistance to diclofop and also low-level cross-resistance to ALS herbicides. When this same study was performed with annual ryegrass, the resistance factors were approximately 40-fold after the same number of selections.

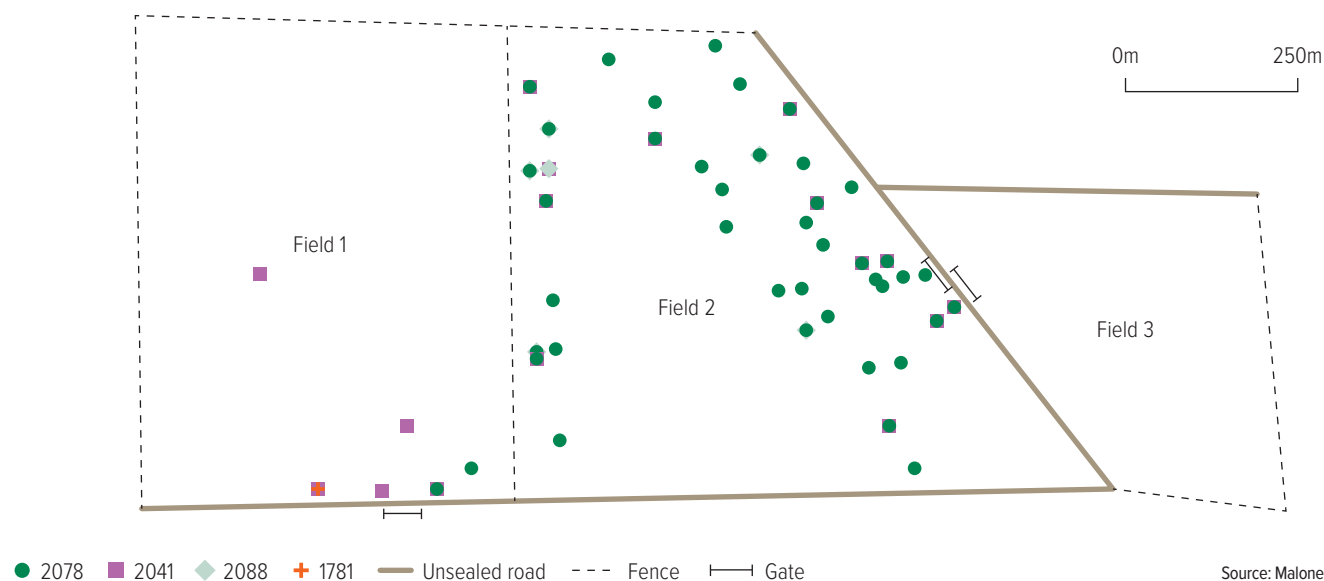
This high level of genetic diversity in annual ryegrass is now contributing to multiple resistance mechanisms developing within the same individual. Stacking of resistance mechanisms typically results in plants that are unable to be controlled by increasing application rate. In a recent study (Han, et al., 2016a), 33 resistant field populations of annual ryegrass were selected from a weed survey conducted in Western Australia in 2010. On analysis, 79% demonstrated enhanced levels of metabolism of diclofop acid; 91% had one or more target site substitution(s) conferring ACCase resistance (50% had a single substitution); and 70% demonstrated both target site and metabolic resistance. In the same paper, the authors also referenced a study of >2000 *Lolium* individuals from 301 populations collected in France. In this study, only 28% of the resistant plants contained only target site resistance; with the other 72% displaying metabolic resistance to diclofop alone, or in combination with target site resistance.

Figure 6A: Differences in ACCase inhibitor sub-Group 2 binding sites.



Source: From Jang, et al. (2013)

Figure 6B: Resistance distribution of ACCase resistant annual ryegrass in three fields at Roseworthy, SA. The location and amino acid substitution(s) in ACCase of each resistant individual is shown.



Source: Malone, et al. (2014)

In the annual ryegrass study above (Malone, et al., 2014), adjacent cropping fields at Roseworthy, South Australia were evaluated to understand the frequency and extent of ACCase resistance (Figure 6B). Despite a history of cropping and Group 1 herbicide use across all paddocks, different resistance patterns were detected. In Field 1, 9% of annual ryegrass plants collected were resistant to Group 1 herbicides (locations and target site substitutions indicated), 34% of plants were resistant in Field 2; however no resistant plants were detected in Field 3.

This example demonstrates that herbicide-resistant populations can vary extensively both within and between paddocks and underpins the need for detailed herbicide testing. It also demonstrates the potential for different test results, depending on which areas or plants were sampled.

For weeds other than annual ryegrass, there has not been enough genomic research conducted in Australia to understand which substitutions are likely to be selected in field populations. It is largely unknown if a 'fop till you drop' strategy would also be likely to work in these other species.

Why does clethodim sometimes continue to work on annual ryegrass after the fops have failed in the field?

A complex interaction of the specific substitutions involved; the obligate cross-pollination nature of annual ryegrass leading to substitution homozygosity and application rate appear to be the key drivers.

The 2041 substitution appears to be one of the most common (potentially first) substitutions frequently encountered in annual ryegrass in Australian fields following initial selection with ACCase herbicides (Malone, et al., 2014).

When occurring alone, this 2041 substitution confers high-level resistance to most fop herbicides (>100 fold) but has only a lower level effect on dim herbicides (5 to 7-fold) and pinoxaden (13 fold; Jang, et al., 2013). As a result, field populations with the common 2041 substitution are likely to be controlled at commercial rates of dim or den herbicides, but not fops.

Other common substitutions appear to be substitutions at the 1781 and 2078 locations. It appears that single 1781 substitution confers significant resistance across fops and dims, with field application rates probably unlikely to control weeds containing this substitution.

In the case of the 2078 substitution, resistance factors appear higher across all classes of ACCase inhibitor herbicides, with control reduced at field rates. However, a study (Vila-Aiub, et al., 2015) demonstrated reduced ACCase activity and associated plant growth in annual ryegrass plants containing this substitution, without any Group 1 herbicide challenge. This would suggest that a potential fitness penalty may occur with homozygous Asp-2078-Gly substitution. This fitness penalty was not observed with the Ile-1781-Leu substitution in the same study.

This demonstrates that different substitutions can display different levels of field control with different herbicide groups.

In addition, annual ryegrass is diploid (two copies of each chromosome) and is an obligate out-croser. Where a single substitution occurs on one chromosome, the level of resistance

may be low, depending on the substitution involved, and not able to be detected following commercial herbicide application as plants may still be controlled.

As annual ryegrass is an out-croser, there is a high level of genetic diversity and potential for selection of individuals that are homozygous for the substitution (that is, the substitution occurs on both chromosomes). Plants that were homozygous for the 1781 substitution displayed resistance factors 6 to 18 times higher than the susceptible individuals for diclofop and haloxyfop acid, tralkoxydim and clethodim; such that field failures would occur at commercial application rates (Yu, et al., 2007a).

Homozygote 2078 or 2088 individuals displayed even higher levels of resistance, in the order of 32 to 53x and 38 to 75x respectively for the two substitutions for the same range of herbicides. In the same study, (Yu, et al., 2007a) also identified that individuals with two separate substitutions (Ile-1781-Leu plus Trp-2027-Cys or Ile-1781-Leu plus Ile-2041-Asn) also showed increased resistance ratios of 7x and 13x respectively, therefore likely being able to survive field rates.

Herbicide application rate is also important. In the study above, clethodim dose response to susceptible and 1781, 2078 and 2088 homozygous mutants were established. Under laboratory conditions, an application rate of 7.5gai/ha controlled all susceptible individuals, with 4.4gai/ha being calculated as the LD₅₀ (lethal dose required to kill 50% of the population). This corresponds to 'original' clethodim product labels that recommended application rates of 36–60gai/ha. For the homozygous 1781, 2078 and 2088 mutants, LD₅₀ values were calculated as 98, 105 and 115gai/ha respectively (Yu, et al., 2007a). In recent years, the commercial application rate on clethodim labels has been increased to 120gai/ha. This would suggest that at this rate some effect on these homozygous mutant individuals may still be observed.

The final piece of the puzzle is that often there has been simultaneous selection for other non-target site resistance mechanisms, which has occurred alongside selection for target site substitutions. Often these non-target site mechanisms have conferred low order resistance factors, and therefore may not have been easily identified in early studies. It now appears that these other resistance mechanisms have often been underestimated and may be providing additive effects, further exacerbating field performance of the Group 1 herbicides.

ACETOLACTATE SYNTHASE INHIBITORS

Group 2 (ALS enzyme inhibitors) herbicides are also highly susceptible to field failures resulting from target site substitution within weeds. As with the Group 1 (ACCase enzyme inhibitors) example above, there are also multiple sub-groups of Group 2 herbicides that have differential binding to the ALS enzyme, and can exhibit different resistance levels when exposed to different target site substitutions (Table 6D). The three primary classes of ALS inhibitor herbicides commonly used in Australian grain production are the triazopyrimidine sulfonamides (TPS), sulfonyleureas (SUs) and imidazolinones (IMIs).

In an Australian study, substitutions Pro-197-Ala, Pro-197-Arg, Pro-197-Gln, Pro-197-Leu, Pro-197-Ser and Trp-574-Leu were reported from field collections of annual ryegrass collected in Western Australia (Yu, et al., 2008). The Pro-197 substitutions conferred resistance to the sulfonyleurea herbicide sulfometuron, whereas the Trp-574-Leu substitution conferred resistance to both sulfometuron and the imidazolinone herbicide imazapyr.

Table 6D: Known amino acid substitution in ALS(AHAS) endowing resistance to herbicides (as at October 2016).

Amino acid substitution	Relative resistance of ALS to herbicides			
	No. of species (includes BL weeds)	Triazolopyrimidine sulfonamides (TPS)	Sulfonylureas (SUs)	Imidazolinones (IMIs)
Ala-122-Val	2	S	R	R
Ala-122-Thr	6	S/R	S	R
Ala-122-Tyr	1	R	R	R
Pro-197-Thr	12	MR/R	MR/R	S/MR
Pro-197-His	8	S/MR/R	R	S/MR/R
Pro-197-Arg	4	MR	R	S/MR
Pro-197-Leu	12	S/MR/R	R	S/MR/R
Pro-197-Gln	7	S/R	R	S/MR
Pro-197-Glu	1	R	R	R
Pro-197-Ser	25	MR/R	R	S/MR
Pro-197-Ala	10	MR/R	R	S/MR
Pro-197-Ile	1	R	R	MR
Pro-197-Tyr	1		R	
Pro-197-Asn	1	MR	R	
Ala-205-Val	5	S/MR	S/MR/R	MR/R
Ala-205-Phe	1	R	R	R
Asp-376-Glu	12	MR/R	MR/R	MR/R
Arg-377-His	1	R	R	
Trp-574-Leu	35	R	R	R
Trp-574-Gly	1		R	
Trp-574-Met	1		R	
Ser-653-Ile	1		MR	R
Ser-653-Thr	5	S	S/MR	R
Ser-653-Asn	6	S/MR		R
Gly-654-Glu	1			R
Gly-654-Asp	1		MR	R

Relative resistance: S = Susceptible biotype, MR = Moderate resistance (< 10-fold relative to sensitive biotype), R = High resistance (> 10-fold), blank = Not determined.

Multiple entries in cells above indicate the range reported across studies.

Source: Adapted from Tranel, et al. (2016)

PHOTOSYSTEM II (PSII) INHIBITORS

In the case of target site substitution conferring resistance to the Group 5 (photosystem II inhibiting) triazine herbicides, a common serine to glycine amino acid substitution at the 264 location (Ser-264-Gly) has been recorded globally across more than 60 different weed species. This substitution confers a high level of resistance to triazines, however it comes at a cost of reduced photosynthetic efficiency, and therefore imparts a fitness penalty to the plant (Powles & Yu, 2010).

This fitness penalty typically means that 'resistant' weeds are less likely to be able to compete with the crop, or other susceptible weeds. The lack of diversity of substitution conferring triazine resistance, in conjunction with a significant fitness penalty, could be argued to be the reason why target site resistance to triazines has not 'exploded' compared to other modes of action affected by target site substitutions.

Table 6E: Amino acid substitutions conferring resistance to photosystem II herbicides selected in weeds.

Amino acid substitution	Confers resistance to
Ser-264-Gly	Triazines
Ser-264-Thr	Triazines and ureas (for example, diuron)
Val-219-Ile	Diuron and metribuzin
Asn-266-Thr	Bromoxynil
Ala-251-Val	Metribuzin
Phe-255-Ile	All photosystem II herbicides

Source: Powles & Yu (2010) and Cobb & Reade (2010)

This Ser-264-Gly substitution is the same substitution that has been selected in 'Triazine Tolerant' (TT) canola varieties. The fitness penalty described above explains the resultant reduction in yield typically seen with 'triazine tolerant' canola varieties commonly grown in Australia that also contain this substitution.

A Ser-264-Thr substitution has also been recorded that confers resistance to both the triazine and urea sub-classes of PSII inhibitor herbicides. In addition, Val-219-Ile, Asn-266-Thr, Ala-251-Val and Phe-255-Ile substitutions have been shown to provide varying cross-resistance patterns within PSII herbicides; Table 6E (Powles & Yu, 2010).

5-ENOLPYRUVYLSHIKIMATE-3-PHOSPHATE SYNTHASE (EPSPS) INHIBITORS

Target site substitution is one of the resistance mechanisms affecting glyphosate (Sammons & Gaines, 2014). Four single substitutions at the 106 site that confer resistance to glyphosate (Table 6F) are known to exist globally across a range of weeds (Pro-106-Ala, Pro-106-Ser, Pro-106-Thr and Pro-106-Leu). Unlike many examples of target site substitution for other herbicides where resistance is typically high order, these single Pro-106 substitutions conferring resistance to glyphosate are typically weak (~2 to 10x resistance factors).

In addition to those listed in the study below (Table 6F), more recent target site substitutions have also been recently described in feathertop Rhodes grass (Hereward, 2016) via a Pro-106-Ser mutation, while a double Pro-106-Ser and Pro-106-Leu substitution has also been identified in this species (Ngo, et al., 2018a).

Glyphosate-resistant awnless barnyard grass with double Pro-106-Thr and Pro-106-Leu target site substitution that showed up to 2x resistance has recently been identified from a field-collected population (Han, et al., 2016b). This population was still able to be controlled with typical field glyphosate application rates (450gai/ha) – albeit applied under laboratory conditions.

While most cases of target site amino acid substitution conferring glyphosate resistance have low order resistance factors, a field-collected population of crowsfoot grass from Malaysia (Yu, et al., 2015) was shown to have developed a double substitution (Thr-102-Ile and Pro-106-Ser [identified as TIPS]). This resistance development is significant in that it has now been identified from a field-selected population, as this TIPS substitution is the same substitution that was genetically engineered into the first glyphosate-tolerant commercially grown maize crops. This population of crowsfoot grass displayed more than a 180x resistance factor and cannot be controlled by a commercial application of glyphosate. The paper proposes that the second substitution at the 102 location would only be possible after the population was previously selected for the 106 event.

While single substitutions conferring glyphosate resistance are being discovered more frequently, they are on occasion still able to be controlled by commercial application rates in the field. Usually this is associated with increasing application rate and targeting very small weeds under excellent growing conditions. When double substitutions conferring resistance occur, or when a single target site substitution is combined with a non-target site mechanism, this often leads to higher levels of resistance and is often responsible for in-field glyphosate failure.

Table 6F: Reported Pro106 target-site substitutions in EPSPS endowing glyphosate resistance in weed species.

Species	Pro106 to	Fold resistance	Other mechanisms detected?
<i>Eleusine indica</i>	Ser	2–4	No
<i>Amaranthus tuberculatus</i>	Ser	5	Yes, reduced translocation
<i>Amaranthus tuberculatus</i>	Ser	5	No, Ser substitution did not fully account for resistance
<i>Echinochloa colona</i>	Ser	6.6	No
<i>Lolium multiflorum</i>	Ser	2–5	No
	Ala	5–15	
<i>Eleusine indica</i>	Ser	3	No
	Thr	3	
<i>Digitaria insularis</i>	Thr	4	Yes, reduced absorption and reduced translocation
<i>Eleusine indica</i>	Ser	2	No
<i>Lolium rigidum</i>	Leu	1.7	Yes, unknown mechanism
<i>Lolium rigidum</i>	Ser	6–8	Yes, reduced translocation
	Thr	8–11	Yes, reduced translocation
<i>Lolium multiflorum</i>	Ser	5	Yes, reduced translocation
<i>Lolium multiflorum</i>	Ser	5	No, reduced translocation detected in different population
<i>Lolium rigidum</i>	Thr	2–3	No
<i>Lolium rigidum</i>	Ser and Leu	16–21	No, but other mechanisms suspected
<i>Lolium rigidum</i>	Ser	—	No
<i>Lolium rigidum</i>	Ala	14	Yes, reduced translocation

Source: Adapted from Sammons & Gaines (2014)

Table 6G: EPSPS gene amplification reported in glyphosate-resistant weed species.

Species	Population origin	EPSPS relative genomic copy number range
<i>Amaranthus palmeri</i>	USA (Georgia)	40–100
<i>Amaranthus palmeri</i>	USA (North Carolina)	20–60
<i>Amaranthus palmeri</i>	USA (New Mexico)	2–10
<i>Amaranthus palmeri</i>	USA (Mississippi)	33–59
<i>Amaranthus tuberculatus</i>	USA (Missouri, Illinois)	4
<i>Lolium multiflorum</i>	USA (Arkansas)	15–25
<i>Kochia scoparia</i>	USA (Kansas, Colorado)	3–9
<i>Amaranthus spinosus</i>	USA (Mississippi)	26–37

Source: Adapted from Sammons & Gaines (2014)

6.3.1.2 Gene amplification

Another resistance mechanism that involves modifying the target site is 'gene amplification', which has been shown to confer resistance to glyphosate in some species.

Plants with the gene amplification resistance mechanism to glyphosate have been shown to have multiple copies of the 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) gene along the chromosome. Resistant species have been identified with between two and more than 100-fold duplication of the EPSPS gene (Sammons & Gaines, 2014).

Multiple copies of the EPSPS gene leads to over-production of EPSPS. When glyphosate is applied, it 'knocks out' many binding sites, however the sheer number of sites means that not all binding sites are able to be saturated, and those unaffected by the glyphosate continue to accept the enzyme and plant growth continues. At low levels of gene multiplication, it may be possible to increase control by increasing application rate, however at higher levels of amplification this is impractical.

Until recently, EPSPS gene amplification had only been detected in limited species from North America (Table 6G).

In 2015, (Malone, et al., 2015) identified two field populations of brome grass (*Bromus diandrus*) from Victoria and South Australia that appear to also have gene amplification as the mechanism of resistance to glyphosate. In this study, individuals from the two field populations averaged 13.5 and 29 copies of EPSPS respectively. EPSPS expression ranged from a 2 to 12.3-fold increase across individuals, however the correlation between EPSPS copies and expression was not high.

Additionally, gene amplification in windmill grass has also been detected (Ngo, et al., 2018b). In this study, windmill grass contained between 32 to 48 more copies of the EPSPS gene than the susceptible plants and were 2.4 to 8.7-fold more resistant and accumulated less shikimate after glyphosate treatment than susceptible plants.

To date, gene amplification has only been identified as a mechanism conferring resistance to glyphosate.

6.3.2 Non-target site resistance

In addition to target site resistance, many other mechanisms of herbicide resistance also exist. Often, these are collectively grouped as 'non-target site resistance', however their mechanisms of resistance are typically diverse and may be non-related.

Typically, but not exclusively, a feature of non-target site resistance mechanisms is that these mechanisms often confer lower level resistance (often less than 10x), at least in the early stages of selection. The practical outcome of this is that sometimes it may be possible to still achieve a level of commercial control by increasing application rate. However, as selection pressure continues, often populations are selected for higher order resistance mechanisms, or multiple mechanisms within the same plant. Eventually these combinations are likely to express as spray failures.

Key non-target site resistance mechanisms include:

- Reduced translocation via vacuole sequestration;
- Metabolic resistance; and
- Changes in weed morphology or ecology.

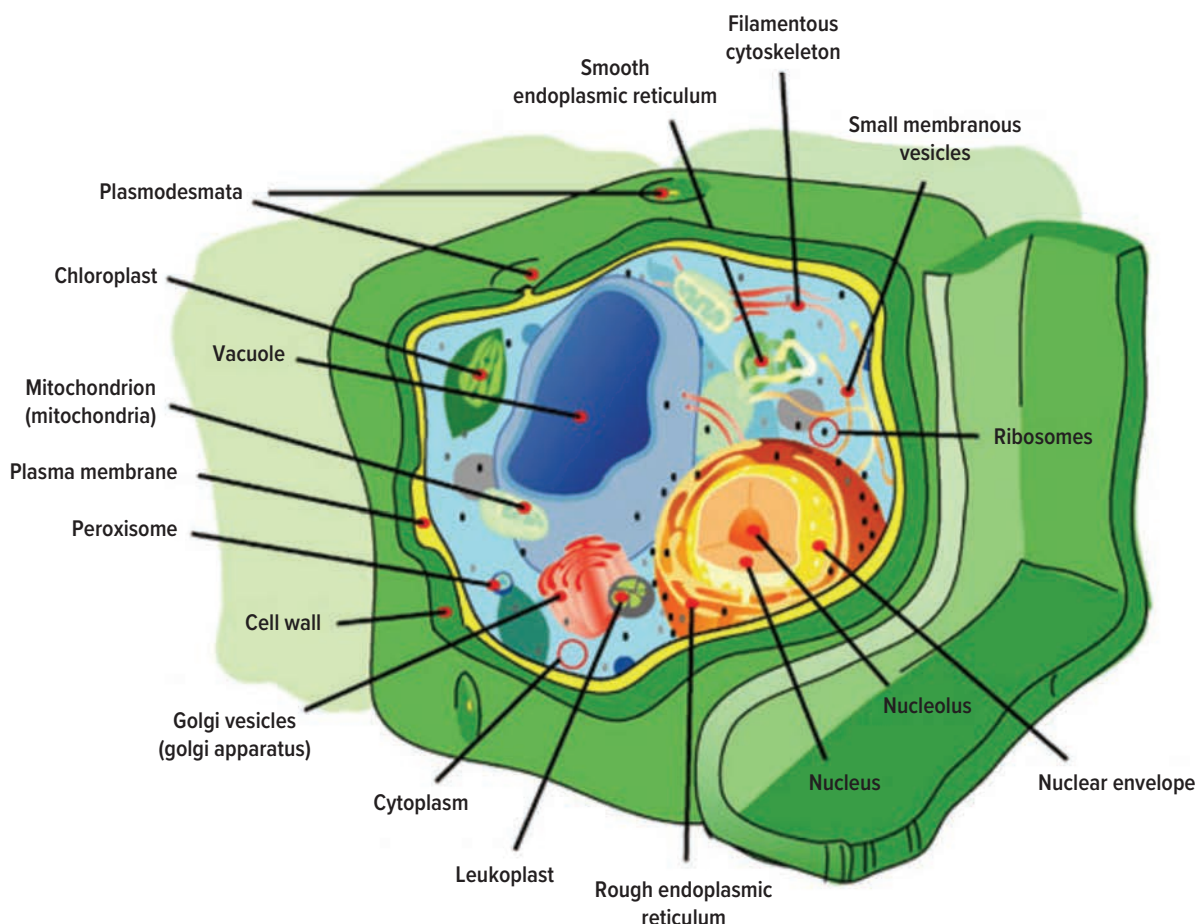
6.3.2.1 Reduced translocation via vacuole sequestration

Some of the first examples of glyphosate resistance in annual ryegrass in Australia were due to reduced translocation (Wakelin, et al., 2004; Preston & Wakelin, 2008; Adu-Yeboah, et al., 2014). Resistant plants can limit the movement of foliar-applied herbicide reaching the target site that is, the chloroplasts for herbicides such as glyphosate and paraquat, which are affected by this resistance mechanism.

It appears that resistant plants primarily do this by vacuole sequestration, that is, moving herbicide into the vacuole within the cell where it cannot access the target enzyme system located in the chloroplasts.

Resistant plants with this mechanism actively transport the glyphosate across the cell membrane, however divert some of the herbicide away from the chloroplast and into the vacuole. Once inside the vacuole, the herbicide is ineffective. The transport mechanisms involved appear to only work in one direction, so that once the herbicide is contained within the vacuole, it does not appear able to be released. In glyphosate-resistant Canadian fleabane from North America, approximately 10x more glyphosate accumulated in the vacuole of resistant individuals. In further work with Canadian fleabane, vacuole sequestration was shown to be temperature-dependent, with significantly reduced sequestration at cold (8°C) temperatures (Sammons & Gaines, 2014). In addition to Canadian fleabane, vacuole sequestration of glyphosate has also been demonstrated to confer resistance in Johnson grass and *Lolium* species (Ge, et al., 2012).

Figure 6C: Plant cell structure.



Source: Smith (2016)

Vacuole sequestration has also been shown as a key resistance mechanism conferring resistance to paraquat in annual ryegrass in Australia (Yu, et al., 2010; Yu, et al., 2007b). Vacuole sequestration of paraquat is also temperature-dependent (Purba, et al., 1995).

6.3.2.2 Metabolic resistance

A resistance mechanism increasingly implicated in field herbicide failures is metabolic resistance. In a recent Western Australian review (Han, et al., 2016a), almost 80% of resistant annual ryegrass was identified to have some level of enhanced metabolism of diclofop.

Plants (and all living organisms) contain many enzymes responsible for modifying various chemicals within the plant, which are required for a wide range of metabolic functions. Metabolism is typically achieved by either hydrolysing (breaking the herbicide apart) or conjugating (adding to the chemical structure) the substrate, however other processes are also involved (Table 6H). These metabolic enzymes are usually from the cytochrome P450 monooxygenases (P450), glucosyl transferases (GT) or glutathione S-transferase (GST) super-families, each containing hundreds of different enzymes that all have specific roles.

Metabolic resistance occurs where the plant upregulates genes responsible for the production of one or more of these enzymes that are able to degrade the herbicide in question (Yu & Powles, 2014). Higher levels of these enzymes within resistant plants intercept the herbicide after entering the cell and render it inactive before the herbicide reaches its target binding site.

In susceptible weeds, the levels of P450 or GST enzymes are typically not at levels high enough to prevent sufficient herbicide reaching the site of activity intact and causing plant death. Continual herbicide selection, and in particular with low application rates, has been shown to increasingly select individuals with increased upregulation of P450 or GST production. With higher levels of these enzymes, more herbicide can be degraded before reaching the target site and weed survival may result.

In the early stages of selection for metabolic resistance, resistance factors may be low. Growers often find that they require continual increasing application rates to achieve the same level of weed control as the population is repeatedly challenged by herbicides. With continued selection, individuals with higher levels of P450 or GST production +/- other forms of resistance will be selected and eventually the weeds will no longer be controlled by commercial application rates.

What is extremely concerning is that these enzyme families are not specific to individual herbicides. They are often able to metabolise different herbicides, both from within the same mode of action sub-group, between sub-groups within the same mode of action, but critically also between some entirely different modes of action (Hidayat & Preston, 2001; Christopher, et al., 1994). It is worth noting that some modes of action (for example, Group 22, Group 9) appear to be not able to be metabolised within plants, and therefore these are unaffected by metabolic cross-resistance.

An example of metabolic cross-resistance occurs in wild oats. As can be seen in Table 6I, resistant plants (R1) showed much faster conversion of the toxic diclofop-acid to non-herbicide polar

metabolites, than the herbicide-susceptible individuals. (Target site substitution was eliminated as a source of resistance. Data not shown here).

From commercial resistance testing, it has been observed that many populations of wild oats (above 40% of those tested) appear to also be resistant to the arylaminopropionic acid (Group O) herbicide flumetraler-methyl, despite many fields having no, or very little previous use of this herbicide. These populations appear to have developed metabolic resistance to overcome Group 1 (ACCase) or Group 2 (ALS) herbicides, with the metabolic resistance also conferring cross-resistance to flumetraler-methyl.

Metabolic resistance is not limited to post-emergent herbicides. Laboratory studies (Busi & Powles, 2013; Busi & Powles,

2016) have shown that metabolic cross-resistance between pyroxasulfone, prosulfocarb/s-metolachlor and tri-allate could be generated with 3 or 4 'low-rate' selections where survivors were crossed with other survivors.

Metabolic resistance is of great concern, as it is likely to confer resistance to other herbicide mode of action groups, including some herbicide modes of action that have not as yet even been developed.

Interestingly, when some other chemicals are also present within the plant, the levels of cytochrome P450s have been shown to be either increased or decreased, depending on the chemical involved. Certain chemicals inhibit/reduce the plants' production of P450 enzymes. In weeds, this has been demonstrated using the

Table 6H: Common metabolic breakdown pathways and herbicide groups affected.

Metabolic breakdown	Herbicide groups affected
Conjugation Reaction with another compound (for example, glucose, glutathione, aspartic acid) to form a larger molecule	Many herbicides. Glutathione s-transferase (GST) usually involved.
Dealkylation Removal of alkyl (C_nH_{2n+1}) side chain(s)	Triazines, substituted ureas, carbamates, thiocarbamates, dinitroaniline herbicides.
Deamination Removal of amine (NH_2) group	Metribuzin.
Decarboxylation Removal of the $-COOH$ group	Many phenoxy and benzoic acids, substituted ureas.
Hydrolysis Splitting of the molecule by the addition of water. May activate or deactivate herbicides.	Carbamates, thiocarbamates, substituted ureas, sulfonyleureas, triazines. Common process for many herbicides applied as esters to convert to the parent acid for example, aryloxyphenoxypropionates.
Hydroxylation Addition of $-OH$ group (often associated with removal or movement of a chlorine atom)	Many phenoxy and benzoic acids, triazines.
Oxidation Attachment of an oxygen.	Many herbicides. Usually driven by cytochrome P450. Often followed by conjugation to a glucose or other sugars and then often moved to the vacuole or cell wall.

Source: Varshney & Sondhia (2008)

Table 6I: Metabolism of [^{14}C]-diclofop-methyl by susceptible (S) and resistant (R1) wild oat plants 48 and 72 hours after treatment.

Population	Time after treatment (hrs)	Radio-labeled [^{14}C] (% recovered in the extracts)		
		Diclofop metabolites	Diclofop-acid	Diclofop-methyl
S	48	42.5 a	53.6 a	3.8 a
R1	48	71.5 b	26.4 b	2.01 b
S	72	59.7 a	37.0 a	3.3 a
R1	72	78.1 b	20.5 b	1.5 b

Letter in italics designated statistical significance.

Source: Ahmad-Hamdani, et al. (2013)

Table 6J: Examples of safeners used in Australian grains production.

Safener	Use pattern
cloquintocet-mexyl	Formulated with Group 1 herbicides clodinafop and fenoxaprop, and also Group 27 herbicides bicyclopyrone and topramezone so that they can be applied to wheat.
mefenpyr-diethyl	Formulated with Group 1 herbicides containing sethoxydim and fenoxaprop; Group 2 herbicides containing iodosulfuron and mesosulfuron; and Group 27 herbicides containing pyrasulfotole when used in various cereal crops.
oxabtrinil or metcamifen	Applied as a seed treatment on sorghum to increase the crop's metabolism of s-metolachlor.

organophosphate insecticides malathion and phorate. In insects, piperonyl butoxide (PBO) and other organophosphates have also been shown to suppress P450 production.

For example, in laboratory studies on weeds, the addition of malathion has been shown to reduce the activity of the P450 enzymes in some metabolic resistant populations (Christopher, et al., 1994; Hidayat & Preston, 2001; Owen, et al., 2012), while an alternative organophosphate insecticide, phorate, also has been shown to influence P450 production in some weed populations with metabolic resistance (Busi, et al., 2017). In this study, phorate increased control of chlorsulfuron (Group 2), pyroxasulfone (Group 15) and trifluralin (Group 3) of metabolic resistant annual ryegrass while decreased control with prosulfocarb and triallate (both Group 15) was observed. The annual ryegrass population was resistant to all these modes of action.

Other chemicals result in the opposite effect (that is, increasing the activity of P450 or GST genes) and thereby decreasing the herbicide performance (Riechers, et al., 2010; Davies, 2001).

Some chemicals, often referred to as crop safeners, have been shown to increase the rate of herbicide degradation within the plant, by increasing metabolic enzyme performance and are used commercially to safen particular herbicides (Table 6J).

In addition to these chemicals used specifically as safeners, research has shown that the addition of 2,4-D can also increase the activity of P450 enzymes (Han, et al., 2013), thereby decreasing the effectiveness of several Group 1 and Group 2 herbicides when applied close to the 2,4-D application. This is the mechanism responsible for the biological incompatibility of phenoxy and Group 1 herbicides and cautions against this mixture are found on a number of herbicide labels. This increased P450 activity also explains the safening effect often seen in cereals when phenoxy herbicides are tank-mixed with sulfonylurea herbicides.

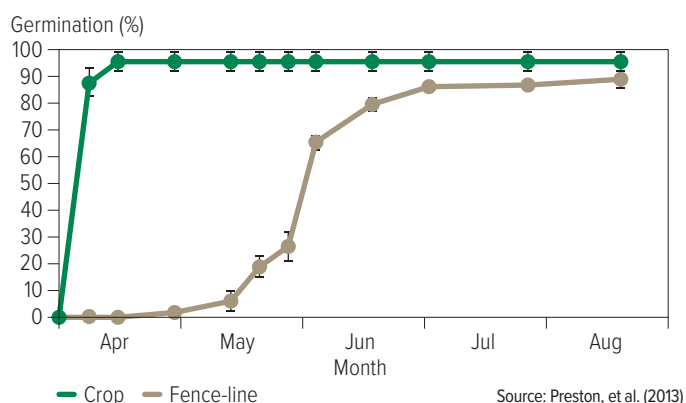
6.3.2.3 Changes in morphology or ecology of the species

Some species have been shown to modify their leaf surface in response to continued herbicide challenge, for example by increasing cuticle thickness or increasing leaf hairiness, which reduces the ability of the herbicide to penetrate the leaf, leading to herbicide failure.

Another example of species adaptation can be seen in populations of barley grass and brome grass throughout South Australia and the Victorian Mallee. Typically, these species have historically been reported as having little seed dormancy and emerging largely as a single cohort, following season-breaking rainfall in the autumn. Growers have typically relied on a range of tactics to control these weeds, including pre-season knockdowns and tillage, pre-emergent herbicides applied at planting and early season in-crop herbicides. In response to prolonged early season control measures, populations of barley grass in South Australia have been selected for individuals that contain a vernalisation gene, which requires a period of chilling before germination (Fleet & Gill, 2010). This delay of germination until later in winter typically means that germination occurs after the effects of pre or early season treatments have finished, allowing these individuals containing the gene for vernalisation to be able to survive and dominate the population.

Changes (delay) in germination have also been reported in annual ryegrass (Owen, et al., 2015) following intensive herbicide selection over a number of seasons.

Figure 6D: Brome grass populations from cropped fields have higher levels of seed dormancy than those from fence lines.



For further information on herbicide resistance mechanisms and how they work, the Australian Herbicide Research Initiative has produced an excellent set of online learning modules that can be accessed at learninghub.weedsmart.org.au/ (site registration required).

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