

Causes of poor ryegrass results & paraquat & glyphosate resistance- 2020 season- Dubbo

Peter Boutsalis^{1,2}, John Broster³ & Christopher Preston¹

¹ *School of Agriculture, Food & Wine, University of Adelaide*

² *Plant Science Consulting P/L*

³ *Charles Sturt University*

Keywords

glyphosate and paraquat resistance, annual ryegrass, barnyard grass, feathertop Rhodes grass, optimising control, testing, random weed survey, double knock.

GRDC code

UCS00020

Take home messages

- Glyphosate resistance in annual ryegrass continues to increase whereas resistance to paraquat remains very low
- Significant glyphosate resistance in fleabane, awnless barnyard grass and feathertop Rhodes grass, especially in samples from Queensland
- Reduced control occurs if herbicides are applied to stressed weeds
- Improving herbicide efficacy by good application can reduce selection for herbicide resistance.

Incidence of resistance in NSW

The GRDC continues to fund random weed surveys in cropping regions to monitor for changes in resistance levels in key weed species. The methodology involves collecting weed seeds from paddocks chosen randomly at pre-determined distances. Plants are tested in outdoor pot trials during the growing season. The majority of annual ryegrass populations in NSW are resistant to Group A 'fop' and Group B herbicides with some variability between the surveyed regions (Table 1). No populations have been found that are resistant to the newer pre-emergent herbicides although this has been reported in other states. Of particular concern is the number of populations resistant to glyphosate in some of the regions.

Table 1. Extent (percentage) of herbicide resistance in annual ryegrass populations collected in NSW random surveys (resistance defined as populations with >20% survival)

	NSW (2015 - 2019)	2019 eastern NSW	2015 western NSW	2016 NSW northern	2016 NSW plains	2017 southern NSW	2018 NSW slopes
diclofop	59	92	16	32	65	84	77
clethodim	2	12	1	1	1	3	0
sulfometuron	50	82	30	22	35	74	70
imazamox/imazapyr	47	83	8	22	39	75	76
trifluralin	1	2	2	0	0	1	1
prosulfocarb + S-metolachlor	0	0	0	0	0	0	0
pyroxasulfone	0	0	0	0	0	0	0
glyphosate	5	14	6	5	0	7	3
Samples	608	53	117	94	111	128	105

Among the other species resistance was much lower. 29% of wild oats populations across NSW were resistant to Group A 'fop' herbicides (Table 2). Group B 'SU' resistance was common for sow thistle (43%) and Indian hedge mustard (27%) across the state, with this rising to 75% of sow thistle populations in eastern NSW resistant (data not shown). Three populations (1%) of sow thistle from northern NSW were resistant to glyphosate. Of the wild radish populations surveyed 38% were resistant to diflufenican and 23% to 2,4-D amine (Table 2).

Table 2. Extent (percentage) of herbicide resistance in populations of the other species collected in NSW random surveys (resistance >20% survival, * herbicide not tested or not applicable for species)

Herbicide group	Wild oats	Barley grass	Brome grass	Sow thistle	Wild radish	Indian hedge mustard
diclofop	29	0	0	*	*	*
clethodim	1	0	0	*	*	*
sulfometuron	4	1	7	43	4	27
imazamox/ imazapyr	*	*	2	*	0	8
atrazine	*	*	*	*	4	0
diflufenican	*	*	*	*	38	4
2,4-D Amine	*	*	*	1	23	2
trallate	0	*	*	*	*	*
paraquat	*	3	*	*	*	*
glyphosate	0	*	0	1	0	0
Samples	511	133	110	202	28	71

An additional survey collected weed species across northern NSW and Queensland in summer 2016/17. The species collected during this survey included awnless barnyard grass, feathertop Rhodes grass, fleabane and some additional sowthistle samples. These samples were screened for resistance to glyphosate, with a significant percentage of the populations for all species (except for sowthistle) resistant to this herbicide (Table 3).

Table 3. Extent (percentage) of glyphosate resistance for weed species collected in 2016 summer survey (Includes sowthistle collected in northern NSW and Queensland winter survey)

	Northern NSW		Queensland	
	% Resistant	Populations tested	% Resistant	Populations tested
Awnless barnyard grass	0	5	37	37
Feathertop Rhodes grass	50	2	70	60
Fleabane	100	25	100	36
Sowthistle	7	45	3	62

Incidence of glyphosate resistance in NSW

Bayer CropScience provides free access to the Resistance Tracker website consisting of thousands of weed samples from resistance testing across Australia (<https://www.crop.bayer.com.au/tools/mix-it-up/resistance-tracker>). This website enables the searching of resistance according to weed species, mode of action herbicide, postcode and closest town with data presented from 2003 (Figure 1).

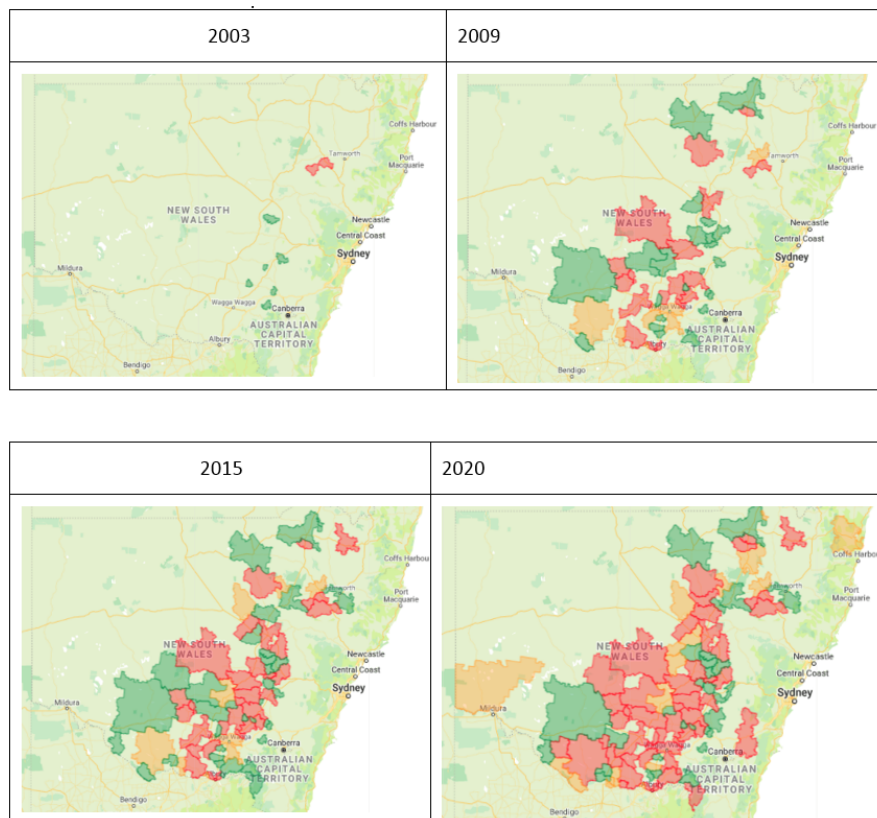


Figure 1. Occurrence of glyphosate resistance in annual ryegrass in NSW in 2003, 2009, 2015 and 2020. Dark green shading = postcode regions where testing has not detected glyphosate resistance in ryegrass, orange shading = postcodes where glyphosate resistance is developing and red shading = postcodes where resistance has been detected.

2020 season: The early break in 2020 across most southern cropping regions resulted in an opportunity for knockdown weed control. Multiple applications of glyphosate and paraquat were possibly targeting multiple flushes of weeds, in particular ryegrass from early autumn prior to sowing. Plants surviving glyphosate from WA, SA, Vic and NSW were sent to Plant Science Consulting for testing using the Quick-Test method to verify whether herbicide resistance had contributed to survival in the field. The data presented in Figure 2 indicates that 43%, 70% and 78% of ryegrass samples sent from SA, Vic and NSW in 2020 respectively, were confirmed resistant to glyphosate. This highlights that in a majority of cases, glyphosate resistance has contributed to reduced control in the paddock.

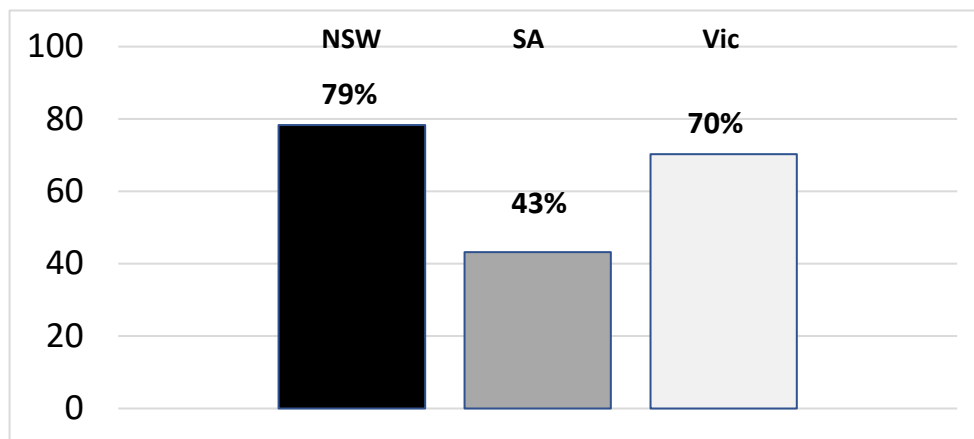


Figure 2. Percent (%) resistance to glyphosate confirmed in farmer ryegrass samples originating from 83 NSW, 37 SA and 74 Vic cropping paddocks treated with glyphosate in autumn 2020. Testing conducted by Plant Science Consulting using the Quick-Test.

Discrepancy between resistance testing and paddock failures to glyphosate

In some cases, plants that survived glyphosate in the paddock are not resistant. Reasons for the discrepancy between the paddock and a resistance test can include poor application or application onto stressed plants, incorrect timing, sampling plants that were not exposed to glyphosate, antagonistic tank mixes, inferior glyphosate formulation, poor water quality, incorrect adjuvants, or a combination of the above.

Evolution of glyphosate resistance

Glyphosate was first registered in the 1970s and rapidly became the benchmark herbicide for non-selective weed control. Resistance was not detected until 1996 in annual ryegrass in an orchard in southern NSW (Powles et. al. 1998). Only a few cases of resistance were detected in the following decade (refer to Bayer Resistance Tracker). The fact that it required decades of repeated use before resistance was confirmed indicated that the natural frequency of glyphosate resistance was extremely low.

There are several contributing factors for the increasing resistance in ryegrass and other weed species to glyphosate with generally more than one factor responsible. Reducing rates can increase the selection for resistance, particularly in an obligate outcrossing species such as ryegrass, resulting in the accumulation of weak resistance mechanisms to generate individuals capable of surviving higher rates. This has been confirmed by Dr Chris Preston where ryegrass hybrids possessing multiple resistance mechanisms were generated by crossing parent plants with different resistance mechanisms. Differences in the level of resistance has also been detected in self-pollinating species such as awnless barnyard grass (Figure 3).

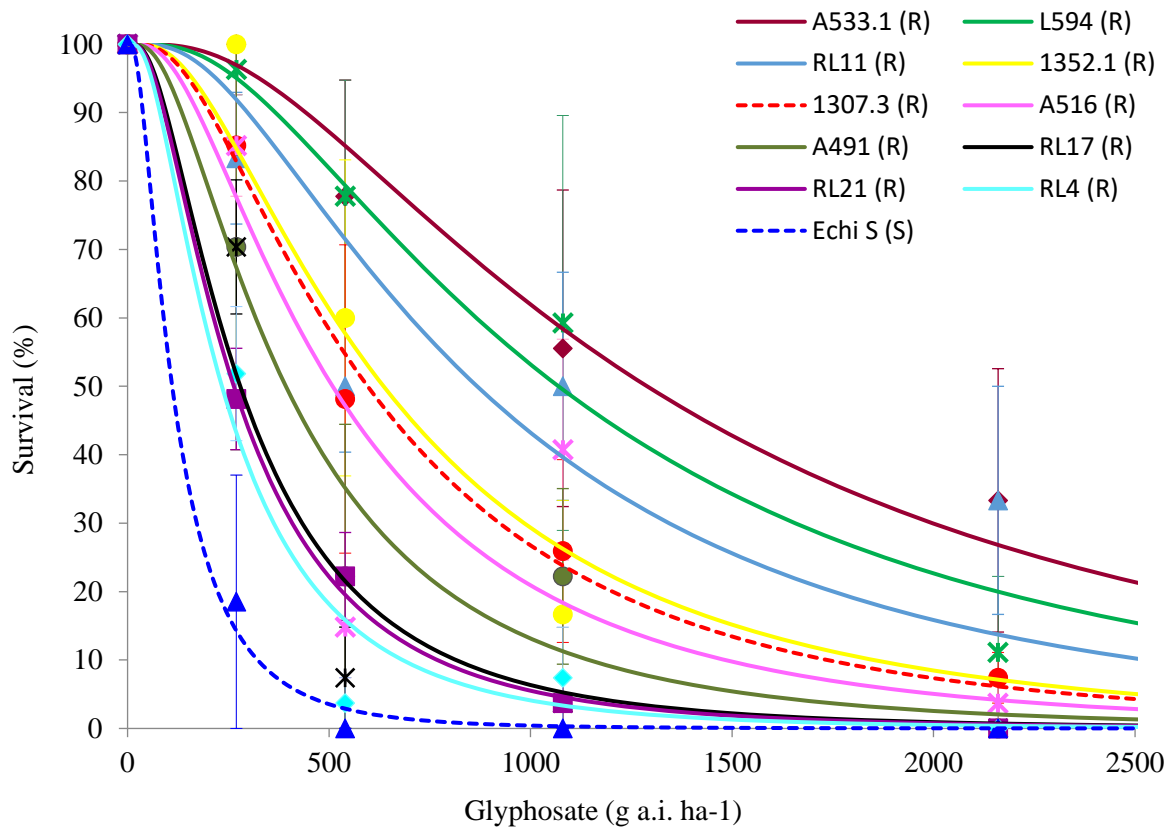


Figure 3. Response of 10 resistant awnless barnyard grass biotypes to increasing rates of glyphosate (The University of Adelaide). 'Echi S' is the susceptible control population.

Other factors that can select for glyphosate resistance by reducing efficacy include:

1. Using low quality glyphosate products and surfactants. Currently there are over 500 glyphosate products registered in Australia. Numerous trials have confirmed significant differences in activity between some glyphosate products on various weed species. In a recent outdoor pot trial conducted over summer nine glyphosate formulations were tested on susceptible barnyard grass, feathertop Rhodes grass, blackberry nightshade and glyphosate resistant sowthistle at equivalent g ai/ha rates. Significant differences were observed between most glyphosate products with some products providing significant control of glyphosate resistant sowthistle at the registered rate of 750g ai/ha. One of the most likely reasons for the difference between products is likely to be the quality of inbuilt surfactants.

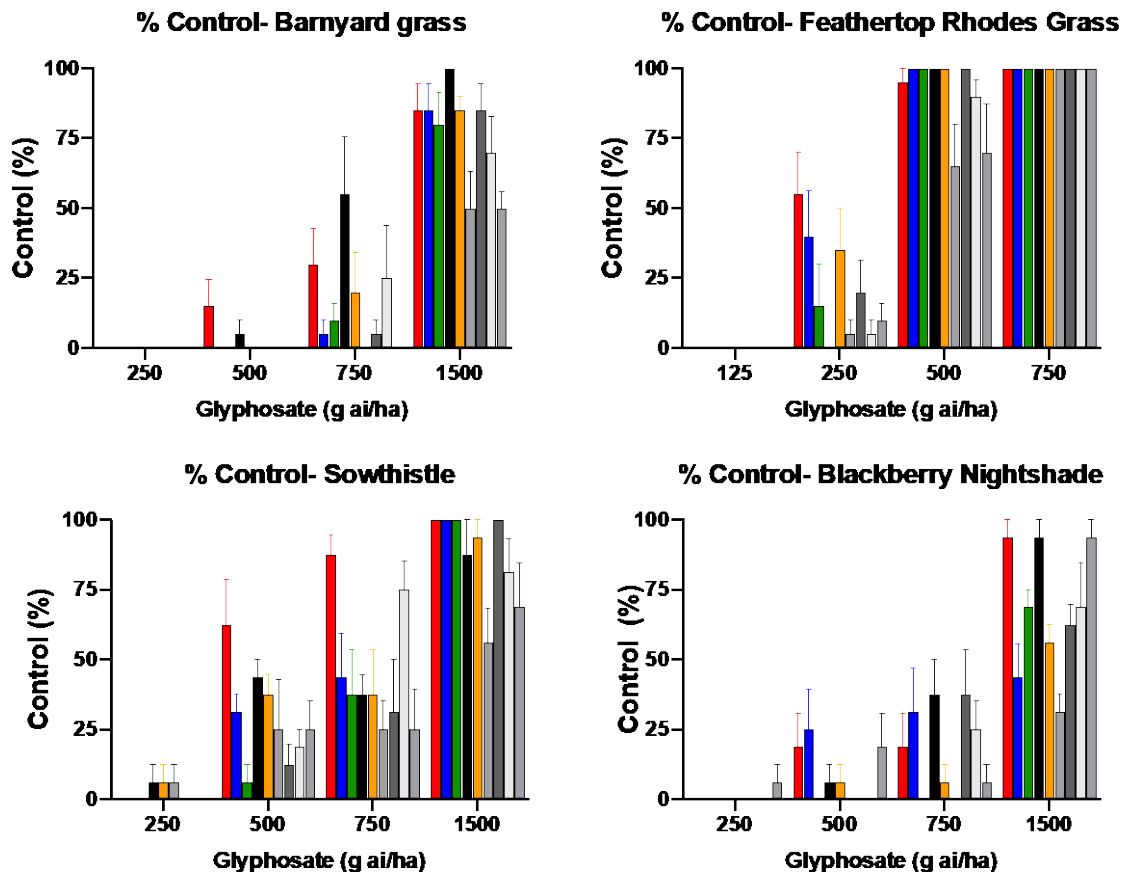


Figure 4. Control of four summer weed species with nine different glyphosate formulations. (Plant Science Consulting)

2. Mixing glyphosate with too many other active ingredients resulting in antagonism, particularly in low water volumes
3. Using low quality water, particularly hard water. Glyphosate is a weak acid and binds to positive cations (i.e. magnesium, calcium and bicarbonate) that are in high concentration in hard water (i.e. >200 ppm)
4. Applying glyphosate during periods of high temperature and low humidity, resulting in the rapid loss of glyphosate from solution on leaf surfaces thereby reducing absorption,
5. Translocation of glyphosate in stressed plants can be reduced. Optimising glyphosate performance requires the translocation to the root and shoot tips. While this can occur readily in small seedlings, in larger plants, glyphosate is required to translocate further to the root and shoot tips to maximise control
6. Shading effects reducing leaf coverage resulting in sub-lethal effects
7. Applying glyphosate onto plants covered with dust can result in reduced available product for absorption as glyphosate strongly binds to soil particles
8. Application factors such as speed and nozzle selection, boom height can reduce the amount of glyphosate coverage
9. A combination of the above factors can reduce control and increase selection for resistance.

Optimising glyphosate performance

The selection of glyphosate resistance can be minimised by considering the points above. A number of important pathways to improve glyphosate performance include:

Avoid applying glyphosate under hot conditions.

A trial spraying ryegrass during the end of a hot period and a following cool change was conducted in October 2019. Ryegrass growing in pots were sprayed at 8am, 1pm and 8pm with temperature and Delta T recorded prior to each application. Control of well hydrated plants ranged between 0% and 40% when glyphosate was applied during hot weather (30 to 32.5°C) and high Delta T (14 to 16.7) with the lowest control when glyphosate was applied at midday (Figure 5). In contrast, glyphosate applied under cool conditions just after a hot spell resulted in significantly greater control (65%-80%), indicating that plants can rapidly recover from temperature stress provided moisture is not limiting.

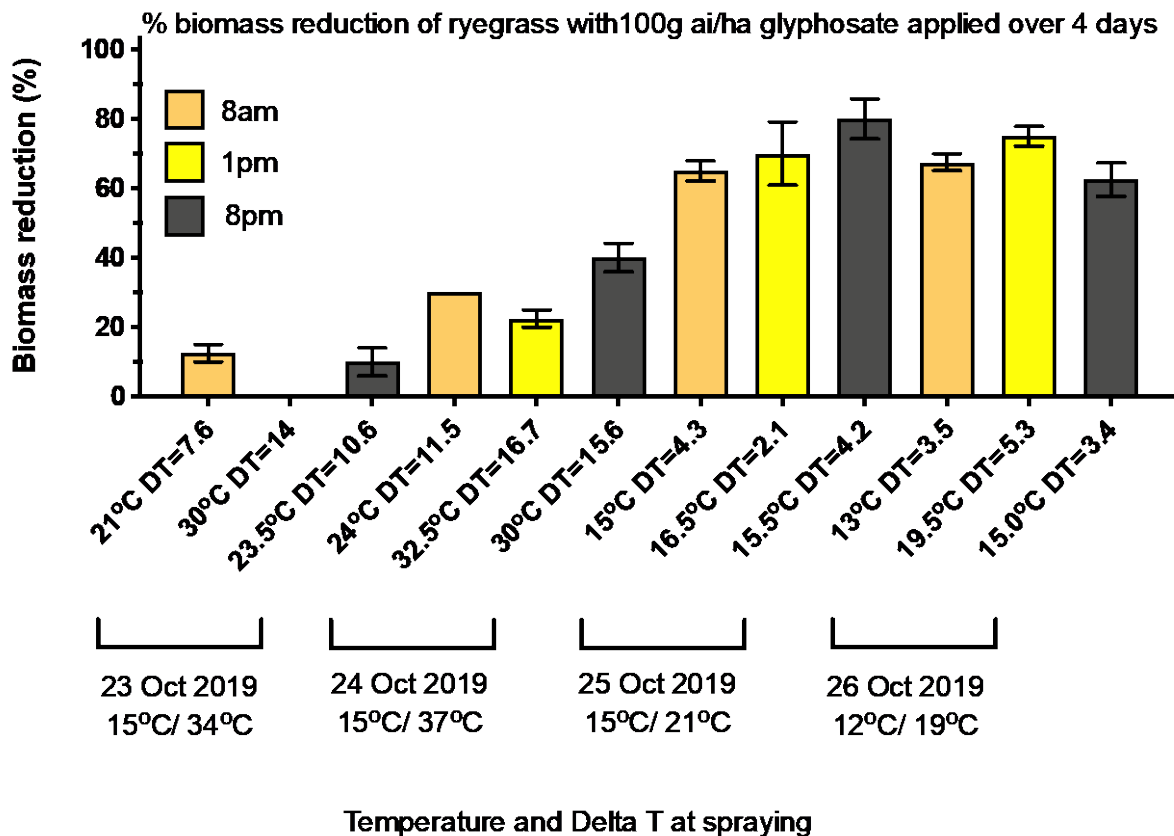


Figure 5. Effect of temperature & Delta T on glyphosate for ryegrass control (Plant Science Consulting)

(A sub lethal rate was used to differentiate between treatment differences. Plants were grown and sprayed under optimum conditions).

Reduced control can occur if plants are water stressed. In an outdoor summer pot trial, the effect of water stress on glyphosate activity on barnyard grass was assessed. A sub-set of plants were water stressed 2 days prior to application with glyphosate. Plants at three growth stages were included. Control of stressed plants (drought treatment) was significantly less than of non-stressed plants at all growth stages (Figure 6).

Barnyard grass vs glyphosate vs water stress vs growth stage
Sprayed early Feb 2020 @ 32°C

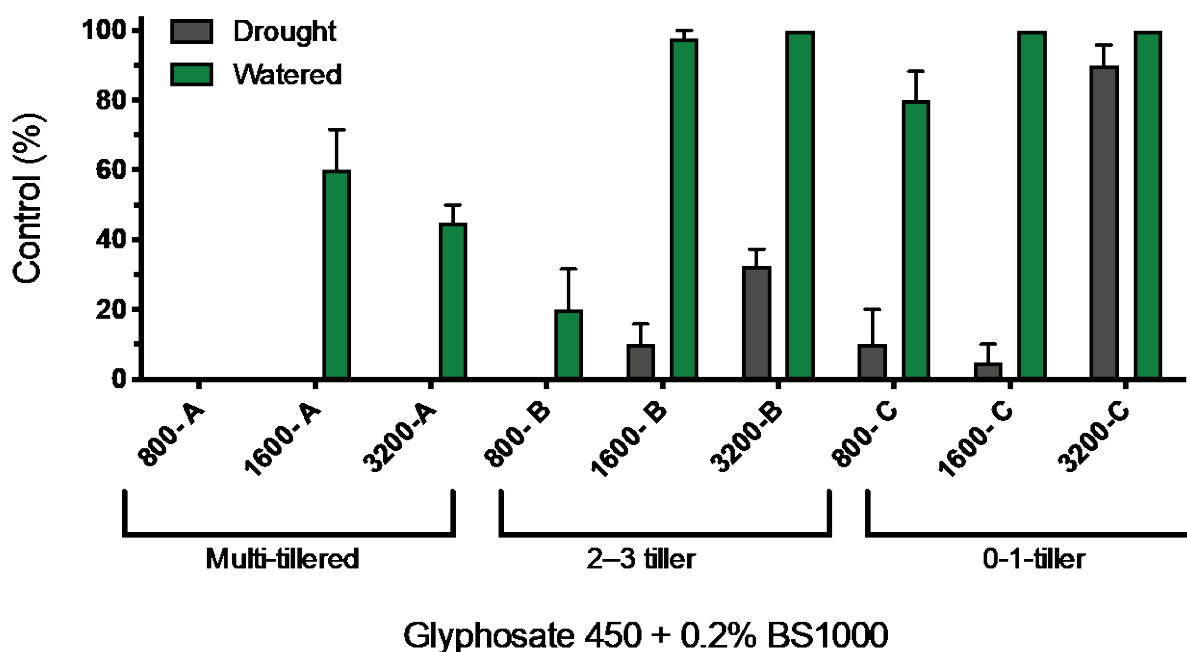


Figure 6. The effect of three rates of glyphosate on three growth stages of barnyard grass, half not water-stressed and the other half water-stressed (Plant Science Consulting)

Improving water quality and glyphosate activity by using ammonium sulfate (AMS)

The addition of AMS has several functions. One is to soften water by combining to positively charged ions such as magnesium and calcium common in hard water. The negative charged sulfate ions combine with the positive cations preventing them from interacting with glyphosate and reducing its solubility and leaf penetration. Additionally, AMS has been shown to independently improve glyphosate performance, as the ammonium ions can work with glyphosate to assist cell entry, increasing uptake and activity. In a pot trial conducted with soft water, ammonium sulfate was shown to significantly improve control of ryegrass with 222 mL/ha (100 g ai/ha) of glyphosate 450 (Figure 7). As a general rule, growers using rainwater (soft) should consider 1% of a liquid AMS formulation, if using hardwater (i.e. bore, dam) 2% AMS is recommended. The addition of a wetter resulted in a further improvement of herbicide efficacy.

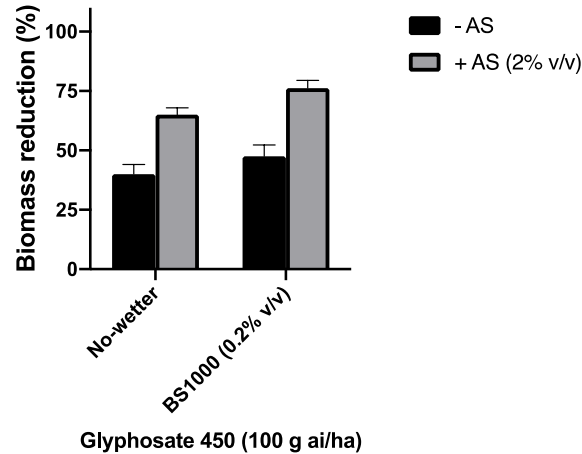


Figure 7. Effect of ammonium sulfate (AS) and wetter on glyphosate for ryegrass control (A sub lethal rate was used to differentiate between treatment differences. Plants were grown and sprayed under optimum conditions).

Herbicide activity can vary at different growth stages.

In a pot trial investigating the effect of glyphosate at 4 ryegrass growth stages (1-leaf to 4-tiller), good control was achieved at the 3 older growth stages but not on small 1-leaf ryegrass (Figure 8). Most glyphosate labels do not recommend application of glyphosate on 1-leaf ryegrass seedlings. Very small seedlings (i.e. 1-leaf) are still growing on seed reserves and have not yet commenced sugar production via photosynthesis. As a consequence, little glyphosate is translocated downwards with the sugars to the growing point of shoots and roots (meristem), reducing efficacy.

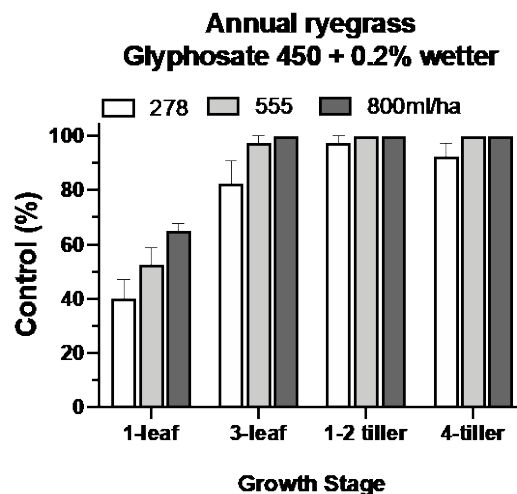


Figure 8. Effect of ryegrass growth stage on glyphosate activity (A sub lethal rate was used to differentiate between treatment differences. Plants were grown and sprayed under optimum conditions).

Double knock

A double knock strategy is defined as the sequential application of two weed control tactics directed at the same weed cohort (germination). The most common double knock strategy is glyphosate followed by paraquat. This has been widely adopted to prevent or combat glyphosate resistance in several weed species, including ryegrass. The first 'knock' with glyphosate controls the majority of

the population, with the second 'knock' (paraquat) intended to kill any individuals that have survived glyphosate. Trial work conducted by Dr Christopher Preston (Figure 9) showed that control was optimised when the paraquat was applied 1-5 days after the glyphosate for two glyphosate resistant ryegrass populations. (However optimal timing depends on weed size and growing conditions, with at least 3-5 days often being required for full glyphosate uptake and translocation, especially in larger plants). In this study, when the glyphosate resistant plants were left for 7 days before the paraquat application they can stress, resulting in the absorption of less paraquat, reducing control with the second tactic. If growing conditions are poor or plants large, the stress imposed by glyphosate maybe further delayed.

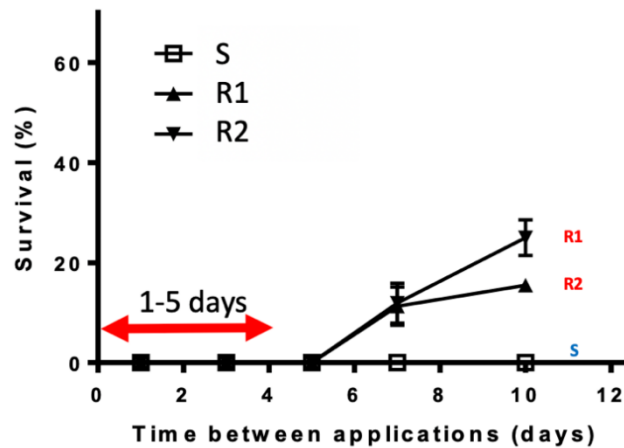


Figure 9. Double knock timing. Glyphosate applied onto a susceptible (S) and two glyphosate resistant ryegrass biotypes (R1 & R2) followed by paraquat 1, 3, 5, 7 and 10 DAA. Trial work conducted by Dr Christopher Preston (The University of Adelaide).

Incidence of paraquat resistance

Resistance to paraquat has been detected in a few ryegrass populations from WA, SA, Vic. They have originated along fencelines, non-cropped farm areas, lucerne/clover seed production paddocks and vineyards (Figure 10). Detection has been via random weed surveys and samples sent to Plant Science Consulting and Charles Sturt University following reduced control in the field. While the number remains low it is important to use paraquat according to label recommendations with emphasis on rate, growth stage and population size. The first case of paraquat resistance in ryegrass detected globally was in South African orchards after decades of use on advanced growth stages resulting in sub-lethal effects (Yu *et. al.* 2004). More locally, a sample of perennial ryegrass was confirmed highly resistant to paraquat from a vineyard in the Adelaide Hills in 2019 following application of sublethal rates of paraquat for many years to keep the ryegrass suppressed but maintain ground cover (P. Boutsalis). This sample is also highly resistant to glyphosate.

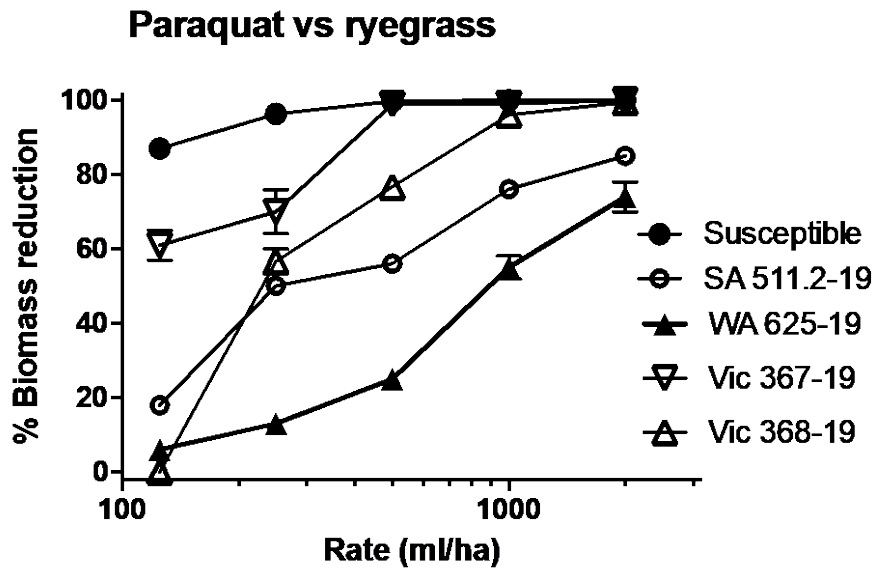


Figure 10: Efficacy of the first confirmed cases of paraquat resistance in annual ryegrass from SA, Vic and WA. Error bars indicate variation. Study conducted by Plant Science Consulting.

Additionally, two populations of barley grass resistant to paraquat and one developing resistance (10-20% survival) have been collected during the NSW random surveys.

Summary

The number of cases of glyphosate resistant weed populations continues to rise particularly for annual ryegrass, barnyard grass, feathertop Rhodes grass and sowthistle. The early break in autumn 2020 resulted in the testing of about 200 ryegrass populations prior to sowing with over half confirmed resistant to glyphosate. Decades of strong selection pressure resulting from repeated use coupled with application under sub-optimum conditions have contributed to increasing resistance levels. More efficient use of glyphosate combined with effective IWM strategies is required to reduce further increases in resistance.

Although paraquat resistance remains very low, it is concerning that it has been detected in annual ryegrass.

Acknowledgements

The information for the random weed surveys was undertaken as part of GRDC projects UCS00020 and UCS00024.

References

- Powles SB, Lorraine-Colwill DF, Dellow JJ, Preston C. (1998). Evolved resistance to glyphosate in rigid ryegrass (*Lolium rigidum*) in Australia. *Weed Science*. 46:604–607
- Yu Q, Cairns A, Powles S. (2004). Paraquat resistance in a population of *Lolium rigidum*. *Functional Plant Biology*. 31:247–254

Contact details

Peter Boutsalis

Plant Science Consulting P/L

University of Adelaide, Waite Campus, Glen Osmond SA 5064

Herbicide resistance website: www.plantscienceconsulting.com.au

@PBoutsalis

Email: peter.boutsalis@adelaide.edu.au

John Broster

Charles Sturt University, Wagga Wagga, NSW 2678

Email: jbroster@csu.edu.au

@JohnCBroster