Modelling the effectiveness of glyphosate resistance prevention strategies in Australian sub-tropical farming systems

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Summary Glyphosate resistance is a threat to the viability of sub-tropical Australian farming systems. Effective strategies to avoid or delay resistance are thus of substantial value. We used our glyphosate resistance model to perform 250 simulations of glyphosate resistance evolution in common sowthistle and awnless barnyard grass under a range of resistance prevention strategies, starting from different levels of resistance.

From these, we observed four key factors that affected strategy effectiveness. These factors should be considered when developing resistance management or prevention strategies.

1. Weed ecology. Sowthistle emerges year-round, and because less of the population is affected by any given selection event, time to resistance was longer than in barnyard grass.
2. Starting time. Waiting until the population is more than 0.01% resistant was predicted to make any preventative strategy less effective.
3. Frequency of non-glyphosate tactics. We tested a range of herbicide rotation frequencies from annually to one year in four. Few treatments that were not applied at least biennially were effective.
4. Intensity. Treating all cohorts that emerge in a given season was predicted to be much more effective than treating one cohort, even if it was the largest per year. While the efficacy of control of glyphosate survivors was important, the number of cohorts treated was more influential.

Keywords Glyphosate, herbicide resistance, modelling, *Echinochloa colona*, *Sonchus oleraceus*.

INTRODUCTION

Weed control in north-eastern Australia's sub-tropical farming systems has become substantially less diverse over recent decades due to the imperatives of minimising tillage for soil and water conservation, regulatory and environmental pressures, and post-patent price reductions of the system's most valuable herbicide, glyphosate. Both cotton and grain production in the region now rely primarily on glyphosate for weed control, and this has resulted in the selection of glyphosate-tolerant weeds and glyphosate-resistant biotypes of originally susceptible weed species (Werth *et al*. 2010). Resistance to glyphosate now threatens profitability and sustainability of cropping in this region.

While some growers may now be dealing with resistant populations, the majority are likely to be in the position of trying to maximise the time remaining before glyphosate susceptibility is lost in key weeds. We used a glyphosate resistance model (Thornby and Walker 2009) to predict the number of years of susceptibility remaining to growers taking various kinds of delaying action, starting from a range of estimated current frequencies of glyphosate resistance in two key weed species–awnless barnyard grass (*Echinochloa colona* (L.) Link) and common sowthistle (*Sonchus oleraceus* L.) We evaluated the ability of each weed control strategy for avoiding or (more likely) delaying resistance, and for the effects of inter-species differences on each strategy's effectiveness.

MATERIALS AND METHODS

Glyphosate resistance model To simulate the evolution of resistance in north-eastern Australian farming systems, we updated DAFF's glyphosate resistance model to include all weed control options used in both transgenic and conventional cotton, sorghum, wheat, sunflowers and mungbeans in the region. The model is an age- and stage-structured population model implemented in Vensim 5 (Ventana Systems, Inc.), linked to the Agricultural Production SIMulator (Keating *et al*. 2003) (APSIM). Code in both Vensim and APSIM facilitates modelling of resistance genetics as a partially dominant, single-gene.

We developed a set of parameters for APSIM's generic weed module in order to simulate additional species, including sowthistle (not shown).

Simulations We developed a set of over 250 different 30-year simulations of resistance evolution. We report here on three simulation categories:

a) intensive two-year seed bank reduction programs for summer fallows in winter grains;
b) controlling glyphosate survivors in transgenic cotton crops; and

c) integrated weed management (IWM) with non-glyphosate tactics in summer fallows in non-irrigated transgenic cotton.

In the model, awnless barnyard grass emerges only between September and March and is controlled with glyphosate after each flush in fallows if the population is greater than one plant per square metre, plus other controls as specified for each simulation. Sowthistle can emerge year-round, and is controlled with glyphosate and a broadleaf selective herbicide after emergence in fallow, if the population is above one plant per square metre, plus other controls as specified. In-crop controls are specified for each (cotton) simulation.

Two-year intensive programs In these simulations, we tested the effects of applying various combinations of double knock (glyphosate followed by paraquat a few days later), pre-emergent herbicides and non-glyphosate knockdown herbicides either on the main flush or all flushes of weeds per year for two years, with glyphosate used alone for the next three years. These simulations tested the effects of reducing the seed bank of summer fallow weeds to low levels periodically in winter cropping. Given that growers in the region have almost certainly applied glyphosate intensively for some years, separate simulations were conducted with the delaying strategy not applied until 0, 0.01, 0.1, 1, 10, or 100% resistance thresholds were reached.

Controlling glyphosate survivors We used a set of transgenic cotton simulations to test the effects of applying a follow-up tactic to control survivors of either one or all in-crop glyphosate applications at different frequencies in the rotation. This follow-up action was either inter-row tillage (80% kill rate) or inter-row tillage plus manual removal (99.9% kill rate). We tested irrigated (one crop per year) and non-irrigated (one crop per two years) rotations.

IWM in cotton In this set we varied the number of non-glyphosate actions in crop and fallow in non-irrigated cotton (one crop every two years). The in-crop non-glyphosate tactics were pre-plant and mid-season pre-emergent herbicides, full disturbance tillage at planting and/or between rows. Summer fallow strategies were glyphosate alone, or an early residual and a double knock plus glyphosate if required, designated 'IWM' hereafter. More robust IWM could certainly be devised; this represents a working minimum of non-glyphosate tactics.

RESULTS

Two-year intensive programs Nine different strategies were tested for barnyard grass and eight for sowthistle, being combinations of pre-emergent herbicides, double knocks and non-glyphosate knockdown herbicides. A subset of results is shown in Table 1. Substantial differences are predicted between species: sowthistle is slower to reach 100% resistant, and more responsive to all but the most effective two-year

Table 1. Glyphosate resistance evolution and seed bank density in barnyard grass (BYG) and sowthistle (ST) under several seed bank management strategies used for two years in every five after a threshold of resistance is reached.

Seed bank reduction strategy		
Strategy starting point	Years to >99% resistant	Long-term seed bank density* (seeds m^{-2})
Glyphosate alone		
0%	13 (BYG)	3697 (BYG)
	18 (ST)	2185 (ST)
Pre-emergent followed by glyphosate		
0%	13 (BYG)	3553 (BYG)
	20 (ST)	885 (ST)
1%	13 (BYG)	3519 (BYG)
	20 (ST)	1109 (ST)
100%	13 (BYG)	3519 (BYG)
	18 (ST)	1542 (ST)
Double knock A followed by glyphosate		
0%	13 (BYG)	3253 (BYG)
	21 (ST)	577 (ST)
1%	13 (BYG)	3301 (BYG)
	20 (ST)	733 (ST)
100%	13 (BYG)	3056 (BYG)
	18 (ST)	1304 (ST)
Double knock A + pre-emergent fb paraquat		
0%	15 (BYG)	2912 (BYG)
	29 (ST)	18 (ST)
1%	13 (BYG)	3393 (BYG)
	27 (ST)	34 (ST)
100%	13 (BYG)	3432 (BYG)
	18 (ST)	855 (ST)
Double knock B followed by paraquat		
0%	24 (BYG)	128 (BYG)
1%	13 (BYG)	362 (BYG)
100%	13 (BYG)	383 (BYG)

*Long-term seed bank density is the average number of seeds per square metre predicted in years 20–30.
Double knock A: glyphosate followed by paraquat
Double knock B: grass selective followed by paraquat

intensive tactics. In general, these two-year seed bank reduction tactics were not able to provide long delays in glyphosate resistance in barnyard grass, although the double knock with a grass selective and paraquat used on subsequent flushes provided good long-term seed bank control. For sowthistle (and in other species not shown), there is substantial benefit in acting before resistance becomes a visible problem.

Controlling glyphosate survivors The model predicted that substantial reductions in the rate of evolution of glyphosate resistance can be obtained by deliberately controlling the survivors of glyphosate applications (Tables 2 and 3). Unlike in the previous simulation set, barnyard grass was more able to respond to these delaying tactics than sowthistle, especially in irrigated systems where actions were included in every summer. Long-term seed bank control required the use of multiple applications of survivor-control methods per season in non-irrigated systems.

IWM in cotton The frequency of non-glyphosate tactics in summer fallows between non-irrigated cotton crops was predicted to be of key importance in both time to resistance and long-term control of the seed bank (Table 4). Glyphosate-only summer fallows acted against the benefit of all in-crop treatments substantially.

Pre-emergent herbicides used alone were less effective at delaying resistance and managing seed banks than a combination of pre-emergent and

Table 2. The effects of deliberately controlling barnyard grass glyphosate survivors at high or moderate efficacy at different frequencies in cotton.

Treatments used to control glyphosate survivors	Years to >99% resistant		Long-term seed bank density (seeds m^{-2})	
	Irrig	D/L	Irrig	D/L
Nil	19	13	1497	5354
IRT multiple times per crop	>30	17	1	2565
IRT+man multiple per crop	>30	25	<1	4
IRT once per crop	22	13	815	3488
IRT+man once per crop	24	14	4	3439
IRT once per two crops	19	13	1498	3488
IRT+man once per two crops	19	13	771	3439

IRT – Inter-row tillage at 80% efficacy
IRT+man – Inter-row tillage followed up with manual removal at 99.9% efficacy
Irrig – irrigated cotton, one crop per year
D/L – dryland (non-irrigated) cotton, one crop per two years

Table 3. The effects of deliberately controlling sowthistle glyphosate survivors at high or moderate efficacy at different frequencies in cotton.

Treatments used to control glyphosate survivors	Years to >99% resistant		Long-term seed bank density (seeds m^{-2})	
	Irrig	D/L	Irrig	D/L
Nil	18	18	2480	1970
IRT multiple times per crop	21	19	1378	930
IRT+man multiple per crop	>30	29	2	4
IRT once per crop	18	18	2229	1741
IRT+man once per crop	22	19	349	1329
IRT once per two crops	18	18	2496	1911
IRT+man once per two crops	20	18	2040	1671

PPPE – pre-planting pre-emergent herbicide
Layby – pre-emergent herbicide applied between rows mid-cropping
IRT – inter-row tillage

Table 4. Glyphosate resistance evolution and seed bank density of barnyard grass under several IWM strategies used in non-irrigated cotton crops with and without IWM in fallow.

Additional tactics used in crop	Years to >99% resistant	Long-term seed bank density (seeds m^{-2})
Glyphosate-only summer fallows		
Glyphosate only	13	5354
PPPE	15	5065
PPPE+layby	17	2907
PPPE+2xIRT	21	780
PPPE+2xIRT every second crop	17	2891
IWM summer fallows		
Glyphosate only	17	3392
PPPE	19	3437
PPPE+layby	20	37
PPPE+2xIRT	>30	8
PPPE+2xIRT every second crop	17	18

inter-row cultivation. Less frequent use of the most robust strategy in crop resulted in good seed bank control where IWM was used in fallows, but was of only moderate benefit for delaying resistance.

DISCUSSION

It is not surprising that more frequent use of non-glyphosate tactics equates generally to greater delay before populations become dominated by resistance, and to better long-term seed bank control of resistant populations. There are several nuances revealed in the simulations we carried out, however, regarding the possibilities for glyphosate resistance management in sub-tropical agriculture.

The differences between sowthistle and barnyard grass demonstrate that species ecology is of real importance in resistance management. In the system studied here, summer-dominant species appear to be both more susceptible to glyphosate resistance and more responsive to management by robust and diverse systems. This is likely due to interactions between sowthistle's propensity to emerge at any time of year, crop rotation and in-crop management, and the use of selective herbicides in fallow. Indeed, barnyard grass was also best managed by treatments that included a grass selective in fallows (Table 1). Strategies for managing resistant populations must clearly be devised with reference to species ecology, rather than generically.

Growers in sub-tropical northern Australian cropping are almost certainly not dealing with completely unselected weed populations – that is, they may have some non-trivially elevated level of resistance-conferring alleles in weed populations even where glyphosate resistance is still effectively invisible. Simulations of different starting times for the two-year intensive programs (Table 1) paint a grim picture, suggesting that glyphosate resistance will be hard to avoid entirely, but show that long-term seed bank management is possible where a robust management strategy is applied before the population becomes mostly or entirely resistant, rather than waiting until glyphosate is entirely ineffective. Sowthistle simulations showed this most clearly here, but it is also apparent in other species we modelled (data not shown).

The efficacy of non-glyphosate treatments was important to the effectiveness of resistance-delaying strategies: in particular, strategies that relied only on residual herbicides (which, due to variations in timing between application and germination, often had relatively low efficacies) were less effective than those including knockdown herbicides or tillage. However, the results for controlling-survivors simulations in cotton show that applying inter-row tillage multiple times per season is a better option than using more effective tactics less often, especially in non-irrigated systems.

Simulations such as these can be used to test a large number of permutations of influential factors and possible answers to management problems – permutations that would be difficult to test in real-world experiments. Our simulations suggest the following important points for strategy development:

1) devise resistance prevention and management plans with reference to species characteristics, including seed bank dynamics;
2) start managing resistance before it is common enough to be visible or dominant in order to gain control of resistant seed banks in the longer term;
3) apply routine non-glyphosate tactics at least biennially to control resistant seed banks; and
4) managing multiple cohorts in 'intensive' years is more effective than spreading non-glyphosate actions over several predominantly glyphosate-centric years.

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