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Managing glyphosate resistance in Australian cotton farming: modelling shows how to delay evolution and maintain long-term population control

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Abstract. Glyphosate resistance is a rapidly developing threat to profitability in Australian cotton farming. Resistance causes an immediate reduction in the effectiveness of in-crop weed control in glyphosate-resistant transgenic cotton and summer fallows. Although strategies for delaying glyphosate resistance and those for managing resistant populations are qualitatively similar, the longer resistance can be delayed, the longer cotton growers will have choice over which tactics to apply and when to apply them. Effective strategies to avoid, delay, and manage resistance are thus of substantial value. We used a model of glyphosate resistance dynamics to perform simulations of resistance evolution in *Sonchus oleraceus* (common sowthistle) and *Echinochloa colona* (awnless barnyard grass) under a range of resistance prevention, delaying, and management strategies.

From these simulations, we identified several elements that could contribute to effective glyphosate resistance prevention and management strategies. (*i*) Controlling glyphosate survivors is the most robust approach to delaying or preventing resistance. High-efficacy, high-frequency survivor control almost doubled the useful lifespan of glyphosate from 13 to 25 years even with glyphosate alone used in summer fallows. (*ii*) Two non-glyphosate tactics in-crop plus two in-summer fallows is the minimum intervention required for long-term delays in resistance evolution. (*iii*) Pre-emergence herbicides are important, but should be backed up with non-glyphosate knockdowns and strategic tillage; replacing a late-season, pre-emergence herbicide with inter-row tillage was predicted to delay glyphosate resistance by 4 years in awnless barnyard grass. (*iv*) Weed species' ecological characteristics, particularly seed bank dynamics, have an impact on the effectiveness of resistance strategies; *S. oleraceus*, because of its propensity to emerge year-round, was less exposed to selection with glyphosate than *E. colona*, resulting in an extra 5 years of glyphosate usefulness (18 *v.* 13 years) even in the most rapid cases of resistance evolution.

Delaying tactics are thus available that can provide some or many years of continued glyphosate efficacy. If glyphosate-resistant cotton cropping is to remain profitable in Australian farming systems in the long-term, however, growers must adapt to the probability that they will have to deal with summer weeds that are no longer susceptible to glyphosate. Robust resistance management systems will need to include a diversity of weed control options, used appropriately.

Additional keywords: cotton, *Echinochloa colona*, glyphosate, herbicide resistance, modelling, *Sonchus oleraceus*.

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Introduction

Weed control in Australian cotton farming has become substantially less diverse over recent decades (Werth *et al*. 2008), owing to the imperatives of minimising tillage for soil and water conservation, regulatory and environmental pressures, post-patent price reductions of the system's most useful herbicide (glyphosate), and the broad adoption of glyphosate-resistant crops. All of these factors have increased the use and importance of glyphosate for weed control in Australian cotton production, and at the same time reduced the use of other herbicides and tillage. Cotton production in Australia is especially reliant on glyphosate for weed control; well over 90% of the area planted to cotton each year carries a glyphosate-resistance trait. In recent years, cotton has been planted on up to 600 000 ha per season in New South Wales and Queensland (ABARES 2012). Crops may be planted annually if irrigation is available, or biennially or less frequently without irrigation. In dryland situations, cotton has typically been grown in rotation with other crops, usually with winter cereals (Walker *et al*. 2005). Summer fallows are used between winter crops, and between cotton crop years in dryland cropping, to allow incorporation and retention of soil moisture for use by subsequent summer crops. Where summer fallows are included in the cropping rotation, glyphosate is also the main method of weed control (Walker *et al*. 2005; Osten *et al*. 2007). Therefore, whether summer cropping is annual, biennial, or even less frequent, summer-active weeds are under substantial selection pressure for glyphosate resistance. Loss of control of summer annual weed species, including through resistance, is a substantial threat to cotton production in this system.

Over-reliance on glyphosate in subtropical Australian farming has resulted in the selection of both glyphosate-tolerant weeds (Werth *et al*. 2010) and glyphosate-resistant biotypes of *Echinochloa colona* (L.) Link. (awnless barnyard grass), a usually susceptible summer annual grass (Nguyen Thai *et al*. 2012). Both resistant and tolerant species make good control of the weed spectrum with glyphosate difficult, and since managing weeds with a diversity of methods is both more complex and more expensive than using glyphosate alone, resistance (and indeed even the need for strategies to delay the onset of resistance) threatens the long-term viability of many cotton farms.

Australian cotton is predominantly grown in subtropical north-eastern Australia. Although some growers in this region are now faced with glyphosate-resistant populations of *E. colona* (Preston 2012), in the majority of fields, growers are still likely to be trying to maximise the time remaining before glyphosate susceptibility is lost in this weed. There are also various other locally important species that are not yet resistant. Therefore, attempting to create substantial delays in the onset of resistance remains a viable goal for most growers for at least some fields, even while the management of resistant populations is rapidly becoming the major imperative.

In Australia, transgenic glyphosate-resistant cotton cropping is subject to regulation via a Crop Management Plan (CMP) (Monsanto Australia 2013). The CMP mandates that growers must take action to prevent seed set in any weeds that survive glyphosate applications, but does not include specific instructions on how this is done. Substantial variability likely exists in how dedicated and effective individuals are at finding survivors, and when and how they attempt to kill them. We used an updated version of our glyphosate resistance model (Thornby and Walker 2009) to explore how this variability might affect the usefulness of the survivor-control approach.

Through mode-of-action labelling on products, the CMP, and best management practice advice in general, growers are encouraged to use a variety of methods to control weeds in an integrated weed management (IWM) program (McGillion and Storrie 2006). Several pre-emergent and post-emergent herbicides applied at various timings, and inter-row cultivation, are available in-crop, and tillage plus a wider range of herbicides can also be used in summer fallows (Maas 2012). We used our updated model to predict the effectiveness of the range of IWM tools available in cotton cropping systems in Australia, both in-crop and in-fallow.

In order to explore the effects of these variations on evolution of glyphosate resistance, we simulated the number of years of glyphosate susceptibility for two key weed species, *E. colona* and *Sonchus oleraceus* L. (common sowthistle), under a range of different management strategies in irrigated and non-irrigated cotton systems. Both of these weeds are frequently observed to be among the most prevalent species in subtropical north-eastern Australia generally (as in Osten *et al*. 2007), or on Australian cotton farms specifically (Werth *et al*. 2010). Thus, loss of control of these weeds would be (or is, in the case of *E. colona*) a widespread threat to the sustainable use of glyphosate-resistant cotton in Australia. Both species scored in the top six of all weeds listed on glyphosate product labels in Australia for propensity to evolve herbicide resistance (Werth *et al*. 2011). We evaluated the outcome of each weed control strategy in terms of delaying resistance, the effects of ecological differences between species on the effectiveness of each strategy, and the impact on long-term weed population density. We aimed to use the model's predictions to develop a set of strategies that could guide grower decision-making towards minimising the rate of evolution of glyphosate resistance in *E. colona* and *S. oleraceus*, and maximising weed population control over the long-term, even where resistance occurs.

Materials and methods

Glyphosate resistance model

To simulate the evolution of resistance in Australian cotton farming systems, we updated our glyphosate resistance model to include representations of all the typical weed control options used in herbicide-resistant and conventional cotton in subtropical north-eastern Australia. The model is an age- and stage-structured population model that simulates weed population density and changes in allele frequencies for a resistance-conferring gene. The model is implemented in Vensim 5 (Ventana Systems, Inc., Harvard, MA, USA), linked to the Agricultural Production Systems Simulator (APSIM; Keating *et al.* 2003). Through APSIM, the model runs on a daily time-step and allows for variation in weather conditions and therefore weed control requirements from year to year.

Various APSIM modules are used to provide functionality around plant growth, competition between weeds and crops, and resource availability for growth and development such as water and nutrients. The APSIM manager module also provides for on-event scripting for weed management and flags for mating and germination events. The Vensim extensions (as outlined in Thornby and Walker 2009) model resistance genetics, mating between genotypes, and seed bank dynamics, since APSIM does not directly model population persistence between seasons. Resistance is assumed to be due to a single, partially dominant gene conferring moderate levels of resistance, as has been found in the resistant *E. colona* populations studied to date (Nguyen Thai *et al.* 2012) and in other species with glyphosate resistance (Powles and Preston 2006). Figure 1 provides a schematic view of the breakdown between population dynamics equations in Vensim and APSIM's plant growth and development code and management scripting. A summary of the model is given below, summarised from Thornby and Walker (2009).

There are three seed bank fractions, defined as age classes: <1 year old; 1–2 years old; $\geq$3 years old; and three genotypes (homozygous susceptible, homozygous resistant, heterozygous). The size S of the seed bank fractions i for genotype g in any year n is determined as:

$$S_{ig(n)} = S_{ig(n-1)} + E_{g(n-1)} - [G_{ig(n)} + M_{ig(n)} + T_{ig(n)}] \quad (1)$$

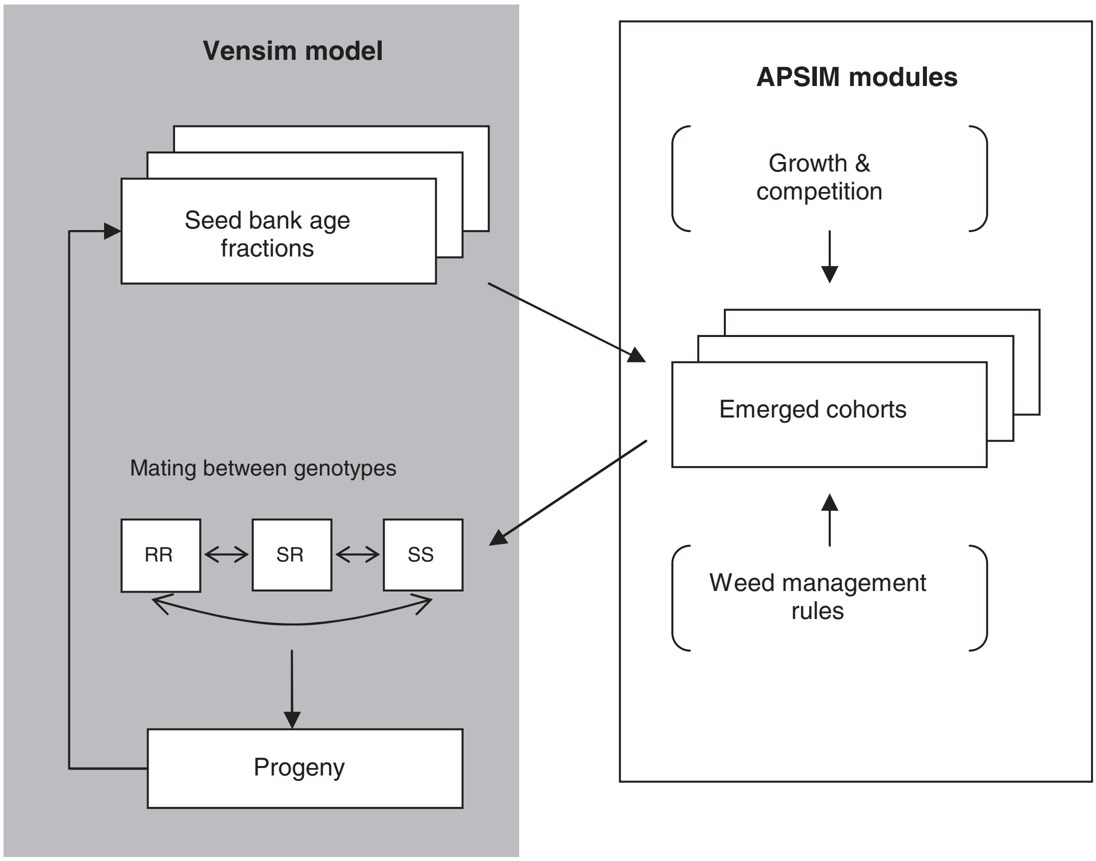

Fig. 1. Schematic view of the DAFF glyphosate resistance model, showing information flows within and between population dynamics (Vensim) and plant growth and weed management sub-models (APSIM).

where E is the number of seeds entering the seed bank fraction, either from seed rain for the first fraction or as transport from younger fractions (that is, ageing) for fractions two and three; G is the number of germinants from a seed bank fraction; M is the seed bank mortality for a fraction; and T is the transport out from a fraction to the next oldest fraction (again simulating ageing), if applicable, at the end of the season. Seed bank mortalities are applied as a simple multiplier per fraction.

Seedling cohorts are germinated as a coarse estimated accumulation of staggered germinations over time. Up to four cohorts may germinate per year for any species, each on a single day in the model, with contributions from each age fraction of the seed bank for each genotype. The size of a cohort C of genotype g emerging from seed bank age fraction i is determined thus:

$$C_{gi} = C_{\mathrm{gmax}} \cdot D_{\mathrm{rain}} \cdot D_{\mathrm{therm}} \cdot D_{\mathrm{seed}\,i} \qquad (2)$$

where C_{gmax} is the maximum number of seeds that could germinate in a cohort (a fixed proportion of the current seed bank); D_{rain} is a discount factor for rainfall amounts accumulated in APSIM over 5 days that fall between the minimum and the optimum; D_{therm} is a discount factor for thermal time in degree-days (base 12°C) accumulated since the beginning of the season or the time of the last cohort; and D_{seed} is a discount factor that reduces potential germination in older seed bank age fractions. All discount factors D have values $0 < D \leq 1$ (Table 1).

As in the previous version of the model, we developed a set of parameters for APSIM's generic weed module in order to model additional species, including *S. oleraceus* (Table 1). Parameter values for *E. colona* were also altered to reflect more recent data.

Simulations

We developed a range of scenarios of resistance evolution in cotton, in three categories: (*a*) crop rotations; (*b*) frequency and efficacy of controlling glyphosate survivors in transgenic cotton crops; and (*c*) IWM strategies, varying diversity of weed management actions in crops and summer fallows.

In each of these simulations, the population is assumed to have had no prior selection by glyphosate. Each scenario is simulated over 30 years. A baseline strategy of glyphosate-only applications in annual glyphosate-resistant cotton crops is common to all three sets of simulations *a*, *b*, and *c*.

In all simulations, *E. colona* emerges after rainfall between September and March and is sprayed with glyphosate after each flush in fallows if the population is greater than the population density threshold of 0.5 plants m^{-2} (Table 1), plus other controls as specified for each simulation (see Tables 2, 3, 4). *Sonchus oleraceus* can emerge year-round (Widderick *et al*. 2010), and is controlled after emergence in fallows with one application of glyphosate plus a broadleaf-selective herbicide (a common

Table 1. Biological parameter values for *Echinochloa colona* and *Sonchus oleraceus* used in APSIM and Vensim modules to simulate evolution of glyphosate resistance

Parameter	Units	Value per species	
		Echinochloa colona	*Sonchus oleraceus*
Initial weed seed bank density	seeds m^{-2}	40	60
Frequency of resistance alleles in unselected population		1×10^{-8} (typical estimate)	1×10^{-8} (typical estimate)
Mean maximum seed production per plant	seeds $plant^{-1}$	42 000 (Mercado and Talata 1977)	5500 (Salisbury 1942)
Annual mortality of ≤1-year-old seed		0.1 (S. Walker, H. Wu, J. Werth, unpubl.)	0.2 (Chauhan *et al*. 2006; Widderick *et al*. 2010)
Annual mortality of 1–2-year-old seed		0.4 (Martinkova *et al*. 2006)	0.6 (M. Widderick, S. Walker, unpubl.)
Annual mortality of seed >2 years		0.95 (Walker *et al*. 2006; Uremis and Uygur 2005)	0.95 (M. Widderick, S. Walker, unpubl.)
Fraction of seed rain entering the seed bank		0.7 (S. Walker, H. Wu, D. Minkey, unpubl., predation studies)	0.6 (M. Widderick, S. Walker, unpubl., seed fragility)
Proportion of flowers self-pollinated		0.95 (Madsen *et al*. 2002)	0.95 (Boutsalis and Powles 1995)
Maximum density of weeds reaching reproduction	plants m^{-2}	2000 (D. Thornby, J. Werth, unpubl. estimate)	600 (D. Thornby, M. Widderick, unpubl. estimate)
Maximum germination from one cohort as a proportion of current seed bank (*Cgmax*)		0.5 (Wu *et al*. 2004; S. Walker, J. Werth, unpubl.)	0.4 (Dorado *et al*. 2009; M. Widderick, unpubl. estimates)
Discount factor for germination from 1–2-year-old seed bank fraction ($Dseed_2$)		0.2 (as above)	0.2 (D. Thornby, M. Widderick, unpubl.)
Discount factor for germination from >2-year-old seed bank fraction ($Dseed_3$)		0.15 (as above)	0.15 (D. Thornby, M. Widderick, unpubl.)
Base temperature for degree days calculations	°C	12 (Martinkova *et al*. 2006)	7 (Steinmaus *et al*. 2000)
Minimum accumulated temperature required since start of season for germination	degree-days	120 (Werth 2007)	40 (D. Thornby, M. Widderick, unpubl.)
Optimal accumulated temperature since start of season for germination	degree-days	300 (Werth 2007)	2000 (Dorado *et al*. 2009)
Minimum accumulated rainfall for germination of a cohort	mm rain accum. over 4 days	40 (Werth 2007; J. Werth, D. Thornby, unpubl.)	20 (estimated from Widderick 2002)
Optimal accumulated rainfall for germination of a cohort	mm rain accum. over 4 days	100 (Werth 2007; J. Werth, D. Thornby, unpubl.)	90 (estimated from Widderick 2002)
Plant canopy height	mm	200 (D. Thornby, unpubl.)	500 (D. Thornby, unpubl.)
Fallow weed control application threshold (minimum)	Plants m^{-2}	0.5	0.5

Table 2. List of cropping rotations simulated with a glyphosate resistance model to test the evolution and population dynamics of *Echinochloa colona* and *Sonchus oleraceus* under a range of different rotations that include cotton

GRC, Glyphosate-resistant cotton; CC, conventional cotton

Rotation	Scenario	Summer 1	Winter 1	Summer 2	Winter 2	Summer 3	Winter 3	Summer 4	Winter 4	Summer 5	Winter 5	Summer 6	Winter 6
Annual GRC	1	GRC	Fallow										
Biennial GRC	2	GRC	Fallow	Fallow	Fallow								
Annual GRC/CC	3	GRC	Fallow	GRC	Fallow	CC	Fallow						
Biennial GRC/CC	4	GRC	Fallow	Fallow	Fallow	GRC	Fallow	Fallow	Fallow	CC	Fallow	Fallow	Fallow
GRC/sorghum	5	GRC	Fallow	Fallow	Fallow	Sorghum	Fallow	Fallow	Fallow				
Wheat/GRC	6	Fallow	Wheat	Fallow	Wheat	Fallow	Wheat	Fallow	Wheat	Fallow	Fallow	GRC	Fallow

control measure in the real system), followed by glyphosate alone at 4-week intervals, if the population is >0.5 plants m^{-2}, plus other controls as specified for each scenario. In all simulations, the non-glyphosate controls were applied regardless of weed density.

In all three sets of simulations, we analysed annual and biennial cotton cropping. In biennial cropping, the non-cotton summers remain fallow. Management of summer fallows is critically important for maximising crop yields in this system, so we have incorporated scenarios to predict the difference between using glyphosate alone and glyphosate plus other tactics in summer fallows.

Crop rotation

We used the model to analyse several subtropical, north-eastern Australian, mixed-crop rotations for comparison with cotton-only rotations (Table 2). Many of this region's non-irrigated growers are highly responsive to changes in commodity prices

Table 3. Scenarios of different frequencies and efficacies of controlling survivors of glyphosate applications in annual or biennial continuous glyphosate-resistant cotton to test evolution of glyphosate resistance and population dynamics of *Echinochloa colona* and *Sonchus oleraceus*

IWM, Integrated weed management, here referring to one pre-emergence herbicide application followed by a double-knock (paraquat plus glyphosate) followed by glyphosate. Ticks indicate the use of IWM or glyphosate-only tactics in summer fallows between biennial cotton crops. Values indicate the number of times survivor control occurs per crop, with 1/2 indicating one application every second crop

Scenario	80% Survivor control	99.9% Survivor control	IWM summer fallows	Glyphosate-only summer fallows
7	3×			
8		3×		
9	1×			
10		1×		
11	1/2			
12		1/2		
13	3×		✓	
14		3×	✓	
15	1×		✓	
16		1×	✓	
17	1/2		✓	
18		1/2	✓	
19	3×			✓
20		3×		✓
21	1×			✓
22		1×		✓
23	1/2			✓
24		1/2		✓

Table 4. List of scenarios of different frequencies and types of integrated weed management (IWM) strategies including various non-glyphosate tactics in annual or biennial continuous glyphosate-resistant cotton to test evolution of glyphosate resistance and population dynamics of *Echinochloa colona* and *Sonchus oleraceus*

IWM here refers to one pre-emergence herbicide application followed by a double-knock (paraquat plus glyphosate) followed by glyphosate; layby, a mid-season pre-emergence herbicide treatment applied between planting rows. In-crop tactics are those added in addition to normal glyphosate applications in glyphosate-tolerant cotton. Ticks indicate the use of tactics in each crop, and/or each summer fallow between biennial cotton crops where summer fallows are indicated. Values indicate the number of times an application is made per crop, with 1/2 indicating one application every second crop and 1/2, 2× indicating two applications every second crop

Scenario	Pre-plant pre-emergence herbicide	Layby	Inter-row tillage	IWM summer fallows	Glyphosate-only summer fallows
25	✓				
26	1/2				
27	✓	✓			
28	1/2	1/2			
29	✓			✓	
30	1/2			✓	
31	✓	✓		✓	
32	✓				✓
33	1/2				✓
34	✓	✓			✓
35	1/2	1/2		✓	
36	1/2	1/2			✓
37			2×		
38			2×	✓	
39			2×		✓
40			1×		
41			1×	✓	
42			1×		✓
43			1/2		
44			1/2	✓	
45			1/2		✓
46	✓		2×		
47	✓		2×	✓	
48	✓		2×		✓
49	1/2		1/2, 2×		
50	1/2		1/2, 2×	✓	
51	1/2		1/2, 2×		✓

and available soil moisture, and consequently grow diverse rotations including cotton and winter and summer grains and pulses. We tested several rotations as examples of Australian cropping systems, both representative of current practice, and speculative: (*i*) glyphosate-resistant cotton alone; (*ii*) two glyphosate-resistant followed by one conventional cotton; (*iii*) glyphosate-resistant cotton and sorghum in alternate years; (*iv*) four winter wheat crops followed by a long fallow and one glyphosate-resistant cotton crop in the subsequent summer.

Weed-control tactics for the rotation scenarios were as follows. In glyphosate-resistant cotton crops, only glyphosate was used. In conventional cotton, pre-emergence herbicides (i.e. soil-applied herbicides used to control weeds not yet emerged) were applied before sowing and mid-season, and glyphosate was applied at planting. In sorghum crops, a pre-emergence herbicide and glyphosate were used before sowing, and glyphosate was applied before harvest. In wheat, glyphosate was applied at sowing, and one broadleaf herbicide application was made in-crop. In summer fallows between wheat crops, a pre-emergence herbicide was used before first emergence of summer weeds, and glyphosate was applied to subsequent emergences over the population density threshold. In each case of the use of pre-emergence herbicide, we assumed that the herbicide used was one appropriate to the soil, climate, and plant-back situation in which it is applied. Efficacy was 90% on the day of application, falling to zero over a period of 8 weeks.

Controlling glyphosate survivors

We used a set of simulations to test the effects of applying a follow-up tactic to control survivors of glyphosate applications at different frequencies in glyphosate-resistant cotton cropping (Table 3). The follow-up action was at either 80% kill rate or 99.9% kill rate, and was applied after every glyphosate application, after one glyphosate application per crop, or after one glyphosate application every second crop. We tested annual and biennial cotton rotations as defined above, so that in the least-frequent case, one follow-up application was being applied every 4 years.

In the biennial cotton simulations, we tested the frequency and efficacy of survivor control against a background of either glyphosate-only summer fallows or 'IWM fallows', in which a pre-emergent herbicide is applied shortly before first summer weed emergence, a 'double-knock' (glyphosate followed a few days later by paraquat) is applied to the first-emerging cohort, and glyphosate is used on subsequent emergences if the trigger weed density threshold is exceeded.

Integrated Weed Management in cotton

In this set, we varied the number of non-glyphosate actions in crop and fallow in glyphosate-resistant cotton (Table 4). The in-crop, non-glyphosate tactics were pre-plant and midseason (known as 'layby') pre-emergent herbicides, full disturbance tillage at planting, and tillage between rows (affecting an estimated 80% of the weed population). The layby herbicide is assumed to have minimal impact on the crop's competitiveness with weeds. Potential tillage effects on subsequent emergences or on soil structure are not modelled. Summer fallow strategies used in biennial cotton were either with IWM (a spring-applied, pre-emergence herbicide and a double-knock, plus glyphosate up to every 4 weeks thereafter if required), or with glyphosate alone, as in the survivor control simulations described above.

In all simulations, glyphosate is applied three times over the top of every glyphosate-resistant cotton crop.

Results

Biennial cropping with glyphosate-resistant cotton and without non-glyphosate tactics in summer fallows was, overall, the worst-case scenario for *E. colona* (scenario 1, Table 5). Glyphosate resistance was predicted to evolve in 13 years, and long-term seed-bank density was the highest of all scenarios. Results for *S. oleraceus* were less clear; biennial cropping with glyphosate alone resulted in resistance after 18 years, but several other scenarios had similar results and two were slightly faster (see below).

Crop rotations

In annual cotton cropping scenarios, switching between glyphosate-resistant and conventional cotton varieties had little effect on either rate of resistance evolution or long-term seed bank population size, adding only a year at most to the useful lifespan of glyphosate (from 18 to 19 years to resistance in *S. oleraceus*, scenarios 1 and 3, Table 5). In biennial situations, moderate benefits for slowing resistance were predicted for both weeds (scenarios 2–6). Two years were added to time-to-resistance where glyphosate was used alone in summer fallows (up to 15 years in *E. colona* and 20 years in *S. oleraceus*). Greater benefits were obtained by adding a pre-emergence herbicide and a double-knock to summer fallows, however. Switching between glyphosate-resistant and conventional cotton and using two non-glyphosate actions in the summer fallows increased the predicted useful lifespan of glyphosate on *E. colona* to 20 years, an increase of almost 50% over the worst case, and on *S. oleraceus* by >60% (scenario 3). Seed bank density was similarly reduced; very low-density seed banks were predicted for *S. oleraceus* when non-glyphosate actions were added to summer fallows (7–187 seeds/m^2). Seed banks were reduced by ~40% for *E. colona*, compared with the worst case, although the levels achieved (>3000 seeds m^{-2}) would still be termed inadequate long-term control.

Switching to a cotton–sorghum rotation (scenario 5) was not predicted to offer any benefits over glyphosate-resistant cotton alone for *S. oleraceus*, but was of substantial benefit for long-term *E. colona* control, reducing long-term seed bank density from >5000 to <500 seeds m^{-2}, and delaying resistance by up to 7 years, where non-glyphosate actions were used in summer fallows. The cotton–sorghum rotation was the only one of this set of scenarios in which satisfactory long-term control of *E. colona* seed banks was obtained.

The wheat–cotton rotation was marginally better than cotton alone for *E. colona* (scenario 6). For *S. oleraceus*, long-term seed bank control was particularly improved (down to 4–287 seeds m^{-2}), and where IWM measures were used in summer fallows, this was one of only a few simulations in the whole experiment in which resistance did not come to dominate the population within 30 years.

Table 5. Changes in rate of evolution of glyphosate resistance and long-term seed bank population density (mean of years 20–30 of 30-year simulations) of *Echinochloa colona* and *Sonchus oleraceus* under several different cropping rotations with cotton, sorghum, and wheat

GR, Glyphosate resistant; IWM, integrated weed management. Ratios indicate the frequency of listed crops in a rotation

Scenario	Rotation	Years to >99% resistant		Long-term seed-bank density (seeds m^{-2})	
		E. colona	*S. oleraceus*	*E. colona*	*S. oleraceus*
	Annual cropping				
1	GR cotton alone	19	18	1497	2840
3	GR and conventional cotton, 2 : 1	19	19	1531	2174
	Biennial cropping, glyphosate-only summer fallows				
2	GR cotton alone	13	18	5354	1970
4	GR and conventional cotton, 2 : 1	15	20	4768	1724
5	GR cotton and sorghum, 1 : 1	15	17	2326	1816
6	Wheat and GR cotton, 4 : 1	13	18	3187	287
	Biennial cropping, IWM summer fallows				
2	GR cotton alone	17	24	3392	187
3	GR and conventional cotton, 2 : 1	20	29	3422	7
5	GR cotton and sorghum, 1 : 1	24	24	429	10
6	Wheat and GR cotton, 4 : 1	19	>30	2859	4

For both weeds, there was a substantial difference between scenarios with glyphosate-only summer fallows and those with IWM used in summer fallows. Resistance evolution was delayed by up to 9 years in *E. colona* and ≥9 years in *S. oleraceus*. Long-term average seed-bank densities were reduced by ~10–80% in *E. colona* and by 90–99% in *S. oleraceus*, just by adding two non-glyphosate actions to summer fallows.

Controlling glyphosate survivors

The model predicted that substantial reductions in the rate of evolution of glyphosate resistance could be obtained by deliberately controlling the survivors of glyphosate applications (Table 6). *Echinochloa colona* was more likely to respond to these delaying tactics than *S. oleraceus*, including delays of up to 12 years (almost doubling the worst-case lifespan of glyphosate) in biennial cropping with glyphosate-only summer fallows (scenario 14), and delays in resistance beyond 30 years in annual cropping scenarios (7 and 8) and one biennial cropping scenario with IWM tactics used in summer fallows (scenario 20). Long-term seed bank control in biennial cropping was adequate for *E. colona* (reduced from ~5000 to 1000 seeds m^{-2}) or very good (down to 3 seeds m^{-2}) where multiple applications of survivor-control methods were made per season, for glyphosate-only and IWM summer fallows, respectively (scenarios 19 and 20). Similar seed-bank size predictions were found for *S. oleraceus* except that summer fallow IWM added much greater benefits in biennial cropping scenarios. While efficacy was important in delaying resistance and providing long-term control of population size, using any tactic at high frequency was better than using very high efficacy tactics infrequently.

Integrated Weed Management in cotton

The model predicted that varying levels of success could be obtained by using combinations of non-glyphosate tactics in-crop and in summer fallows (Table 7). Notably, there were substantial differences between annual and biennial cropping in the case of *E. colona*. In annual cropping scenarios, every strategy using more than one non-glyphosate action in every crop resulted in resistance evolution being pushed beyond the 30-year time limit of the model (scenarios 27, 37, 40, 46, 49). Where non-glyphosate controls were applied in every crop (scenarios 27, 37, 40, 46), long-term seed-bank control was excellent (<60 seeds m^{-2}) except in the case of adding a single pre-planting, pre-emergence herbicide (scenario 25), in which case, long-term seed-bank control was still substantially better than using glyphosate alone, at 876 seeds m^{-2}. Results for *S. oleraceus*, conversely, were not substantially different from the use of glyphosate alone, except for some seed-bank size reduction where two inter-row tillage operations were combined with an early pre-emergence herbicide (scenario 46).

Where summer fallows in biennial cropping contained only glyphosate, results from using any type, combination, or

Table 6. Effects on rate of evolution of glyphosate resistance and long-term seed-bank density (mean of years 20–30 of 30-year simulations) of deliberately controlling *Echinochloa colona* and *Sonchus oleraceus* glyphosate survivors at high or moderate efficacy at different frequencies in cotton

IWM, Integrated weed management

Scenario	Efficacy and frequency of treatments used to control glyphosate survivors	Years to >99% resistant		Long-term seed bank density (seeds m^{-2})	
		E. colona	*S. oleraceus*	*E. colona*	*S. oleraceus*
	Annual cropping				
1	None	19	18	1497	2480
7	80% kill after every glyphosate application	>30	21	1	1378
8	99.9% kill after every glyphosate application	>30	>30	<1	2
9	80% kill after first glyphosate application	22	18	815	2229
10	99.9% kill after first glyphosate application	24	22	4	349
11	80% kill after first glyphosate application in alternate crops	19	18	1498	2496
12	99.9% kill after first glyphosate application in alternate crops	19	20	771	2040
	Biennial cropping, glyphosate-only summer fallows				
2	None	13	18	5354	1970
13	80% kill after every glyphosate application	17	19	2565	930
14	99.9% kill after every glyphosate application	25	29	4	4
15	80% kill after first glyphosate application	13	18	3488	1741
16	99.9% kill after first glyphosate application	14	19	3439	1329
17	80% kill after first glyphosate application in alternate crops	13	18	3488	1911
18	99.9% kill after first glyphosate application in alternate crops	13	18	3439	1671
	Biennial cropping, IWM summer fallows				
2	None	17	24	3392	187
19	80% kill after every glyphosate application	24	>30	1045	3
20	99.9% kill after every glyphosate application	>30	>30	3	2
21	80% kill after first glyphosate application	18	24	3436	10
22	99.9% kill after first glyphosate application	18	28	3297	11
23	80% kill after first glyphosate application in alternate crops	17	24	3437	8
24	99.9% kill after first glyphosate application in alternate crops	17	24	3342	10

Table 7. Rate of evolution of glyphosate resistance and long-term seed bank density (mean of years 20–30 of 30-year simulations) of *Echinochloa colona* and *Sonchus oleraceus* under several weed management strategies used in cotton crops
PPPE, Pre-planting, pre-emergence herbicide; layby, a midseason pre-emergence herbicide treatment applied between planting rows; IRT, inter-row tillage; IWM, integrated weed management

Scenario	Additional tactics used in crop	Years to >99% resistant		Long-term seed bank density(seeds m^{-2})	
		E. colona	*S. oleraceus*	*E. colona*	*S. oleraceus*
	Annual cropping				
1	Glyphosate only	19	18	1497	2480
25	PPPE	20	17	876	2218
26	PPPE every second crop	19	18	1496	2219
27	PPPE + layby	>30	20	<1	2552
28	PPPE + layby every second crop	24	18	54	5523
37	2 × IRT	>30	20	<1	1479
40	IRT	>30	18	<1	2146
43	IRT every second crop	19	17	1274	1932
46	PPPE + 2× IRT	>30	19	<1	579
49	PPPE + 2 × IRT every second crop	>30	19	1	1272
	Biennial cropping, glyphosate-only summer fallows				
2	Glyphosate only	13	18	5354	1970
32	PPPE	15	18	5065	1817
33	PPPE every second crop	15	17	5315	1989
34	PPPE + layby	17	18	2907	1970
36	PPPE + layby every second crop	13	17	3437	1838
39	2 × IRT	22	19	1021	1532
42	IRT	17	18	1881	1740
45	IRT every second crop	13	18	3506	1741
48	PPPE + 2 × IRT	21	18	780	1466
51	PPPE + 2 × IRT every second crop	17	18	2891	1697
	Biennial cropping, IWM summer fallows				
2	Glyphosate only	17	24	3392	187
29	PPPE	19	26	3437	10
30	PPPE every second crop	17	21	3432	121
31	PPPE + layby	20	28	37	16
35	PPPE + layby every second crop	20	27	3178	11
38	2 × IRT	25	28	10	6
41	IRT	21	24	815	11
44	IRT every second crop	18	24	3391	211
47	PPPE + 2 × IRT	>30	29	8	7
50	PPPE + 2 × IRT every second crop	17	24	18	10

frequency of non-glyphosate tactics were diluted substantially. In particular, none of the simulations with glyphosate used alone in fallows predicted good long-term control of resistant seed banks of either weed; seed banks varied between ~3400 and 5000 seeds m^{-2} for *E. colona* and between 1800 and 2000 seeds m^{-2} for *S. oleraceus* where only pre-emergence herbicides were added in-crop (scenarios 32, 33, 34, 36). Some reasonable reductions over the worst-case scenario were found for *E. colona* where frequent inter-row tillage was used (scenarios 39 and 42), down to as low as 780 seeds m^{-2} where inter-row tillage was combined with an early-season, pre-emergence herbicide (scenario 48).

The most robust strategy (an early-season, pre-emergence herbicide plus two inter-row cultivations) when used annually resulted in good long-term seed-bank control in concert with IWM in fallows (scenario 47), and was predicted to offer substantial increases (of >17 years for *E. colona* and 11 years for *S. oleraceus*) in the useful lifespan of glyphosate.

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 control of resistant populations. In order to develop useful strategies for cotton growers, however, we must look for nuances in the simulation results.

Best management strategies are those that deliver good results (i.e. long-term delays in resistance evolution) and fit well with the cropping system. Some authors posit that it is more efficient to use up all of the available susceptibility to a given herbicide and then switch to another option. This is particularly the case in bioeconomic analyses of resistance where herbicide susceptibility is assumed to be a non-renewable resource simply or linearly depleted by every application of the herbicide (as discussed in Weersink *et al.* 2005). It is not clear from our results that the system is so simply reducible, particularly when frequent, highly effective, non-glyphosate weed control tactics are used. Furthermore, the bioeconomic position assumes that the only difference between glyphosate and other options is price, which is demonstrably untrue; glyphosate has advantages over all currently available alternatives in one or more of ease and safety of use, broadness of spectrum, environmental credentials, and reliability. One application of glyphosate foregone now could be of greater value if made later, depending on changes in weed flora, crop rotation, or even the regulatory environment. Retaining the ability for 'emergency control' of weeds that for reasons of climate or crop timing are unable to be controlled with 'less forgiving' herbicides than glyphosate is of substantial practical value. A system that maximises the number of available control options for the maximum amount of time can be demonstrated to be optimal if the values to be optimised are not solely price-related, and thus maximising the length of time before populations become mostly glyphosate-resistant is consistent, in our view, with a best management approach.

Controlling survivors: timing and efficacy

Deliberate, well-timed control of the survivors of glyphosate sprays is predicted to be the best tactic for delaying and managing resistance (Table 6). This was true for both weed species, although several permutations of IWM tactics used on *S. oleraceus* were almost as effective (Table 7). In annual cotton cropping, frequent use of even relatively low-efficacy options for controlling survivors was predicted to be sufficient to provide good long-term control. Where crops are grown in alternate years (and by extrapolation, less frequently), higher efficacy and the addition of some non-glyphosate actions in fallows are required to provide good results (Table 6). We tested at 80% and 99.9% efficacy levels, which could be implemented as inter-row cultivation or shielded spraying with a knockdown herbicide (80%), or as some combination of those with additional chipping, hand pulling, or spot spraying (99.9%).

The efficacy of non-glyphosate treatments was important to the effectiveness of resistance-delaying strategies. In particular, strategies that relied only on pre-emergence herbicides (which, due to variations in timing between application and germination, often had quite low efficacies) were less effective than strategies including knockdown herbicides (used as double-knock applications here) or tillage. However, the results for controlling-survivor simulations in cotton (Table 6) show that applying moderately effective control multiple times per season is better than using more effective tactics less often.

Crop frequency, summer fallows and IWM

The simulations investigating the effects of varying IWM tactics in crop (Table 7) demonstrate the importance of also including non-glyphosate actions between crops. Summer fallows where only glyphosate is used seriously dilute the beneficial effects of even the most frequent in-crop, non-glyphosate tactics. The particular tactics used in-crop had some bearing on the outcome; inter-row tillage, while not the most efficacious treatment available, could be performed at any time in the model, catching late germinations more effectively than pre-emergence herbicides. Pre-emergence herbicides were also prone to losing efficacy if germinations did not occur close to the time of application, so in general terms, while the best strategies used a mixture of tactics, inter-row tillage was a more robust option especially late in-crop.

The substantial differences for *E. colona* outcomes between annual and biennial cropping indicate that annual cropping (i.e. irrigated cropping) is predicted to receive large benefits from adding non-glyphosate actions in-crop. This is likely to be due to a combination of the increased frequency of non-glyphosate tactics in these scenarios and the effects of annual, substantial crop competition on *E. colona* seed production.

Species differences

The differences between *S. oleraceus* and *E. colona* demonstrate that species ecology is of real importance in resistance management. In the cotton system studied here, summer-dominant species such as *E. colona* appear to be both more susceptible to glyphosate resistance and more responsive to strategies that incorporate some use of non-glyphosate tactics, especially in the case of annual cropping. Since *S. oleraceus* emerges in both winter and summer in the model, a substantial proportion of the annual emerged weed population is either not affected by weed control (for simulations with winter cropping) or affected by winter applications of glyphosate plus a selective broadleaf herbicide. Thus, interactions between biology and agronomy appear to result in different rates of resistance evolution. Strategies for managing resistant populations should therefore be devised with reference to species ecology, rather than generically.

Long-term management

Growers in subtropical 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 invisible. For non-irrigated cotton growers in Australia who have been using reduced tillage practices and planting glyphosate-resistant cotton for >10 years, our simulations paint a grim picture, suggesting that only a few years, if any, of efficacy may be left on key species. However, they also show that long-term seed-bank management is possible where a robust strategy is applied before the population becomes mostly or entirely resistant. In particular, biennial cropping scenarios showed that using IWM in summer fallows resulted in consistently lower long-term seed-bank densities of both weeds.

Developing strategies

Models are particularly effective tools for refining and developing strategies for herbicide resistance prevention and management, since they can be used to test much larger numbers of situations and permutations than could be done in the field, and much more quickly. Where current resistance management strategies are largely generic and qualitative, strategies developed using simulation models such as this one can be quantified and specific to particular regions and industries.

Our simulations suggest the following are important parts of a robust strategy for glyphosate resistance management in subtropical broadacre farming:

(1) Deliberately seeking out and controlling glyphosate survivors is the most robust approach to delaying or preventing resistance. Infrequent but very high-efficacy survivor control is not a good substitute for monitoring and controlling survivors frequently.
(2) Two non-glyphosate tactics in crop plus two in summer fallows provide long-term delays in resistance evolution. It is especially important to avoid glyphosate-only summer fallows.
(3) Pre-emergence herbicides are an important tool, but should be backed up with non-glyphosate knockdowns and strategic tillage, especially for controlling late-season germinations.
(4) Resistance prevention and management plans should be devised with reference to species characteristics, particularly including seed bank dynamics.

Monitoring herbicide efficacy after spraying is a critical component of any strategy for weed control and resistance prevention, and our recommendations for controlling survivors rely on robust monitoring practices. It is equally important to follow these guidelines in non-cropped areas on farms as well as in fields.

Our simulations are not spatially explicit. If, in future real incidences of glyphosate resistance in Australian cotton cropping, patches of resistant plants can be identified while still manageably small, zonal management approaches could be taken to minimise the cost of responding to the resistant weed biotype. Future modelling work could usefully aim at describing the spatial dynamics of resistance in patches, to define appropriate, spatially explicit management recommendations.

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