

Simulation modelling to understand the evolution and management of glyphosate resistance in weeds

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Abstract

BACKGROUND: A simulation model is used to explore the influence of biological, ecological, genetic and operational (management) factors on the probability and rate of glyphosate resistance in model weed species.

RESULTS: Glyphosate use for weed control prior to crop emergence is associated with low risks of resistance. These low risks can be further reduced by applying glyphosate in sequence with other broad-spectrum herbicides prior to crop seeding. Post-emergence glyphosate use, associated with glyphosate-resistant crops, very significantly increases risks of resistance evolution. Annual rotation with conventional crops reduces these risks, but the proportion of resistant populations can only be reduced to close to zero by mixing two of three post-emergence glyphosate applications with alternative herbicide modes of action. Weed species that are prolific seed producers with high seed bank turnover rates are most at risk of glyphosate resistance evolution. The model is especially sensitive to the initial frequency of R alleles, and other genetic and reproductive parameters, including weed breeding system, dominance of the resistance trait and relative fitness, influence rates of resistance.

CONCLUSION: Changing patterns of glyphosate use associated with glyphosate-resistant crops are increasing risks of evolved glyphosate resistance. Strategies to mitigate these risks can be explored with simulation models. Models can also be used to identify weed species that are most at risk of evolving glyphosate resistance.

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Keywords: glyphosate resistance; modelling; transgenic crops; herbicide mixtures

1 INTRODUCTION

Since it was first documented in the mid-1990s,¹ evolved resistance to glyphosate has been confirmed in 12 weed species distributed across six continents.² These glyphosate-resistant biotypes have occurred in a number of agricultural situations. The earliest confirmed cases were from broad-area crop production in Australia,^{1,3} where glyphosate was used repeatedly for weed control prior to crop seeding. In the late 1990s and early 2000s, glyphosate resistance was also reported in orchards and vineyards where glyphosate was being used for year-round weed control.^{2,4,5} More recently, the majority of newly reported glyphosate-resistant species have been from agroecosystems where glyphosate-resistant crop varieties are being grown.^{2,6,7}

In common with all examples of pesticide and antibiotic resistance, the development of glyphosate resistance in weed species is an evolutionary phenomenon. Herbicides exert very intense selection pressures, and, in the presence of genetic variability for herbicide susceptibility within weed populations, the evolution of resistance is a likely and predictable outcome.

The rate of, and propensity for, resistance evolution in pest populations is moderated by a number of factors,^{8,9} and these were categorised by Georghiou and Taylor¹⁰ as genetic, biological/ecological and operational factors. Genetic factors include the frequency of resistance (R) alleles in populations, the spontaneous mutation rate to resistance, the mode of inheritance of R alleles and their dominance and fitness in the presence and absence of herbicide. In all cases where mechanism and genetics have been determined, glyphosate resistance is inherited as a (predominantly) single, incompletely dominant nuclear gene.¹¹ In the absence of glyphosate, there is evidence that resistance is associated with a small fitness cost,^{12,13} although further research is required to confirm this for other field-evolved biotypes. For weed species, biological and ecological factors include the breeding system (outcrossing versus selfing), fecundity (seed production capacity), seed bank turnover rate and extent of gene flow within and between populations.^{8,9} There will be interactions between these factors, and parameter values will tend to be species specific, meaning that different species may present different risks of resistance. Operational factors relate to herbicide chemistry

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(Received 16 March 2007; revised version received 1 June 2007; accepted 13 June 2007)

Published online 13 December 2007; DOI: 10.1002/ps.1495

and persistence, frequency of use (within and between growing seasons), herbicide dose applied and the pattern of herbicide use (i.e. use as a component in mixture and sequence programmes). All other weed management practices that affect weed population dynamics come under the umbrella of operational factors.

Simulation models allow the influence of these many factors on the rate and probability of glyphosate resistance evolution to be integrated. In doing so, they can prove useful in two important ways. They provide insight into the relative importance of various genetic, biological and ecological factors to the evolution of resistance. This insight can be valuable in highlighting areas where knowledge about resistance is lacking, and therefore where future research efforts should be concentrated. Simulation models also provide a means to compare the effectiveness of resistance management strategies, providing rapid results without the need for lengthy and often impractical field trials. Models are not, however, a panacea for resistance management studies, and they require a good deal of quantifiable knowledge of the biology of the species in which resistance is being simulated and of the genetics and inheritance of the resistance trait. Here, as in all modelling exercises, the maxim of ‘garbage in, garbage out’ applies.

In the early 2000s, in response to the emerging threat posed by glyphosate resistance, Neve *et al.*^{14,15} developed a simulation model to explore management options to mitigate risks of resistance in *Lolium rigidum* Gaud. (annual ryegrass) in Australian cropping systems. In the present study, a modified version of this model has been used to explore the influence of a number of operational, genetic, biological and ecological factors on the rate and probability of glyphosate resistance evolution. The analysis presented is not specific to any cropping system, region or weed species. Instead, the model is used to demonstrate the utility of the modelling approach to glyphosate resistance management wherever resistance is a threat.

2 MATERIALS AND METHODS

2.1 Modelling framework

The model used is adapted from Neve *et al.*¹⁴ and is implemented in STELLA™ version 9.0 software (isee systems, inc., Lebanon, NH, USA). Specific details of the model structure and parameterisation as they relate to the analysis presented in this paper are given below. Further model details can be found in Neve *et al.*¹⁴

2.1.1 Weed life cycle

Seed bank dynamics. At the beginning of all simulations, the weed population exists as a stock of viable, ungerminated seeds (the soil seed bank). This finite ‘population’ of seeds is defined as all of the seeds of a weed species found in a single cropping field. The size

of the population is the product of the field size (m^2) and the initial weed seed density (m^{-2}). For all simulations presented here, the default field size is 20 ha ($200\,000\text{m}^2$) and the default initial seed bank density is 5000 seeds m^{-2} (initial population size = 1×10^9 seeds).

A predefined fraction of seeds germinate and emerge from the seed bank in each year according to a seasonal emergence curve. The default annual germination proportion is 0.4. The effects of low (0.05) and high (0.8) annual germination fractions on glyphosate resistance evolution are compared in some simulations. It is assumed that emergence commences on 1 April each year and continues throughout the growing season. A dataset has been created to represent a typical weed emergence pattern. This dataset has been fitted to a three-parameter Hill function to describe emergence (Fig. 1). Weed cohorts are defined in relation to management practices on the basis of this emergence curve. For example, when a broad-spectrum herbicide (often glyphosate) is applied on 25 April, weed cohort 1 is all seedlings emerging on or before that date (more details of cohort structure are given in Section 2.1.3). Ungerminated seeds remain in the soil seed bank, but a proportion of these ‘die’ as a result of processes such as loss of viability, fatal germination and seed predation. The default value for this annual seed loss proportion is 0.5 (50% loss).

Weed seedling survival. The number of emerged seedlings that survive to become reproductively mature adults is determined by weed management (Section 2.1.3). Theoretically, it is possible for the model to predict the survival of less than one plant of a particular genotype during any single year (for example, 99% control of ten emerged plants results in the survival of 0.1 individuals). In mathematical terms, these fractional plants can produce seeds and therefore increase the number of glyphosate R or S alleles in the population. In reality, fractional plants do not exist and 0.1 individuals equates to a 10% probability that a single individual will survive. Where

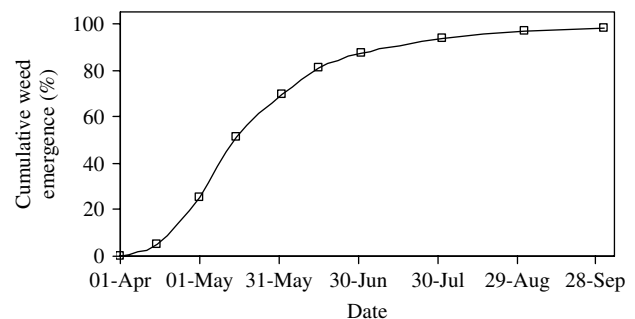


Figure 1. Cumulative weed emergence curve used in model simulations. Emergence percentages are the proportion of total annual emergence. The simulated dataset is fitted to the three-parameter Hill function $y = (ax^b)/(c^b + x^b)$, where a is maximum percentage germination, c is the number of days to reach 50% emergence and b is the slope of the curve at c . Fitted parameter values were $a = 100$, $b = 2.75$ and $c = 44.32$.

fractional plants are predicted, a stochastic routine is implemented to convert values of less than unity to a 0 or 1 (no survivors or a single survivor). Models that do not include this demographic stochasticity will overpredict rates of glyphosate resistance evolution.

Seed production. A hyperbolic model [Equation (1)] based on Massinga *et al.*¹⁶ is used to calculate weed seed production per m²:

$$S = Ad/(1 + Ad/B) \quad (1)$$

where S is weed seed production (m⁻²), d is the density of weed plants (m⁻²), A is the number of seeds produced per plant as d approaches zero and B is maximum seed production (m⁻²). Artificial parameter sets were created for model weed types that are described as having low, intermediate and high levels of per capita and per area seed production (Table 1). Weed cohorts 3 to 6 emerge after the crop (see Section 2.1.3), and surviving individuals from these cohorts will produce fewer seeds owing to greater crop competition. For each 1 day delay in the relative time of crop and weed emergence, it is assumed that weed size and therefore seed production potential will exponentially decrease by 10%. The total density of surviving weed plants for each emergence cohort is modified according to this relationship.

Before the new generation of seeds is returned to the seed bank, a proportion are removed to account for non-viable seeds, predated seeds and seeds that are removed from the field by other processes. This proportion of 'new seed death' has a default value of 0.2.

2.1.2 Genetic parameters

Genetic variability for susceptibility to herbicides is the raw material on which selection acts. This variation may be maintained at low levels in weed populations at polymorphic resistance loci or it may arise *de novo* as a result of spontaneous mutation. The frequency of pre-existing resistant (R) alleles and the *de novo* mutation rate are among the most important parameters in resistance models, yet they are the most difficult to quantify. The average genome-wide rate of base substitution at genetic loci is estimated to be 1×10^{-9} mutations per locus per generation.¹⁷ Currently, two mechanisms of glyphosate resistance are known,¹¹ and it is likely that others are possible, increasing the potential genome-wide mutation rate for glyphosate resistance. In addition to this, glyphosate

resistance may be conferred by other types of mutation (insertions, deletions, duplications). There is evidence suggesting that the frequency of point mutations conferring glyphosate resistance may be orders of magnitude lower than those for other common herbicide resistance alleles.¹⁸ Based on the assumption that reduced mutation rates are balanced by multiple possible glyphosate resistance loci, a genome-wide mutation rate for glyphosate resistance of 1×10^{-9} has been assumed. If glyphosate R alleles are in mutation–selection balance,¹⁷ and assuming a 10% cost for newly arisen R alleles, initial frequencies of glyphosate R alleles will be 10 times higher than the mutation rate (1×10^{-8}).

Glyphosate resistance is modelled as a single, diallelic (R and S) nuclear gene trait, and weed populations are assumed to be in Hardy–Weinberg equilibrium¹⁹ at the beginning of the simulations. The dominance and fitness of the three resistance genotypes (SS, RS and RR) in the presence of glyphosate are reflected in their survival fractions when glyphosate is applied (see Section 2.1.3 and Table 4). All glyphosate applications are at full field rates and result in 95–99% control of susceptible (SS) individuals, 10–20% control of heterozygotes (RS) and 2.5–5% control of homozygote resistant (RR) plants.^{20–22} All glyphosate resistance genotypes are identically susceptible to all other herbicides used during simulations (see Table 4). There is a default assumption in model simulations that there is no difference in the seed production capacity of all three genotypes (no cost of resistance).

Surviving reproductive plants contribute gametes to the gamete pool, and these are recombined according to the breeding system (outcrossing or selfing) of the weed species. The default breeding system is 100% outcrossing, and random, panmictic mating is assumed.

2.1.3 Operational (weed management) factors

All weed management within the model is achieved by herbicides, and operational factors dictate the pattern and timing of herbicide use. A crop is planted on the same day in each year of the simulation. The crop species is not specified, and two crop options exist: conventional and glyphosate resistant. In conventional crops, glyphosate can only be applied prior to crop emergence, while in glyphosate-resistant crops, glyphosate is used as a post-emergence (post-em) selective herbicide. There are five possible timings for herbicide application: one prior to crop seeding (burndown 1), one at the same time as crop seeding (burndown 2) and three sequential in-crop herbicide applications (post-em 1, 2 and 3). For each of the three post-emergence applications it is possible to apply a mixture of two herbicide modes of action. Herbicides are applied at the same time each year (Table 2), and weed cohorts are defined in relation to herbicide application timing (Table 3). The efficacies of various herbicide options vary depending on weed

Table 1. Seed production parameters for model weed species with low, intermediate and high fecundity

Weed fecundity type	Parameter A	Parameter B
Low seed production	500	5000
Intermediate seed production	3000	30 000
High seed production	100 000	200 000

Table 2. Default dates for weed and crop management operations (figures in parentheses are days since commencement of weed emergence)

Weed/crop management operation	Date
Broad-spectrum herbicide – pre-crop seeding (burndown 1)	25 April (25)
Broad-spectrum herbicide – at crop seeding (burndown 2), and pre-emergence herbicide application	10 May (40)
First post-emergence herbicide (post-em 1)	31 May (61)
Second post-emergence herbicide (post-em 2)	15 June (76)
Third post-emergence herbicide (post-em 3)	15 July (106)

Table 3. Weed cohort structure for the default weed type

Weed cohort	Emergence timing	Relative cohort proportion
1	Before burndown 1 (1–25 April)	0.17
2	Between burndown 1 and burndown 2 (26 April – 10 May)	0.26
3	Between burndown 2 and post-em 1 (11 May–10 June)	0.28
4	Between post-em 1 and post-em 2 (11 June–10 July)	0.11
5	Between post-em 2 and post-em 3 (11 July–10 August)	0.1
6	After post-em 3 (11 August–)	0.08

genotype, weed cohort and application timing. For example, glyphosate efficacy against susceptible plants is higher (99%) when it is applied prior to crop

emergence than when it is applied as a post-emergence herbicide (95%). Herbicide efficacies are summarised in Table 4. The pre-emergence herbicide, applied at the time of crop seeding, is a residual herbicide with activity against emerging weeds. Activity is greatest soon after application (90% against cohort 3) and declines until there is no activity against the latest emerging cohort. Herbicides applied at the second post-emergence timing have reduced efficacy versus early emerging cohorts 1 and 2, and the third post-emergence application has no activity versus cohorts 1 and 2 and reduced efficacy versus cohort 3 (Table 4).

2.2 Model simulations

A series of model simulations were performed to explore the influence of operational, biological/ecological and genetic factors on the evolution of glyphosate resistance. As the model contains stochastic elements, it was run 100 times (iterations) for each simulation, and outputs for the proportion of SS, RS and RR genotypes, total seed bank population size and adult plants m^{-2} were saved. Weed populations have been defined as 'resistant' when the combined total of RS and RR individuals is greater than 20%. Results are presented as the cumulative probability of resistance evolving over the 20 year period of the simulations. This approach encompasses some of the stochasticity that is implicit in all evolutionary processes and enables resistance management strategies to be compared according to their relative risks of resistance rather than as strictly deterministic outcomes.

2.2.1 Resistance management strategies

A major purpose of the glyphosate resistance model is to compare risks of various glyphosate use patterns and to explore strategies to mitigate these risks. A

Table 4. Weed control efficacies for all herbicide options included in the model according to resistance genotype and emergence cohort. Other herbicide types are appropriate non-specific products that will generally achieve high control efficacy

		% weed control														
		Cohort 1			Cohort 2			Cohort 3			Cohort 4			Cohort 5		
Application timing	Herbicide type	SS	RS	RR	SS	RS	RR	SS	RS	RR	SS	RS	RR	SS	RS	RR
Burndown 1 (preseeding)	Glyphosate	99	20	5	–	–	–	–	–	–	–	–	–	–	–	–
	Other	99	99	99	–	–	–	–	–	–	–	–	–	–	–	–
Burndown 2 (at seeding)	Glyphosate	99	20	5	99	20	5	–	–	–	–	–	–	–	–	–
	Other	99	99	99	99	99	99	–	–	–	–	–	–	–	–	–
Pre-emergence	Other	–	–	–	–	–	–	90	90	90	60	60	60	30	30	30
Post-em 1																
1st herbicide	Glyphosate	95	20	5	95	20	5	95	20	5	–	–	–	–	–	–
	Other	95	95	95	95	95	95	95	95	95	–	–	–	–	–	–
2nd herbicide	Mixing partner (other)	95	95	95	95	95	95	95	95	95	–	–	–	–	–	–
Post-em 2																
1st herbicide	Glyphosate	70	10	2.5	70	10	2.5	95	20	5	95	20	5	–	–	–
	Other	70	70	70	70	70	70	95	95	95	95	95	95	–	–	–
2nd herbicide	Mixing partner (other)	70	70	70	70	70	70	95	95	95	95	95	95	–	–	–
Post-em 3																
1st herbicide	Glyphosate	0	0	0	0	0	0	50	10	2.5	95	20	5	95	20	5
	Other	0	0	0	0	0	0	50	50	50	95	95	95	95	95	95
2nd herbicide	Mixing partner (other)	0	0	0	0	0	0	50	50	50	95	95	95	95	95	95

series of simulations were performed where the effects of changes in operational practices were explored for a model weed species in which biological and genetic parameters were unchanging. Default parameter values for this model weed have been given in the preceding sections. Resistance risks associated with glyphosate use prior to crop emergence (burndown applications) and post-emergence use in glyphosate-resistant crops were compared. The implications of rotating conventional and glyphosate-resistant crops were examined, as were various herbicide rotational and mixture strategies. Further details of individual simulations are given in the figure captions.

2.2.2 Weed traits and glyphosate resistance

The impacts of changes in key weed biological and ecological traits on glyphosate resistance evolution were investigated for a single glyphosate use strategy and where genetic parameters were unchanging. The biological traits investigated were seed bank turnover rate and seed production capacity (fecundity).

2.2.3 Reproductive and genetic effects

Finally, a set of simulations was performed to examine the influence of changes in the weed breeding system and genetic parameters on rates and risks of glyphosate resistance evolution. The glyphosate use strategy was identical in all simulations. A series of runs varied the initial frequency of the R allele between 1×10^{-9} and 1×10^{-7} . Obligate outcrossing and selfing breeding systems were compared, as were completely dominant and completely recessive resistance traits. The effects of a fitness cost of resistance that reduced the seed production capacity of resistant types were also investigated.

3 RESULTS

3.1 Resistance management strategies

3.1.1 Glyphosate use for weed control prior to crop emergence

Prior to the advent of transgenic glyphosate-resistant crop varieties, the major use for glyphosate in broad-area crop production worldwide was for control of weeds that emerge prior to crop seeding (herein referred to as a burndown application). In simulations, weed emergence commences about 6 weeks before crop seeding and there are two opportunities for burndown herbicide applications (Table 2). The first set of simulations compares three strategies for burndown herbicide use in conventional crop varieties: (i) two annual applications of glyphosate ('glyphosate burndown'); (ii) an annual rotation of glyphosate with an alternative burndown herbicide ('rotate burndown'); (iii) glyphosate application at burndown 1 with an alternative herbicide used at the second burndown timing ('mix burndown'). In general, glyphosate use for weed control prior to crop seeding in conventional (non-glyphosate-resistant) crops has a low resistance risk (Fig. 2). When glyphosate is

used twice annually, resistance is predicted in 11% of model simulations (analogous to 11% of weed populations) and first appears after 9 years. Annual rotation of glyphosate with an alternative broad-spectrum herbicide with identical efficacy slows the evolution of resistance, with the first cases of resistance reported at year 16, but it does not reduce the number of resistant populations over 20 years. When the mixture strategy was applied, resistance was never predicted.

3.1.2 Post-emergence glyphosate use in glyphosate-resistant crops

With the introduction and widespread adoption of transgenic glyphosate-resistant technology, the use of glyphosate as a crop-selective post-emergence herbicide has become a reality. In some parts of the world it is possible continuously to grow glyphosate-resistant crop varieties with little or no soil cultivation and with very heavy reliance on glyphosate. In extreme cases, five glyphosate applications (two burndown and three post-emergence) may be made during the growing season in every year. Simulations have clearly demonstrated for the present model weed species that this is an entirely unsustainable pattern of glyphosate use, leading to predicted glyphosate resistance within 4 years in 100% of model runs (Fig. 3A). Replacing the glyphosate burndown at seeding with application of an alternative burndown herbicide with equal efficacy slows resistance somewhat, reducing the risk of resistance to around 60% over 20 years. When this 'mix burndown' strategy is combined with a strategy annually to rotate a glyphosate-resistant and a conventional crop variety, the rate and risk of resistance evolution is reduced again (Fig. 3A). However, none of these strategies, where glyphosate is used as the sole post-emergence herbicide in glyphosate-resistant crops, is sufficient to reduce resistance risks to reasonable levels.

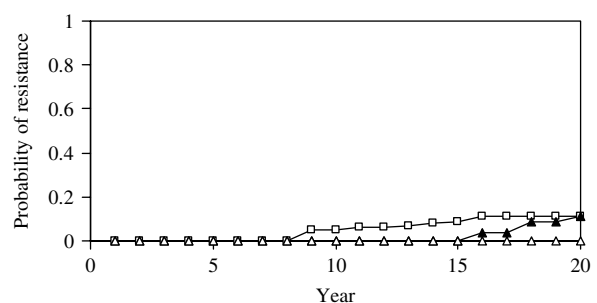


Figure 2. Simulated probabilities of resistance for 'glyphosate burndown' (—□—), 'rotate burndown' (—▲—) and 'mix burndown' (—△—) glyphosate use strategies in a continuous conventional cropping system. Curves represent cumulative probability distributions for resistance evolution over 20 years from 100 independent model iterations. Populations are considered resistant when >20% of individuals possess a resistant phenotype (RS or RR). A pre-emergence herbicide and two post-emergence (post-em 1 and post-em 2) herbicides are applied each year for in-crop weed control. Default biological and genetic parameter values are used.

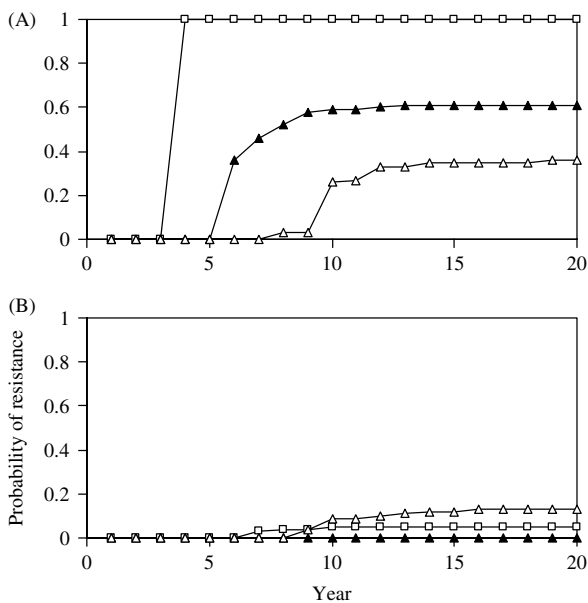


Figure 3. (A) Simulated probabilities of glyphosate resistance when glyphosate-resistant crops are grown every year with two burndown applications of glyphosate each year (□), with a 'mix burndown' strategy (△) and when glyphosate-resistant and conventional crops are grown in 1:1 rotation with a 'mix burndown' strategy (▽). A pre-emergence herbicide is applied every year, and there are three post-emergence herbicide applications (glyphosate or an unspecified 'other' herbicide in glyphosate-resistant and conventional crops respectively) with no mixing partners. In simulations presented in B, the first and second post-emergence glyphosate applications are applied as mixtures with an accompanying herbicide with a completely unrelated mode of action. Glyphosate-resistant crops are grown every year, and pre-emergence herbicides are applied. Comparisons are presented for an annual strategy of two burndown glyphosate applications (□) and a 'mix burndown' strategy (△). A final simulation explores the consequences of a reduction in efficacy of the post-emergence glyphosate mixing partner from 95 to 70% (▽).

The effectiveness of herbicide mixtures, whether applied as sequences or as tank mixtures, has already been demonstrated for burndown herbicide applications (Figs 2 and 3A) and has been shown in other modelling studies.^{15,23} Simulations in Fig. 3B examine the effect of mixing the first and second post-emergence glyphosate application in glyphosate-resistant crops with a second chemically unrelated and highly efficacious (see Table 4) herbicide. The reduction in predicted risks of glyphosate resistance is readily apparent, and resistance is never predicted when this strategy is combined with the 'mix burndown' approach, even though glyphosate-resistant crops are grown every year (Fig 3B). However, in an identical simulation, where the efficacy of the second herbicide is reduced from 95 to 70%, resistance is predicted in 13% of cases (Fig. 3B), clearly demonstrating the importance of ensuring high efficacy of this second herbicide.

3.2 Weed traits and glyphosate resistance

For all simulations in this and the next section a standard strategy is used. This strategy has a continuous use of glyphosate-resistant crops with

two glyphosate burndown applications, no pre-emergence herbicides and three post-emergence glyphosate applications, the first and second of which are accompanied by highly effective (95%) mixing partners.

For any single glyphosate use pattern or management strategy, risks of resistance may vary for different species, depending on their biological and ecological characteristics. Key biological traits include seed bank turnover rate (determined by annual germination fraction and annual seed loss from the seed bank) and seed production capacity. Simulations were performed to investigate the influence of combinations of these weedy traits on resistance evolution in 'model' weed species with low, medium and high levels of seed bank turnover and seed production. In the present default weed type there is a 22% risk of resistance after 10 years, and this risk increases significantly when either or both of the seed bank turnover rate and seed production capacity increase (Table 5).

3.3 Genetic parameters and reproductive strategies

3.3.1 Initial frequency of *R* allele

The importance of realistic estimates of initial frequencies of resistance alleles in simulation models has been highlighted, and the results in Fig. 4 demonstrate this. A decrease by one order of magnitude from the default (1×10^{-8}) to 1×10^{-9} reduces predicted risks of resistance from around 25 to less than 5%, while an increase of one order of magnitude increases risks to 90%. In a finite weed population, this parameter interacts with the population size to determine the actual number of resistance alleles in a population. In fields with high initial seed bank densities, even very low initial frequencies of resistance may result in significant resistance risks.

3.3.2 Weed breeding system and dominance of the resistance trait

In this analysis the weed breeding system is considered as a genetic trait as it interacts with other genetic characteristics of the resistance trait to determine observed rates of glyphosate resistance. When glyphosate resistance is incompletely dominant, as in most field-evolved cases, there is very little difference in simulated rates of resistance evolution between

Table 5. Probabilities (%) of glyphosate resistance at year 10 in a continuous glyphosate-resistant crop rotation for model weed types with different combinations of seed bank turnover and seed production characteristics

Seed bank turnover rate	Seed production capacity		
	Low	Medium	High
Low	0	0	14
Medium	0	22	97
High	9	63	100

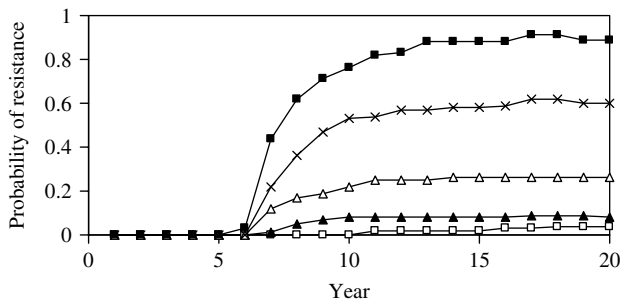


Figure 4. Simulated probabilities of glyphosate resistance when the initial frequency of resistance alleles is set at 1×10^{-9} (\square), 5×10^{-9} (\blacktriangle), 1×10^{-8} (\triangle), 5×10^{-8} (\times) and 1×10^{-7} (\blacksquare). Glyphosate use strategy is a continuous glyphosate-resistant crop, with double glyphosate burndown, no pre-emergence herbicides and three post-emergence glyphosate applications with mixing partners at the first and second application timing.

weed species with obligate outcrossing and obligate selfing breeding systems (Fig. 5). In general, differences in the dominance of the resistance trait make little difference to the rate and probability of resistance in selfing species. Most species in which field-evolved resistance has been documented reproduce predominantly by outcrossing,^{5,7,20,21} and it is in this situation that the dominance of the resistance trait can have the greatest impact on resistance evolution. When glyphosate resistance is completely dominant (identical control of RS and RR genotypes), risks of resistance increase to 45% over 20 years, compared with 26% under the default assumption of incomplete dominance. When resistance is modelled as a completely recessive trait (identical control of RS and SS genotypes), resistance is never predicted under the current glyphosate use strategy in an outcrossing species.

3.3.3 *Fitness costs of resistance*

Where glyphosate resistance is associated with a 20% fitness cost in both heterozygote and homozygote resistant genotypes (the fitness cost is dominant), population level resistance is predicted in 15% of populations in the present standard glyphosate use scenario (see Section 3.2). This represents an 11% reduction compared with simulation where no fitness cost is assumed. Finally, the implications of this 20% fitness cost were explored when glyphosate-resistant and conventional crops were grown in annual rotation with no post-emergence herbicide mixtures (see Fig. 3A for comparison), and risks of resistance were reduced from 36% with no fitness cost to 28%. Even with a moderate cost of resistance and with annual rotation to conventional crops that will favour some selection against glyphosate resistance, in-crop glyphosate use without mixtures is not sustainable for the present model weed species.

4 DISCUSSION

4.1 Modelling glyphosate resistance management

In broad-area crop production, traditional glyphosate use prior to crop seeding has a low resistance risk, even when glyphosate is used annually in systems with no tillage. These modelling-based predictions are corroborated by field observations, as there have been only a few cases of evolved glyphosate resistance associated with pre-crop glyphosate use alone. Reasons for this have been discussed in detail elsewhere¹⁴ and relate to the fact that only that fraction of the population emerged at the time of application is exposed to glyphosate. These low risks

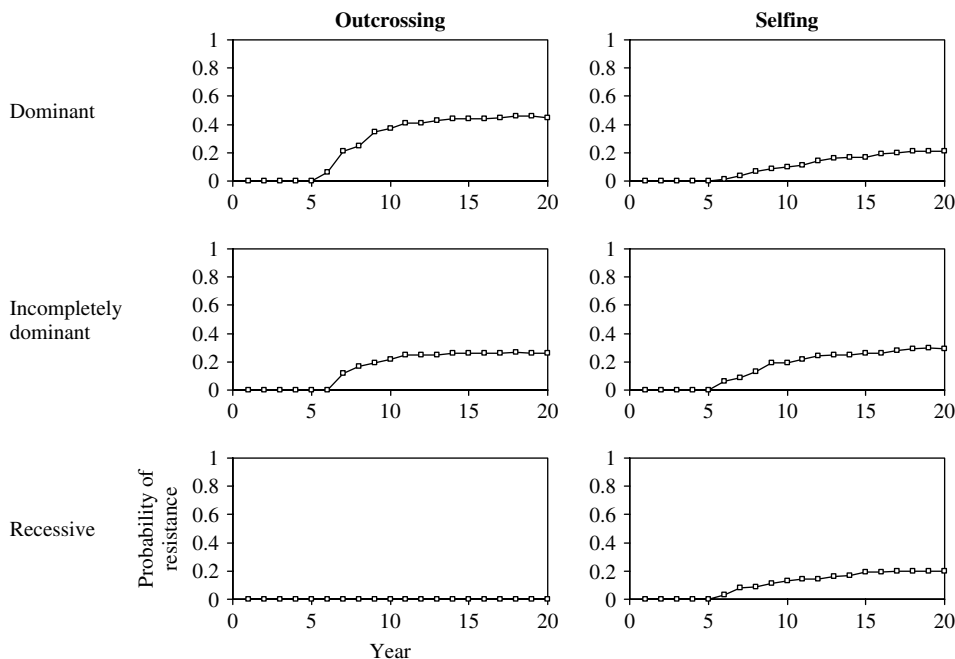


Figure 5. Comparisons of glyphosate resistance risks in model obligate outcrossing and obligate selfing weed species. Glyphosate use strategy is identical to Fig. 4.

can be further reduced by applying an alternative broad-spectrum, highly efficacious herbicide at or before crop seeding (Fig. 2). This ‘mix burndown’ strategy is analogous to the ‘double knockdown’ approach that has been advocated in Australia for the management of glyphosate resistance in *L. rigidum* in no- and minimum-tillage grain production systems.¹⁵ Herbicide mixtures at burndown introduce mode-of-action diversity so that rare surviving glyphosate-resistant genotypes are likely to be controlled by an alternative herbicide at a growth stage where they are most susceptible to this control. Although it has not been explicitly modelled here, it has been shown previously¹⁵ that light soil cultivation prior to or during the seeding operation can have an identical effect to an alternative herbicide providing it gives comparable levels of weed control.

The development of transgenic glyphosate-resistant crop varieties has transformed glyphosate use, making it an inexpensive and highly effective option for broad-spectrum, crop-selective weed control. However, in agroecosystems where this transgenic technology has been adopted, there are escalating numbers of weed species demonstrating the ability to evolve glyphosate resistance.² High risks and rapid evolution of glyphosate resistance are predicted where glyphosate-resistant crops are grown annually. These risks can only be moderately reduced by rotation between conventional and transgenic crops and by deployment of the ‘mix burndown’ strategy (Fig 3A). In common with the previous simulations, it appears that the most effective way to mitigate these high resistance risks is to introduce herbicide diversity in the form of herbicide mixtures. In the analyses presented, mixing partners applied with two of the three post-emergence glyphosate applications were effective in reducing risks, so that glyphosate resistance was never predicted when efficacy of the mixing herbicide was high (95%) (Fig. 3B). The requirement for high efficacy of the second herbicide is paramount and predicates against the use of marginally effective herbicides or herbicide rates (Fig. 3B).

The suspected low mutation rate for glyphosate resistance makes it possible to maintain R allele frequencies at or close to extinction. In finite weed populations, glyphosate resistance management strategies are most effective when they drive R alleles towards extinction and subsequently maintain weed populations at levels where *de novo* mutation to resistance is unlikely. Model outputs demonstrate that this is happening for the most effective management strategies, and therefore it may be possible to prevent glyphosate resistance indefinitely. For resistance to other herbicides, such as ACCase- and ALS-inhibitors, where initial frequencies of R alleles are thought to be orders of magnitude higher,^{18,24} it is less likely that these alleles can be eliminated from populations. In this situation, resistance becomes a more deterministic process, and it is only possible to delay, but perhaps never to prevent, resistance.

Recognition of this key comparative advantage in efforts to contain glyphosate resistance should motivate greater efforts towards glyphosate resistance management.

Finally, it should be acknowledged that simulation models should not be used to provide prescriptive advice on the effectiveness of individual management strategies; for example, strategy *a* will result in resistance in species *x* at year *y*. Rather, they should be used as a means to compare resistance management strategies to provide robust evidence that strategy *a* is *more* effective than strategy *b* in reducing risks of glyphosate resistance.

4.2 Biological and ecological factors

Resistance management attempts to manipulate operational factors to influence the evolution of glyphosate resistance. Biological, ecological and genetic factors are intrinsic to the system (species, population) in which resistance is simulated, and they are less easily manipulated (exceptions such as cultivation to promote germination and the effect of herbicide dose on the dominance of the resistance trait are not considered here). Many weed traits may interact to influence the dynamics of glyphosate resistance evolution, and here only seed bank turnover and seed production capacity were considered, showing that increases in either of these increased risks of resistance (Table 5). In common with the discussion above, the influence of these traits on risks of resistance evolution depends on their ability to increase or decrease the probability of maintaining R alleles at or close to extinction. When, as in these simulations, the annual loss of seed from the seed bank as a result of seed death is constant (50%), increasing the annual germination rate increases the probability that initially rare resistant genotypes will germinate and therefore potentially survive and set seed. Conversely, when the germination fraction is low, a greater proportion of resistant types will succumb to predation, loss of viability and other processes resulting in seed death. A slower seed bank turnover rate also has a buffering effect on the rate of resistance evolution, acting as it does as a ‘genetic memory’ for the population in the form of a reservoir of susceptible seeds.

In populations of weeds that are prolific seed producers (an individual of the most fecund model weed type simulated can produce 100 000 seeds), the success of a single resistant individual in surviving to seed set can ensure that extinction of R alleles from the population is highly unlikely. In fact, in these species it may only be necessary for a single individual to survive to seed set for resistance at the population level to become inevitable. Prolific seed producers also typically have the largest populations that contain the highest number of R alleles, reducing the probability of R allele extinction from the outset.

4.3 Reproductive and genetic factors

Most field-evolved cases of glyphosate resistance exhibit quite similar genetic characteristics.¹¹ This

suggests that glyphosate resistance evolution is favoured by these characteristics or may even be constrained so that it only occurs under this set of parameters. The mutation rate from S to R alleles and the resulting mutation–selection equilibrium that determines R allele frequencies in weed populations are almost impossible to quantify. This crucial parameter has a large influence on the rate and probability of glyphosate resistance evolution (Fig. 4). In the ‘worst-case scenario’ with five annual glyphosate applications and little herbicide diversity (Fig 3A), resistance was predicted in 100% of the weed populations by year 4. Accepting that this practice is reality in some cases and that resistance has not occurred to this extent, it is possible that the default initial R allele frequency of 1×10^{-8} may be a little higher than is actually true. While lack of knowledge of this parameter value may be viewed as a weakness of glyphosate resistance models, it should not influence the relative effectiveness of resistance management strategies. Of course, were the mutation rate to be much lower than assumed here, it may be the case that less costly resistance management strategies could sufficiently reduce resistance risks. In their previous analysis, Neve *et al.*¹⁵ accounted for the uncertainty in this parameter by defining a range and distribution of initial R allele frequencies and randomly sampling from this distribution at each iteration of the model. Finally, it is worth reiterating that the model has only considered the inheritance of glyphosate resistance as a single gene trait. While polygenic glyphosate resistance has not been documented, quantitative responses to other herbicides applied at low dose rates have been reported,²⁵ and this phenomenon remains a possibility for glyphosate resistance also.

Glyphosate resistance evolved most rapidly as a completely dominant trait in obligate outcrossing weed species (Fig. 5) and, under the glyphosate use strategy tested, never evolved in outcrossing species where resistance was recessive. In spite of evidence from field-evolved biotypes that resistance evolves predominantly in outcrossing species, there was no suggestion that self-pollination prevented evolution of resistance. In fact, recessive traits only came to prominence in selfing species. The glyphosate resistance model does not consider spatial structure, and this has implications for overall rates of resistance evolution and for relative rates between outcrossing and selfing species. The assumption of panmixia (random mating) in outcrossing species is a simplification. In reality, limited pollen flow will ensure that mating between neighbours is the most common occurrence. At the same time, where the majority of seed dispersal occurs around the parent plant, these neighbours are likely to be closely related, increasing the extent of positive assortative mating. In selfing species, movement of R alleles within the population only occurs by seed dispersal, which may be quite limited. In the model, newly produced seeds are returned to a homogeneous seed bank and emergence in the following year is

assumed to be spatially uniform, so that each square metre of the field has an equal density of emerging seedlings. In crop fields, weed distributions are not uniform and, where rare resistant individuals survive and produce a new seed generation, these new seeds will be locally distributed, leading to spatial aggregation that will result in density-dependent regulation of seed production and in a reduction in effective outcrossing. This spatial aggregation will result in a reduction in the rates of resistance evolution in both outcrossing and selfing species, although the extent of sib competition may be reduced in outcrossing in comparison with selfing species because of the ability of R alleles to spread as a result of pollen dispersal. Future resistance models should consider the impact of these spatial reproductive and genetic effects.

Fitness costs of resistance, modelled here as a reduction in maximum seed production capacity, can reduce probabilities and rates of resistance evolution by reducing the relative contribution of resistant genotypes to the seed bank. In a field-evolved *L. rigidum* biotype, an 18% reduction in seed production of the resistant phenotype in the absence of competition has been reported.¹³ Baucom and Mauricio¹² reported a 35% difference in seed production between the most susceptible and most tolerant lines of *Ipomoea purpurea* (L.) Roth. In simulations where a 20% cost of resistance was included, the probability of resistance was reduced, although not to a point where resistance risks were mitigated in strategies with continuous or rotational post-emergence glyphosate use. Understanding the magnitude of fitness costs associated with glyphosate resistance should remain a priority, however, as this will provide insight into R allele frequencies in unselected weed populations. Also, management strategies that maximise the impact of fitness costs by targeting vulnerable life history stages may be designed.

4.4 Future use of simulation models for glyphosate resistance management

Models of glyphosate resistance evolution have proved useful in highlighting resistance risks and exploring strategies for mitigation of these risks. In general terms, glyphosate resistance management should aim to reduce glyphosate selection pressure by introducing herbicide (and other management) diversity to cropping rotations and to maintain weed populations at the lowest practical levels to favour the extinction of R alleles. It is important to remember that, in comparison with resistance to other herbicide modes of action, the low mutation rates for glyphosate resistance means that resistance need not be an inevitable outcome of glyphosate use.

Future developments to the existing glyphosate resistance model should incorporate spatial structure and processes so that the implications of spatial aggregation and gene flow within and between

populations can be accounted for. These models should then be used to refine management strategies for glyphosate resistance and, by exploring the range of biological, genetic and operational factors that impact on resistance evolution, should begin to identify the situations and weed species in which future risks of glyphosate resistance are greatest.

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