

Simulating evolution of glyphosate resistance in *Lolium rigidum* I: population biology of a rare resistance trait

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Summary

Despite frequent use for the past 25 years, resistance to glyphosate has evolved in few weed biotypes. The propensity for evolution of resistance is not the same for all herbicides, and glyphosate has a relatively low resistance risk. The reasons for these differences are not entirely understood. A previously published two-herbicide resistance model has been modified to explore biological and management factors that account for observed rates of evolution of glyphosate resistance. Resistance to a post-emergence herbicide was predicted to evolve more rapidly than it did to glyphosate, even when both were applied every year and had the same control efficacy. Glyphosate is applied earlier in the growing season when fewer weeds have emerged and hence exerts less selection pressure on populations. The evolution of glyphosate resistance was predicted to arise more rapidly when glyphosate applications were later in the growing season. In simulations that assumed resist-

ance to the post-emergence herbicide did not evolve, the evolution of glyphosate resistance was less rapid, because post-emergence herbicides were effectively controlling rare glyphosate-resistant individuals. On their own, these management-related factors could not entirely account for rates of evolution of resistance to glyphosate observed in the field. In subsequent analyses, population genetic parameter values (initial allele frequency, dominance and fitness) were selected on the basis of empirical data from a glyphosate-resistant *Lolium rigidum* population. Predicted rates of evolution of resistance were similar to those observed in the field. Together, the timing of glyphosate applications, the rarity of glyphosate-resistant mutants, the incomplete dominance of glyphosate-resistant alleles and pleiotropic fitness costs associated with glyphosate resistance, all contribute to its relatively slow evolution in the field.

Keywords: herbicide, *Lolium rigidum*, model, fitness, glyphosate resistance, ryegrass.

Introduction

The intensive use of herbicides over the last 50 years has resulted in the widespread evolution of herbicide resistance (reviewed in LeBaron & Gressel, 1982; Powles & Holtum, 1994; Powles & Shaner, 2001; Gressel, 2002). Resistance has been documented to almost all herbicide groups in a wide range of cropping systems (Heap & LeBaron, 2001).

The annual graminaceous weed *Lolium rigidum* Gaud. (annual ryegrass) is the most widespread and troublesome weed of Australian agriculture, infesting c. 6 million ha (Alemseged *et al.*, 2001). Its wide distribution,

frequent high densities and high degree of genetic variability, together with a long history of intense selection with herbicides, have resulted in evolution of resistance to herbicides with nine different modes of action in Australia (Heap, 2003). In a random survey in Western Australia, 46% and 64% of *L. rigidum* populations were resistant to diclofop-methyl and chlorsulfuron, respectively, and 37% were resistant to both herbicides (Llewellyn & Powles, 2001).

The introduction and rapid adoption of transgenic glyphosate-resistant crop varieties in North and South America has significantly increased glyphosate use. Notwithstanding frequent use, evolved resistance to

glyphosate remains rare. The first confirmed cases of glyphosate resistance were in *L. rigidum* biotypes from Australia (Powles *et al.*, 1998; Pratley *et al.*, 1999). Subsequently, other resistant *L. rigidum* populations have been found in Australia and South Africa. Resistance in the closely related *Lolium multiflorum* Lam. (Italian ryegrass) has been documented in Chile (Perez & Kogan, 2003). In Australia, in cases where detailed application histories have been available, resistance has evolved in *L. rigidum* following *c.* 15 applications of glyphosate. Yet, in many agricultural and horticultural situations worldwide, in excess of 15 glyphosate applications have already been applied to weed populations without evolution of resistance.

Prior to confirmation of the first glyphosate-resistant *L. rigidum* populations, there was much speculation on biological reasons for the absence of evolved resistance to glyphosate despite intense and prolonged selection pressure (Jasieniuk, 1995; Bradshaw *et al.*, 1997). The latter authors cited the limited success of whole plant selection (with and without mutagenesis) in developing glyphosate-resistant lines of a number of species as evidence for the low probability of field-evolved glyphosate resistance. Jander *et al.* (2003) used ethylmethane sulfonate (EMS) saturation mutagenesis in *Arabidopsis thaliana* (L.) Heynh. to show that mutation rates for resistance to glyphosate were magnitudes of order lower than those for chlorsulfuron and imazethapyr. Glyphosate competitively inhibits the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS), which catalyses the penultimate step in the shikimate pathway. Where transgenic approaches have successfully engineered EPSPS enzymes resistant to glyphosate, these enzymes have commonly had a lower affinity for phosphoenol-pyruvate (PEP), the normal substrate for EPSPS (Padgett *et al.*, 1996). Thus, many mutations potentially conferring target-site-based glyphosate resistance may be lethal or associated with severe fitness costs because of decreased enzyme efficiency.

It is clear that risks of resistance are not the same for all herbicides. In many cases, resistance to the ACCase- and ALS-inhibiting herbicides has evolved after five or less applications (Mallory-Smith *et al.*, 1990; Heap, 1991; Christopher *et al.*, 1992; Gill, 1995). This is in stark contrast to observations for glyphosate. The population genetic and management factors underpinning resistance are important determinants of observed rates of evolution of resistance and vary for different plant species, herbicides and herbicide groups (Maxwell & Mortimer, 1994; Jasieniuk *et al.*, 1996; Diggle & Neve, 2001). Many of these factors have been studied using population-based simulation models (Gressel & Segel, 1978; Maxwell *et al.*, 1990; Mortimer *et al.*, 1992; Cavan *et al.*, 2000).

This paper reports the development of a herbicide resistance model capable of simulating resistance to two chemically unrelated herbicides in a single, age-structured *L. rigidum* population. The model incorporates many aspects of the population dynamics and population genetics of herbicide resistance evolution in *L. rigidum* as well as a wide range of weed management options. The model is used to explore the implications of the timing of glyphosate applications in relation to *L. rigidum* emergence for rates of evolution of resistance. Additionally, the influence of initial allele frequencies, dominance and fitness are explored in an attempt to explain observed rates of evolution of glyphosate resistance in *L. rigidum* in the field. A second, accompanying paper utilizes the model to explore management options which will reduce risks of glyphosate resistance in the future (Neve *et al.*, 2003).

Model development

Overview

The model presented is based on the herbicide resistance model developed by Diggle *et al.* (2003). The model simulates resistance to two herbicides in a single *L. rigidum* population. Resistance or susceptibility to each herbicide is conferred at a single gene locus and there is no cross-resistance or negative cross-resistance between herbicides (resistance at locus Y to herbicide Y has no effect on susceptibility to herbicide Z). Based on the results of Lorraine-Colwill *et al.* (2001), resistance to glyphosate is modelled as a single gene trait. The life cycle and breeding system parameters of the model have been specifically set to represent *L. rigidum*. *Lolium rigidum* is an obligate (100%) outcrossing species and the model assumes completely random panmictic mating between individuals within the population. *Lolium rigidum* populations are finite (a population is defined as all individuals in a single field) and are closed (there is no gene flow into the population from surrounding populations). In these finite populations, extinction of resistance genes may occur when the frequency of resistance alleles is lower than the overall population density (see Diggle *et al.*, 2003). An *L. rigidum* germination and emergence model is used to determine patterns of emergence using historical meteorological data from Wongan Hills in Western Australia (30°52'S, 116°42'E). The output from this emergence model is used to determine the relative size of four discrete cohorts that are incorporated into the life cycle submodel. The competition submodel is substantially modified from Diggle *et al.* (2003) to account for differential competition between discrete *L. rigidum* cohorts and crops. Control efficacies of weed management options are specified on a per cohort basis. The

capacity for *de novo* mutation to resistance within the *L. rigidum* population has also been incorporated. These modifications to the previous model are described below.

Lolium rigidum life cycle model

The life cycle model for *L. rigidum* forms the central element of the overall model which tracks changes in the frequencies of resistant and susceptible genotypes. A comprehensive description of the model structure and the notation used to define parameters is given in the Appendix (Table A1, Fig. A1). In essence, the principles of the life cycle model are as described by Diggle *et al.* (2003). Basically, *L. rigidum* plants (*P*) of genotype *i* (P_i) exist at a number of life history stages: viable seeds in the seedbank (*s*), germinated seeds (*g*), established seedlings (*e*), mature plants (*m*), seed produced on mature plants (*sp*) and seed removed from the seedbank by processes other than germination (*r*). The proportion of individuals progressing from one life history stage to the next (transition probability) is specified on a yearly (generation) basis within the model and is the function of intrinsic population processes and weed management. For example $P_{is} \rightarrow gt_n$ is the fraction of *L. rigidum* seeds of genotype *i* that germinate in year *n*. The addition to the model of four discrete cohorts (c_k) means that the demographics and competitive interactions between each of these cohorts can be accounted separately to represent differences in intrinsic population processes and weed control efficacies in an age-structured population. In particular, the competition submodel has been modified to account for the different competitive abilities and seed production potential of early and late emerging seedlings. The number of *L. rigidum* seeds of genotype *i*, germinating in year *n*, as part of cohort *k* ($P_{igt_n c_k}$) is defined as

$$P_{igt_n c_k} = P_{ist_n} * P_{is} \rightarrow gc_k * P_{germ_{ann}} \quad (1)$$

where P_{ist_n} is the initial seedbank density of genotype *i* in year *n*, $P_{is} \rightarrow gc_k$ is the fraction of the *L. rigidum* seedbank that germinates as cohort *k* and $P_{germ_{ann}}$ is the annual total germination fraction of the seedbank.

Transitions between other life history stages are calculated on a per cohort basis, as described for a single cohort population in Diggle *et al.* (2003). *Lolium rigidum* seed production (P_{ispt_n}) is calculated in a separate and modified competition submodel (Eqn 4 below).

The crop life cycle

A crop (*P'*) of type *i*, either wheat (*Triticum aestivum* L.), oilseed rape (*Brassica napus* L.) or narrow-leaved lupin (*Lupinus angustifolius* L.), is sown in each growing season during the course of the simulation. The crop

sowing date is specified in relation to the start of the growing season and crop emergence is 5 days after sowing. For each crop a sowing rate is specified (P'_{ist_n}) (seeds m^{-2}). Crop density (P'_{imt_n}) is the product of sowing rate and the crop germination fraction ($P'_{is} \rightarrow gt_n$). Crop yield (P'_{ispt_n}) is calculated in the competition submodel.

Seasonality

Seasonality has been incorporated into the model, in order to account for year to year variation in (i) patterns of *L. rigidum* germination and emergence, (ii) competitive interactions between crops and *L. rigidum* and (iii) the range of management options available to producers.

Seasonal aspects of the model have been parameterized from historical daily temperature and rainfall data (1957–1997) from Wongan Hills, a typical intensive grain producing region of the central wheat belt of Western Australia. *Lolium rigidum* germination was assumed to occur when a threshold amount of rain fell over three consecutive days. These thresholds varied on a monthly basis (based on temperature and evaporative potential) and were 36 mm in January, 24 mm in February, 20 mm in March, 16 mm between 1 and 15 April, 12 mm between 16 and 30 April, 8 mm in May and 6 mm in June.

Any rainfall and subsequent *L. rigidum* germination prior to 15 April was defined as out of growing season germination, as typically growers will not sow crops before this date because of the high risk of subsequent drought periods. The majority of *L. rigidum* germinating as a result of this early season rain (subsequently referred to as summer rain) will die as a result of subsequent high temperatures and drought. In years where summer rain is predicted, the summer germination fraction ($P_{germ_{summer}}$) is expressed as a fraction of the total annual germination fraction ($P_{germ_{ann}}$). *Lolium rigidum* seeds that germinate in the summer and that survive to become established seedlings are included in the first cohort of *L. rigidum* emergence during the growing season. The fraction of *L. rigidum* designated as cohort 1 ($P_{is} \rightarrow gt_{n c_1}$) is defined in Eqn 2, where $P_{germ_{gs}}$ is the fraction of the total seedbank that germinates during the growing season and $P_{germ_{c_1}}$ is the fraction of growing season germination which germinates as cohort 1.

$$P_{is} \rightarrow gc_1 = [(P_{germ_{summer}} * P_{germ_{ann}}) * P_{ig} \rightarrow e_{summer}] + (P_{germ_{gs}} * P_{germ_{c_1}}). \quad (2)$$

The start of the crop growing season was calculated for each year as the date of the first significant rainfall event after 15 April. On this basis, the average growing season began on 22 May at Wongan Hills. In order to reduce complexity within the model, seasons were

Table 1 Probabilities of season types and summer rain calculated from Wongan Hills meteorological data (1957–1997)

Season type	Probability of occurrence	Probability of summer rain
Early (before 10 May)	0.1	0.75
Average (10–31 May)	0.6	0.6
Late (after 31 May)	0.3	0.6

grouped into three categories according to the start of the growing season; early, average and late. Growing seasons starting prior to 10 May were defined as early seasons, between 10 and 31 May as average seasons and after 31 May as late seasons. Using meteorological data from Wongan Hills (1957–1997) the probability of each season type was calculated, together with the probability of summer rain for each season type (Table 1). A season type and the occurrence of summer rain are specified during each year of model simulations according to these probabilities.

Lolium rigidum germination and emergence

The periodicity of *L. rigidum* germination and emergence in relation to environmental (climatic, management) and biotic (crop and weed emergence, herbivory) factors is a key determinant of competitive interactions between crops and weeds, and of the timing of weed control strategies. Empirical models that predict the periodicity of seedling emergence for a number of weed species have been developed (Forcella, 1993; Forcella *et al.*, 2000). These models use mean daily soil temperature and rainfall data to predict patterns of weed seed germination and emergence in the field. A version of this model has been developed for *L. rigidum* using *L. rigidum* emergence and meteorological data from field trials across Australia (Archer *et al.*, 2002). A typical representative year for each of the season types at Wongan Hills (early, average and late) was chosen and the daily temperature and rainfall data for each of these three seasons was incorporated into the *L. rigidum* emergence model to provide predictions of daily cumulative seedling emergence for each season type. The data from the emergence model were fitted to a two parameter Hill function (Eqn 3) for each season type. Emergence curves are shown in Fig. 1 and parameter estimates in Table 2.

$$y = \frac{x^b}{(c^b + x^b)} \tag{3}$$

The fitted emergence model in Eqn 3 was incorporated into the herbicide resistance model so that cumulative *L. rigidum* emergence (y) at x days from the start of the growing season could be predicted, where c is

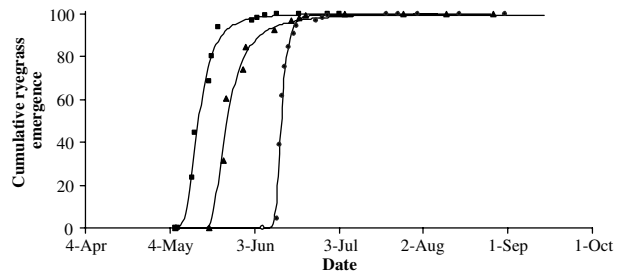


Fig. 1 Predicted (symbols) and fitted (solid lines) values for cumulative *Lolium rigidum* emergence from a germination and emergence submodel (Archer *et al.*, 2002) for a ‘typical’ early (6 May, ■), average (17 May, ▲) and late season (6 June, ●) based on meteorological data from Wongan Hills, Western Australia.

Table 2 Parameter estimates and standard errors (in brackets) for cumulative *Lolium rigidum* emergence curves in a typical early, average and late season at Wongan Hills, Western Australia.

Parameter b is the slope of the curve around c , the time in days since the start of the growing season for 50% of total predicted cumulative emergence to occur

Season type	Parameter estimates	
	b	c
Early	3.12 (0.167)	8.18 (0.159)
Average	2.25 (0.124)	7.28 (0.196)
Late	5.89 (0.237)	6.69 (0.053)

the time until 50% *L. rigidum* emergence (x_{50}) and b is the slope of the curve around x_{50} .

Crop: weed competition submodel

Competition between crops and *L. rigidum* is simulated using a version of the hyperbolic function used by Firbank and Watkinson (1985) and modified by Diggle *et al.* (2003). The addition to this version of the herbicide resistance model of an age-structured *L. rigidum* population and three crop species has required further modifications to this function to account for asymmetric intra-specific competition between different *L. rigidum* cohorts and interspecific competition between *L. rigidum* and the three crop types. As an example, *L. rigidum* seed production (kg ha^{-1}) for cohort 1 plants ($P_i \text{spt}_n c_1$) is calculated as in Eqn 4. Seed production for *L. rigidum* is converted to seeds m^{-2} , assuming a mean seed weight of 0.0025 g,

$$P_i \text{spt}_n c_1 = [P_i m_t n c_1 * k P_i t_n * P_i \text{sp}_{\text{max} t_n}] / [1 + P_i' m_t n * k P_i' t_n * A P_i P_i' * D P_i c_1 P_i' + \sum_{j=1}^9 \sum_{k=1}^4 P_j m_t n c_k * k P_j t_n * D P_j c_1 P_j c_k] \tag{4}$$

A summary and description of the parameters in the competition submodel is given in Table A2 in the Appendix. $P_{mt_n c_1}$ is the density of mature *L. rigidum* individuals of genotype i , cohort 1 at floral initiation and P'_{mt_n} and $P_{jmt_n c_k}$ are the densities of the crop and of competing *L. rigidum* genotypes at the same stage. Parameter estimates for k , sp_{max} , A and D for *L. rigidum* and various crops have been derived from a series of virtual experiments using the crop: weed growth simulation model APSIM (Keating *et al.*, 2003). Values for k and sp_{max} are estimated from the APSIM predicted yield by density relationship of each of the species in monoculture in all seasons (1957–1997) at Wongan Hills when crops were sown and *L. rigidum* emerged at the commencement of the growing season. The k parameters are the inverse of the plant density at which maximum seed production is predicted. For *L. rigidum*, k parameters do not vary between genotypes and hence kPt_n is the value of k in year n . The predicted maximum seed yield for a species in any year is sp_{max} , for example, $P_i sp_{max} t_n$ is the predicted maximum seed yield of *L. rigidum* genotype i in year n . Values for sp_{max} are the same for all *L. rigidum* genotypes unless stated, but may be varied to reflect differences in potential maximum seed yield between susceptible and resistant genotypes (fitness differences).

Values of k and sp_{max} vary according to season, giving rise to a distribution of values for each season type. During each iteration of the model values for k and sp_{max} are randomly chosen for the crop and for *L. rigidum* according to season type. Default values for maximum seed production are based on crop and *L. rigidum* seedlings that are sown or emerge on the first day of the season. In practice, maximum potential seed yields will depend on the time of emergence of *L. rigidum* cohorts and on the crop sowing date. A series of APSIM simulations were run to determine the effect of emergence date and sowing date on the predicted sp_{max} for all crops and *L. rigidum*. Linear regression analyses of relative maximum yield [maximum potential yield at start of season/maximum yield at time x (days after start of season)] were performed to establish parameters to describe the decline in maximum seed yield potential for each species by emergence date (data not shown). The sp_{max} for individual *L. rigidum* cohorts and for the appropriate crop are calculated according to emergence time (days after start of season) during each simulation year.

The parameter A quantifies interference between two competing species when both species emerge on the same day, for e.g. APP'_i is the interspecific antagonism of *L. rigidum* by crop type i when both emerge on the same day. When two individuals of the same species emerge on the same day (and where there are no differences in

the competitive abilities of genotypes), intra-specific antagonism (APP or APP') is one. To estimate A parameters for all species combinations, a series of APSIM simulated competition experiments were run for each crop and *L. rigidum* combination at standard densities when both species emerged synchronously. Predicted crop and *L. rigidum* yields, together with previously estimated values for k and sp_{max} were used to determine A parameters for pairwise species interactions.

When two individuals of the same or different species emerge at different times, competition between the two is modified according to their relative emergence dates. The parameter D modifies interference between two species or two *L. rigidum* cohorts based on their relative emergence dates. Parameter A was calculated as described above from a number of APSIM runs where the two species (or two *L. rigidum* cohorts) emerged at different times. The parameter D was derived by dividing the value of A where species emerge at different times with the A value when individuals emerge synchronously. A function was then fitted by regression analysis to describe the relationship between D and relative emergence date for each combination of species. Fitted regression curves for these relationships for all combinations of species are shown in Fig. 2.

Weed management and cohort structure

Weed management practices regulate weed population dynamics and ultimately weed densities in the field. Essentially, they do so by reducing the probability of any individual moving from one life history stage to the next [i.e. from germinated seeds to established seedlings ($P_{ig} \rightarrow e$)]. The model incorporates a range of herbicidal and cultural weed control options. Cultural control options (soil disturbance at sowing, seed capture at harvest) are selectively neutral for the two loci in the model and as such have equal efficacy against all *L. rigidum* genotypes. During the growing season a range of herbicide options [pre-sowing (pre-sow), pre-emergence (pre-em) and post-emergence (post-em) herbicides] are available. The evolution of resistance to any two herbicides within the population can be simulated at the same time. This is achieved by assigning differential survival to different *L. rigidum* genotypes (resistant and susceptible) following herbicide application. These control efficacies depend on the relative dominance of the resistant and susceptible alleles, on the level of resistance conferred by the resistant alleles and on the rates of herbicides applied.

A number of opportunities exist for *L. rigidum* control prior to crop sowing and cohorts one and two

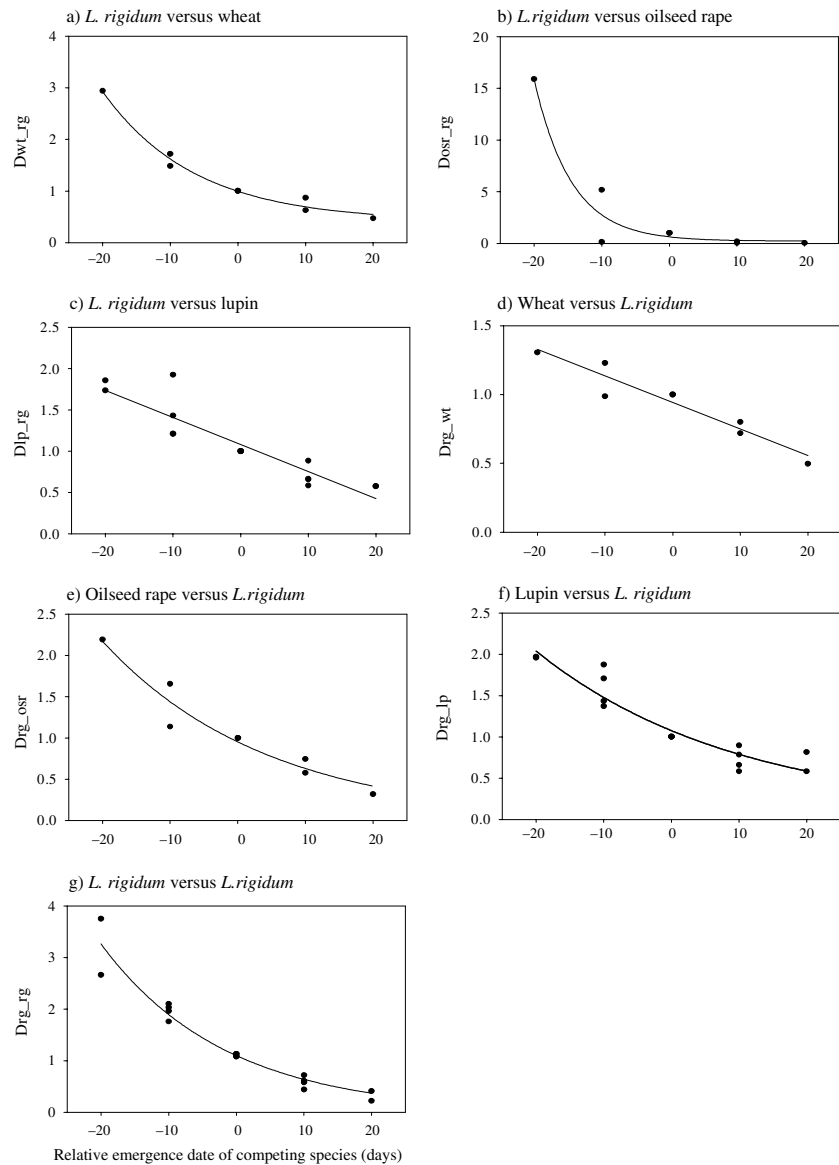


Fig. 2 Fitted regression functions for values of D by relative emergence date for all pairwise interactions of crop species (wheat, oilseed rape and lupin) and *Lolium rigidum*. D is a competition parameter (Eqn 4) that modifies A , the interference between two species, according to the relative emergence date of the two species.

are defined in relation to the timing of these operations. During some seasons it is possible to implement *L. rigidum* control strategies on two occasions before crop sowing. These can take the form of two applications of non-selective herbicides (either of the same or of different herbicides) or a combination of a non-selective herbicide and tillage associated with crop sowing. The timing of the first weed control strategy is specified (days after start of growing season) and the season-specific *L. rigidum* emergence curves (Fig. 1) are used to calculate the number of seedlings emerged to that point. These seedlings form the first *L. rigidum* cohort. All seedlings that emerge between implementation of the first and second pre-sow control strategy are designated cohort 2. *Lolium rigidum* emerging as cohorts 1 and 2 may also be

controlled by tillage associated with the crop sowing operation.

Two crop establishment systems are incorporated into the model, both with no soil cultivation/disturbance prior to crop sowing (direct drilling). In the no-tillage system a tine implement with knife points disturbs a narrow slot of soil into which the seed is sown. This system results in *c.* 15% soil disturbance and 10% control of emerged *L. rigidum* seedlings (no-till). In the minimum tillage system (full width cultivation) a tine implement with overlapping 17.5 cm points results in 100% soil disturbance and 90% control of emerged *L. rigidum* seedlings (min-till).

The proportion of *L. rigidum*, genotype i , cohort 1 which survives and becomes established after crop sowing ($P_{ig} \rightarrow ec_1$) is calculated as shown in Eqn 5.

$$P_{ig} \rightarrow ec_1 = 1 - (P_i\mu_{\text{pre-sow}1}c_1 * P_i\mu_{\text{pre-sow}2}c_1 * P_i\mu_{\text{tillage}c_1}). \quad (5)$$

Efficacies (% mortality) of *L. rigidum* control strategies are represented by μ with a subscript to indicate the particular strategy. Pre-sow 1 and pre-sow 2 are the first and second pre-sowing herbicide applications and tillage refers to soil disturbance at crop sowing. The density of established seedlings of genotype i in cohort 1 (P_{iec_1}) is then calculated as the product of number of germinating seedlings (P_{igc_1}) and $P_{ig} \rightarrow ec_1$.

Similarly, the proportion of *L. rigidum* cohort 2 which survives to become established seedlings after crop sowing ($P_{ig} \rightarrow ec_2$) is 1 minus the product of the proportion controlled by the later application of a non-selective herbicide ($P'_{i\mu_{\text{pre-sow}2}c_2}$) and the proportion controlled by soil disturbance at tillage ($P'_{i\mu_{\text{tillage}c_2}$).

Pre-emergence herbicides with residual soil activity may be applied immediately prior to crop sowing and inhibit germination and emergence of later *L. rigidum* cohorts (cohorts 3 and 4). These herbicides have no activity against *L. rigidum* from cohorts 1 and 2 which have survived pre-sow control and become established as seedlings.

Post-emergence, selective herbicides are usually applied 3–4 weeks after crop sowing. All *L. rigidum* which germinates and emerges between crop sowing and application of post-emergence herbicides is designated as cohort 3. All *L. rigidum* emerging after this time is designated cohort 4. In the absence of density-dependent mortality and herbivory, the proportion of cohort 1 and 2 plants which establish as mature plants within the crop ($P_{ie} \rightarrow mc_k$) is equal to 1 minus the proportion which are controlled by the post-emergence herbicide ($P_{i\mu_{\text{post-em}c_k}$).

For individuals germinating as part of cohort 3, the proportion becoming established is 1 minus the combined efficacy of the pre-emergence and post-emergence herbicides ($P_{i\mu_{\text{pre-em}c_3}$ and $P_{i\mu_{\text{post-em}c_3}$ respectively). Some degree of control of cohort 4 is achieved by the residual activity of soil applied pre-emergence herbicides ($1 - P_{i\mu_{\text{pre-em}c_4}$).

All *L. rigidum* individuals which become established in the crop following the application of post-emergence herbicides become reproductively mature plants and produce seed. One final control option, weed seed collection at harvest, has been incorporated into the model. This option enables a fraction of mature weed seed to be collected as part of the harvest operation. The fraction of seed produced which is returned to the soil seedbank ($P_{isp} \rightarrow s$) is calculated as $1 - P_{\mu_{\text{seed capture}}}$ where $P_{\mu_{\text{seed capture}}}$ is the proportion of *L. rigidum* seed which is collected and removed at harvest.

Simulating glyphosate resistance evolution in *L. rigidum* under continuous cropping

The *L. rigidum* herbicide resistance model described has been used to explore and synthesize some of the key biological factors which may account for the observed rarity of glyphosate resistance. In all simulations presented, unless otherwise stated, glyphosate is applied annually for pre-sowing weed control, crops are sown with no-tillage, pre-emergence herbicides are applied at the time of crop sowing and post-emergence herbicides are applied 21 days after sowing. A crop is grown every year and simulations run for a period of 30 years. For each scenario, 1000 iterations of the model are run. This is equivalent to predicting evolution of resistance in 1000 discrete *L. rigidum* populations and enables results to be presented in terms of a probability of resistance for each of the scenarios. All simulations are run with an average start to the growing season in all 30 years to avoid the confounding effects of seasonal variations in *L. rigidum* emergence. Key default biological and weed management control efficacies are given in Tables 3 and 4, respectively. Resistance and susceptibility to glyphosate is conferred at locus Y and to an unspecified post-emergence herbicide at locus Z. For herbicide applications, 95% control of susceptible genotypes (yy and zz) is assumed and resistance at both loci is completely dominant so that there is 0% control of heterozygote (Yy and Zz) and homozygote (YY and ZZ) resistant individuals. In the first instance, to enable a simple comparison of the effects of herbicide application timing, it was assumed that population genetic parameters (initial allele frequency, dominance and mutation rate) are identical for resistance to glyphosate and the post-emergence herbicide. Selected genetic and biological factors will be varied in later analyses.

Lolium rigidum populations are deemed to be resistant to a herbicide when 20% of the seedbank population is either heterozygous or homozygous resistant. Predicted data for the evolution of resistance are presented as cumulative probability distributions of resistance.

Output from model and discussion

Resistance to glyphosate and a post-emergence herbicide: influence of application timing

Two strategies for glyphosate use were compared. In the first (early application), glyphosate was applied 7 days after the start of the growing season and crops were sown and pre-emergence herbicides applied 3 days after glyphosate application. The post-emergence herbicide was applied 21 days after sowing. In the second strategy (delayed application), glyphosate was applied 18 days

Table 3 Default biological parameter values used in herbicide resistance simulations. Biological parameters are set to these values in all simulations unless stated otherwise in the text. Parameters for the *Lolium rigidum* life cycle that do not have subscript *i* refer to all *L. rigidum* and are not genotype specific

Parameter	Description	Default	Reference(s)
$P_{S_{initial}}$	The initial <i>L. rigidum</i> seedbank density (seeds m^{-2})	500	
$P_{germ_{ann}}$	The annual <i>L. rigidum</i> germination fraction	0.8	Gramshaw (1974); Matthews (1996)
$P_{germ_{summer}}$	The fraction of annual <i>L. rigidum</i> germination emerging during summer rain	0.3	
$P_g \rightarrow e_{summer}$	The fraction of <i>L. rigidum</i> seeds germinating during summer rain which survive to become established seedlings	0.25	
$P_s \rightarrow r_{winter}$	The fraction of ungerminated <i>L. rigidum</i> seed which loses viability or is removed from the seedbank during the growing season	0.1	Pannell <i>et al.</i> (2000)
$P_s \rightarrow r_{summer}$	The fraction of seed present in the seedbank over summer which is lost or removed prior to the growing season	0.3	Pannell <i>et al.</i> (2000)
$P'_{wheat}st_n$	Wheat sowing rate (seeds m^{-2})	100	
$P'_{lupin}st_n$	Lupin sowing rate (seeds m^{-2})	40	
$P'_{osr}st_n$	Oilseed rape sowing rate (seeds m^{-2})	80	
$P'_s \rightarrow gt_n$	Crop germination fraction*	1.0	
λ	Field area (m^2)†	1×10^6	
f_y	The initial frequency of the y allele‡	1×10^{-6}	
f_z	The initial frequency of the z allele	1×10^{-6}	
γ	The mutation rate at y and z loci	1×10^{-8}	

*Crop germination is assumed to be 100%. In addition, all germinating crop seeds establish and become mature reproductive plants (P'_m) so that sowing rate is equal to final crop density (plants m^{-2}).

†The model is based on a finite *L. rigidum* population (Diggle *et al.*, 2003).

‡Initial frequencies of resistance alleles at both loci are specified in year one of simulations. In year one the three genotypes at each locus (i.e. yy, Yy and YY) are assumed to be in Hardy–Weinberg equilibrium, subsequently these are calculated as described in Diggle *et al.* (2003).

after the growing season commenced, sowing was 3 days later and post-emergence herbicides were applied 21 days after sowing. *Lolium rigidum* cohort 1 was defined as all seedlings emerged at the time of glyphosate application, cohort 2 was seedlings emerging between glyphosate application and crop sowing, cohort 3 emerged between sowing and post-emergence herbicide application and cohort 4 was all *L. rigidum* emerging after post-emergence herbicide application. The relative sizes of each cohort for these two strategies was calculated from the emergence curves in Fig. 1 and are shown in Table 5.

Initial allele frequencies for resistance to glyphosate and the post-emergence herbicide were identical (1×10^{-6}) and resistance to both herbicides was assumed to be completely dominant (there were no phenotypic differences between heterozygous and homozygous resistant individuals) (Tables 3 and 4). The 30-year simulations of early and delayed application strategies are compared in Fig. 3, as cumulative probabilities of resistance to glyphosate and the post-emergence herbicide over the 30-year period of the simulation. When the initial frequencies and dominance of resistance alleles were identical, differences in predicted rates of evolution to the two herbicides, such as those in Fig. 3A, were due solely to differential selection pressure related to the

timing of herbicide applications. Where glyphosate was applied 7 days after the start of the growing season, resistance was predicted to occur in 78% of populations after 8 years and in 96% of populations after 9 years. In contrast, resistance to the post-emergence herbicide was predicted after 6 years in 92% of populations and in 7 years in 100%. Rates of evolution of resistance to the post-emergence herbicide were greater because a greater proportion of the population was exposed to the selecting agent (herbicide) resulting in stronger selection pressure.

When glyphosate was applied 18 days after the start of the growing season, almost 90% of total seasonal germination had occurred and hence selection pressure for resistance was greater. Under this scenario, resistance to both herbicides is predicted after only 7 years (Fig. 3B).

In the analyses presented, simultaneous evolution of resistance to two herbicides was simulated. These two processes are not independent. As resistance to the post-emergence herbicide evolves, the overall level of weed control declines, increasing the probability that glyphosate-resistant individuals will survive. In addition, greater overall weed numbers increase the probability of *de novo* mutation to glyphosate resistance. The results presented in Fig. 4 are from simulations where the

Table 4 Default values for weed management control efficacies for each of the four *Lolium rigidum* cohorts. Figures are % control for each weed management practice

Parameter	Genotype (<i>i</i>)*	Cohort			
		1	2	3	4
$P_{i\mu_{pre-sow1}}$	YYZZ	0.95	0.95	–	–
	yyZz	0.95	0.95	–	–
	yyZZ	0.95	0.95	–	–
	Yyzz	0.00	0.00	–	–
	YyZz	0.00	0.00	–	–
	YYzz	0.00	0.00	–	–
	YYZz	0.00	0.00	–	–
	YYZZ	0.00	0.00	–	–
	YYZZ	0.00	0.00	–	–
$P_{i\mu_{no-tillage}}$	ALL	0.10	0.10	–	–
$P_{i\mu_{min-tillage}}$	ALL	0.90	0.90	–	–
$P_{i\mu_{pre-em}}$	ALL	–	–	0.75	0.5
$P_{i\mu_{post-em}}$	yyzz	0.95	0.95	0.95	–
	yyZz	0.00	0.00	0.00	–
	yyZZ	0.00	0.00	0.00	–
	Yyzz	0.95	0.95	0.95	–
	YyZz	0.00	0.00	0.00	–
	YYzz	0.00	0.00	0.00	–
	YYZz	0.95	0.95	0.95	–
	YYZZ	0.00	0.00	0.00	–
	YYZZ	0.00	0.00	0.00	–

*y and Y are alleles for susceptibility and resistance to glyphosate respectively. z and Z are alleles for susceptibility and resistance to an unspecified post-emergence herbicide.

Cohort 1 is all *L. rigidum* emerging before the application of glyphosate, cohort 2 *L. rigidum* emerging between glyphosate application and crop sowing, cohort 3 emerges between crop sowing and application of post-emergence herbicides and cohort 4 emerges after post-emergence herbicide applications.

Table 5 Relative cohort sizes for early and delayed glyphosate use strategies. Cohorts are defined on the basis of crop and weed management (see Table 4)

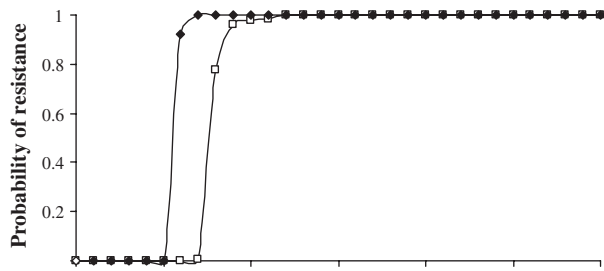
Glyphosate use strategy	Cohort 1	Cohort 2	Cohort 3	Cohort 4
Early	0.478	0.194	0.291	0.037
Late	0.885	0.031	0.065	0.019

Cohort sizes are calculated from an *L. rigidum* emergence curve for an average start to the season at Wongan Hills, Western Australia (Fig. 1).

efficacy of the post-emergence herbicide was maintained for the entire 30-year period.

When glyphosate was applied late, the efficacy of the post-emergence herbicide application (resistance status) had little effect (Fig. 4) as glyphosate selection pressure was sufficiently high to result in relatively rapid resistance to glyphosate, regardless of the control provided by follow-up weed management. However, when glyphosate application was early and continued efficacy of the post-emergence herbicide is assumed,

(A) Early glyphosate application



(B) Delayed glyphosate application

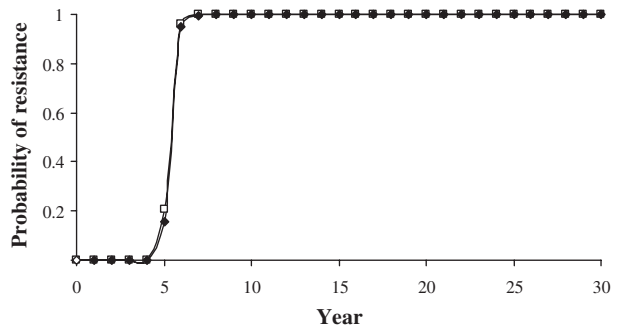


Fig. 3 Cumulative probability distributions for predicted rates of evolution of resistance to glyphosate (—□—) and a post-emergence herbicide (—◆—) in a 30-year cropping simulation when (A) glyphosate is applied 7 days after the start of the growing season and (B) glyphosate is applied 18 days after the start of the growing season. Glyphosate and the post-emergence herbicide are applied every year in a no-tillage cropping system with annual application of a pre-emergence herbicide.

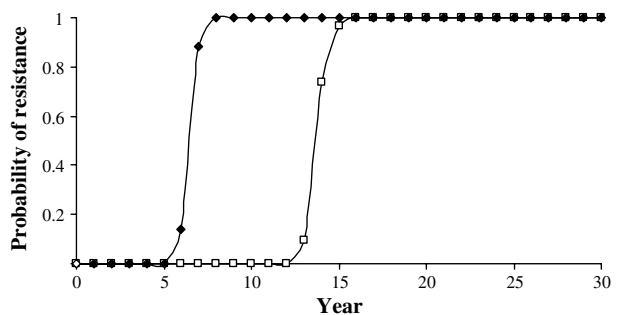


Fig. 4 Cumulative probability distributions for predicted rates of evolution of resistance to glyphosate in a 30-year cropping simulation when glyphosate is applied 7 (—□—) and 18 days (—◆—) after the start of the growing season and post-emergence efficacy is maintained for the entire 30-year period. Glyphosate and the post-emergence herbicide are applied every year in a no-tillage cropping system with annual application of a pre-emergence herbicide.

the rate of predicted glyphosate-resistant evolution was slowed significantly (Fig. 4). Resistance was predicted in 100% of populations after 15 years as opposed to in 96% of populations after 9 years, when post-emergence resistance evolves.

It is not intended that these results be interpreted in terms of potential management strategies to alleviate the risks of glyphosate resistance evolution into the future, though they clearly have some implications for achieving this goal. These issues are explored separately (see Neve *et al.*, 2003). Rather, we attempt to explain the population factors that have resulted in the paucity of field selected glyphosate resistance in *L. rigidum* to date. We have shown that where glyphosate is applied when less than half of the seasonal *L. rigidum* emergence has occurred and where population explosions due to failures of other weed management strategies are avoided, glyphosate resistance is predicted to occur in 15 years in 100% of populations. This compares with evolution of resistance in 6–7 years to post-emergence herbicides with identical initial allele frequencies and dominance. Clearly, within the farming system modelled here, timing of early season glyphosate application alone can have significant impact on rates of evolution of resistance. This result goes some way towards explaining observed differences in the rate and probability of glyphosate resistance and resistance to many commonly used post-emergence herbicides.

In reality, the actual timing of glyphosate application will vary from year to year depending on the season type, crop sown and farm size (on larger farms spraying programmes may be spread over a longer period). With this in mind it is improbable that reduced selection alone accounts for low rates of evolution of glyphosate resistance observed in the field. Further analyses will alternate between early and delayed glyphosate applications to capture this variability whilst exploring the importance of initial allele frequencies, dominance and relative fitness for evolution of glyphosate resistance.

Initial frequency and dominance of glyphosate resistance alleles

Initial frequencies of resistance alleles in unselected weed populations are the product of mutation rates and fitness. While it is almost impossible to measure initial frequencies for glyphosate resistance in unselected wild plant populations, the failure to select glyphosate resistance in EMS-mutagenized *A. thaliana* (Padgett *et al.*, 1996; Jander *et al.*, 2003) suggests that mutations conferring glyphosate resistance are extremely rare. On this basis, we speculate that initial allele frequencies for glyphosate resistance may be considerably less than the 1×10^{-5} to 1×10^{-6} estimated for resistance to other herbicide classes used for selective *L. rigidum* control (Preston & Powles, 2002; Jander *et al.*, 2003). In the following analysis an initial frequency of 1×10^{-8} was assumed.

The dominance of resistance alleles typically depends on the dose of pesticide applied (Georghiou & Taylor,

1986). In most cases, the degree of dominance at commercial field rates is reported. In the first confirmed population of glyphosate-resistant *L. rigidum*, the gene endowing resistance is expressed in an incompletely dominant fashion (Lorraine-Colwill *et al.*, 2001), meaning that survival of heterozygotes at field rates is intermediate between homozygote susceptible and resistant individuals. In the analysis presented in Fig. 5, the percentage control of heterozygotes ($P_{Yy\mu_{pre-sow1}}$) was specified as 0.34 (see Lorraine-Colwill *et al.*, 2001). Control percentages of homozygote susceptible and resistant individuals were as shown in Table 4. The results in Fig. 5 explore predicted rates of evolution of glyphosate resistance when the trait is modelled as incompletely dominant with an initial allele frequency of 1×10^{-8} .

When early and delayed glyphosate applications were alternated and the initial glyphosate resistance gene frequency was 1×10^{-6} , the model predicted that glyphosate resistance would evolve in 100% of *L. rigidum* populations after 10 years of selection (Fig. 5). Reducing the initial frequency of resistant alleles to 1×10^{-8} and modelling glyphosate resistance as an incompletely dominant trait (Lorraine-Colwill *et al.*, 2001) significantly increased the predicted time for resistance to evolve (Fig. 5). In this case, resistance was predicted in <50% of populations after 16 years and does not evolve for 25 years in a small number (2%) of populations. These changes in parameter values, which reduce the initial level of genetic variability and decrease the intensity of selection for heterozygotes (the only resistant phenotypes during the early stages of selection), extend periods for predicted evolution of glyphosate resistance into time-frames which concur with rates of evolution of glyphosate resistance occurring in the field

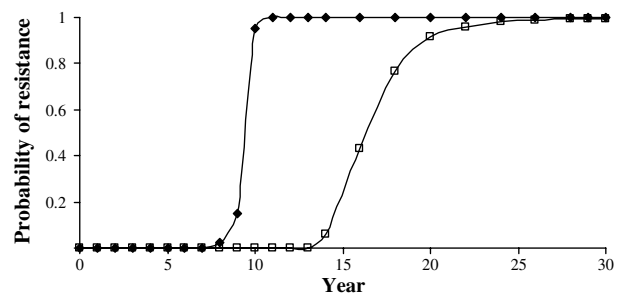


Fig. 5 Cumulative probability distributions for predicted rates of evolution of resistance to glyphosate in a 30-year cropping simulation when glyphosate resistance alleles are completely dominant at an initial frequency of 1×10^{-6} (—◆—) and incompletely dominant at an initial frequency of 1×10^{-8} (—□—). Glyphosate and a post-emergence herbicide are applied every year in a no-tillage cropping system with annual application of a pre-emergence herbicide. Early and late glyphosate applications are alternated.

in Australia. However, it is known that many more populations have had similar levels of exposure and not, as yet, evolved resistance. To this point, relative fitness, which may regulate rates of evolution of resistance and account for different propensities for evolution of resistance in different *L. rigidum* populations has not been considered.

Fitness penalties and observed rates of glyphosate resistance evolution

Fitness costs associated with target-site resistance to the triazine herbicides have been repeatedly demonstrated (reviewed by Holt & Thill, 1994), but can be absent or undetectable for resistance to other herbicide classes. Recent work has measured reduced productivity of resistant compared with susceptible phenotypes in an Australian glyphosate-resistant *L. rigidum* biotype (P. Neve, C. Preston and S. Powles, unpubl. obs.) and field trials have shown a decline in the frequency of glyphosate-resistant phenotypes in this biotype when selection pressure is removed (C. Preston, P. Neve, J. Matthews and J. Dellow, unpubl. obs.). Together, these results suggest that in this biotype the mechanism of glyphosate resistance (as yet unknown) is associated with an ecological fitness cost. In simulations presented in Fig. 6, a fitness penalty associated with glyphosate resistance was inferred by reducing the reproductive output of resistant phenotypes. In the first analysis, maximum seed production ($P_{isp_{max}t_n}$) was reduced by 10% for heterozygotes and by 25% for homozygote resistant individuals. In the second analysis reductions were 25% and 50%, respectively.

Fitness penalties, represented as reduced reproductive output (seed and pollen production) of resistant pheno-

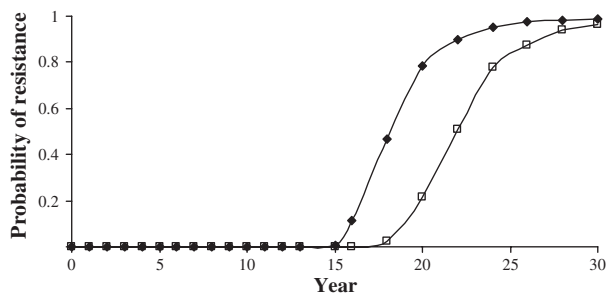


Fig. 6 Cumulative probability distributions for predicted rates of evolution of resistance to glyphosate in a 30-year cropping simulation when maximum seed production is reduced by 10% and 25% (—●—) and by 25% and 50% (—□—) for heterozygous and homozygous resistant individuals, respectively. Glyphosate resistance alleles are incompletely dominant at an initial frequency of 1×10^{-8} . Glyphosate and a post-emergence herbicide are applied every year in a no-tillage cropping system with annual application of a pre-emergence herbicide. Early and late glyphosate applications are alternated.

types, significantly delay the predicted evolution of glyphosate resistance (Fig. 6). In one population where actual costs have been quantified these reflect more closely the lesser of the two penalties simulated and presented in Fig. 6. Under this scenario, resistance evolved in very few (0.6%) populations after 15 years and in 50% of populations after 18 years. These predictions match the limited epidemiological data from the field. These results are clearly dependent on underlying assumptions about the population genetics of glyphosate resistance. However, if we accept that these best estimates are correct we are able to explain many of the reasons for the relatively slow evolution of glyphosate resistance.

Conclusions

Further development of the herbicide resistance model of Diggle *et al.* (2003) has enabled an analysis of the biological and management factors that contribute to the observed lack of evolved glyphosate resistance in weed species in the field. The simulations presented have shown that the timing of herbicide application in relation to *L. rigidum* emergence in the field can have significant impacts on rates of evolution of resistance (Figs 3 and 4). Applications of glyphosate shortly after the commencement of the growing season expose less of the population and consequently exert less selection pressure for resistance. In annual species such as *L. rigidum*, where the majority of germination and emergence occurs early in the growing season, delaying the application of pre-sowing herbicides allows for a significantly greater flush of germination and consequently reduces the differences in selection pressure between pre-sowing and post-emergence herbicides. In species with more protracted, season-long emergence characteristics, selection pressure from pre-sowing herbicides such as glyphosate will remain low compared with herbicides applied later in the growing season. For this reason we can predict that resistance to glyphosate will be slower in these species. For *L. rigidum*, the timing of herbicide application is significant, but does not entirely account for rates of evolution of resistance observed in the field.

Other factors intrinsic to the population genetics of glyphosate resistance were explored and the simulations presented in Figs 5 and 6 have shown that these, together with the factors discussed above, may be responsible for low rates of evolution of glyphosate resistance in *L. rigidum*. The assumptions implicit in these analyses are, however, based on limited empirical data, largely derived from a single glyphosate-resistant *L. rigidum* population (Powles *et al.*, 1998). It is essential that as further resistant populations emerge, detailed ecological, biochemical and genetic studies are

undertaken to characterize the mechanisms of resistance, mode of inheritance and fitness of these populations so that we can better understand the factors that underpin the evolution of glyphosate resistance.

Observed rates of evolution of glyphosate resistance cannot be detached from the ways in which weed populations are managed. The implications for past, present and future use of glyphosate are explored in the accompanying paper, which examines strategies for conserving susceptibility of glyphosate into the future.

Acknowledgements

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Appendix

Table A1 Summary and description of model notation

Descriptor	Definition	Units
P	Total <i>Lolium rigidum</i> plant density	m^{-2}
P_i	Density of <i>L. rigidum</i> genotype i	m^{-2}
P'_i	Density of crop type i	m^{-2}
s	Viable ungerminated seed	
g	Germinated seed	
e	Established seedlings	
m	Mature reproductive plants	
sp	Seed produced	
s_{new}	Seed produced and returned to soil seedbank	
r_{winter}	Seed removed from seedbank during winter other than by germination	
r_{summer}	Seed removed from seedbank during summer other than by germination	
c_k	<i>Lolium rigidum</i> cohort number k	
t_n	Simulation year	

Table A2 Summary and description of parameters used in plant competition submodel

Parameter	Description	Units
$P_{spt_n c_1}$	Seed yield of <i>Lolium rigidum</i> genotype i , cohort 1 in year n	$kg\ ha^{-1}$
$P_{jmt_n c_1}$	Density of mature <i>L. rigidum</i> plants of genotype i , cohort 1 in year n	$Plants\ m^{-2}$
$P_{jmt_n c_k}$	Density of mature <i>L. rigidum</i> plants of genotype j , cohort 1 in year n	$Plants\ m^{-2}$
P'_{jmt_n}	Density of mature crop plants of type i in year n	$Plants\ m^{-2}$
kPt_n	The inverse of <i>L. rigidum</i> mature plant density at which seed yield is half the predicted maximum in year n	
$kP'_i t_n$	The inverse of crop type i mature plant density at which seed yield is half the predicted maximum in year n	
$P_{sp_{max} t_n}$	The predicted maximum seed yield of <i>L. rigidum</i> genotype i in year n	$kg\ ha^{-1}$
$AP_i P'_i$	The interspecific antagonism of <i>L. rigidum</i> of genotype i by crop type i when they emerge on the same day	
$DP_i c_1 P'_i$	Modifies the antagonism of <i>L. rigidum</i> genotype i , cohort 1 by crop type i according to relative time of emergence	
$DP_i c_1 P_j c_k$	Modifies the antagonism of <i>L. rigidum</i> genotype i , cohort 1 by other competing <i>L. rigidum</i> cohorts of genotype j	

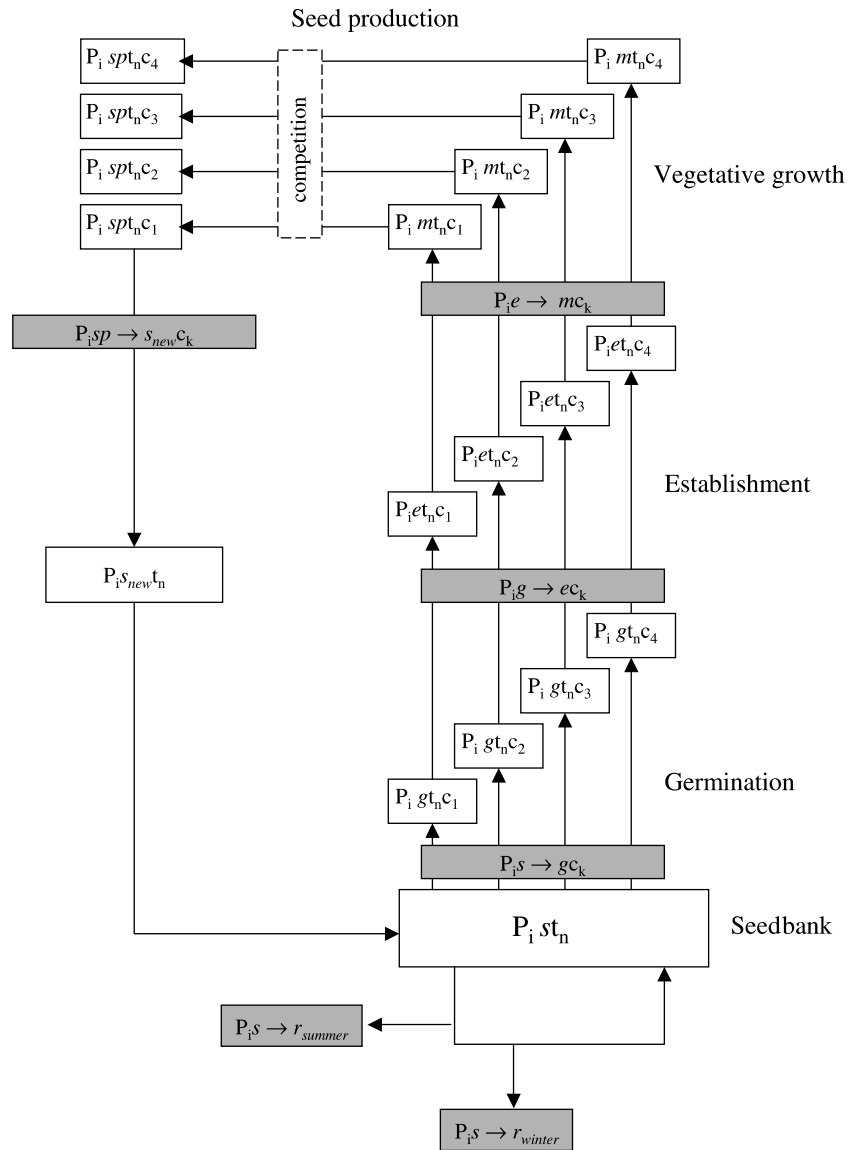


Fig. A1 A schematic flow diagram of the model. This simulates the *Lolium rigidum* life cycle with four discrete emergence cohorts. $P_i s t_n$ is the density (m^{-2}) of *L. rigidum* seeds at the beginning of each model iteration (simulation year, t_n). Transitions from one life history stage to the next are shown by shaded boxes. For example, $P_i s \rightarrow g c_k$ represents seeds of *L. rigidum* (P) of genotype i that germinate as part of cohort k to become $P_i g t_n c_k$, germinated seeds of genotype i in cohort k , year n . The probability of transition from one life history stage to the next is a function of intrinsic population processes and weed management practices. Mature reproductive plants ($P_i m t_n c_k$) produce seed according to a separate competition sub-model (Eqn 3). Seed in the seedbank may be removed in the summer ($P_i s \rightarrow r_{summer}$) or winter ($P_i s \rightarrow r_{winter}$) by processes other than germination (e.g. loss of viability, predation).