# Simulating evolution of glyphosate resistance in *Lolium rigidum* II: past, present and future glyphosate use in Australian cropping

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## Summary

Glyphosate is a key component of weed control strategies in Australia and worldwide. Despite widespread and frequent use, evolved resistance to glyphosate is rare. A herbicide resistance model, parameterized for *Lolium rigidum* has been used to perform a number of simulations to compare predicted rates of evolution of glyphosate resistance under past, present and projected future use strategies. In a 30-year wheat, lupin, wheat, oilseed rape crop rotation with minimum tillage (100% shallow depth soil disturbance at sowing) and annual use of glyphosate pre-sowing, *L. rigidum* control was sustainable with no predicted glyphosate resistance. When the crop establishment system was changed to annual no-tillage (15% soil disturbance at sowing), glyphosate resistance was predicted in 90% of populations, with resistance becoming apparent after between 10 and 18 years when sowing was delayed. Resistance was predicted in 20% of populations after 25–30 years with early sowing. Risks of glyphosate resistance could be reduced by rotating between no-tillage and minimum-tillage establishment systems, or by rotating between glyphosate and paraquat for pre-sowing weed control. The double knockdown strategy (sequential full rate applications of glyphosate and paraquat) reduced risks of glyphosate and paraquat resistance to $<2\%$. Introduction of glyphosate-resistant oilseed rape significantly increased predicted risks of glyphosate resistance in no-tillage systems even when the double knockdown was practised. These increased risks could be offset by high crop sowing rates and weed seed collection at harvest. When no selective herbicides were available in wheat crops, the introduction of glyphosate-resistant oilseed rape necessitated a return to a minimum-tillage crop establishment system.

**Keywords:** *Lolium rigidum*, herbicide, paraquat, model, management, tillage, glyphosate-resistant crops.

## Introduction

Glyphosate, a broad spectrum, non-selective herbicide, is the world's most important and widely used herbicide (Baylis, 2000; Woodburn, 2000). Until recently, its lack of selectivity meant that it could not be used for weed control within crops. However, this is no longer the case as genetic transformation has enabled genes conferring glyphosate resistance to be introduced into a number of crop species (Padgette *et al.*, 1996; Wilcut *et al.*, 1996). In the past few years transgenic glyphosate-resistant *Glycine max* L. (soyabean), *Brassica napus* L. (oilseed rape) and *Zea mays* L. (maize) have been rapidly and widely adopted in North and South America.

The predominant use of glyphosate in current Australian grain production systems is for broad spectrum, non-selective weed control prior to crop sowing. Since the 1970s in Australia, there has been substantial adoption of reduced tillage crop establishment systems and most farmers practice direct drilling in which pre-sowing weed control is achieved with herbicides and soil disturbance occurs at crop sowing only (Pratley & Rowell, 1987). The degree of soil disturbance at sowing varies from 100% in minimum-tillage systems which use

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a tine implement with overlapping shares, to as little as 15% in no-tillage systems that cut a narrow slot in the soil into which seed is placed. These systems considerably reduce cultural weed control and place great emphasis on glyphosate for control of weeds prior to sowing. At the same time, burgeoning resistance to the post-emergence, selective herbicides in Australian populations of *Lolium rigidum* Gaud. (Llewellyn & Powles, 2001) has meant that pre-sowing control with glyphosate has become even more critical.

Glyphosate has a number of other applications in Australian agriculture. Many farm enterprises alternate fields between annual pasture with livestock, and grain production. In the technique known as 'pasture topping', glyphosate may be used in the pasture phase late in the growing season to minimize weed seed production. Glyphosate may also be used to maintain weed-free fields (e.g. fallow) and for total vegetation control in years when drought or severe weed infestations result in crop failure.

Notwithstanding widespread and frequent glyphosate use in Australia and worldwide, resistance to glyphosate in weed species has evolved comparatively slowly and remains rare. The first documented cases of evolved resistance to glyphosate were in *L. rigidum* populations from Australia (Powles *et al.*, 1998; Pratley *et al.*, 1999). More recently glyphosate resistance has been confirmed in *Eleusine indica* (L.) Gaertn. from Malaysia (Lee & Ngim, 2000), *Conyza canadensis* (L.) Cronquist from North America (Van Gessel, 2001) and *Lolium multiflorum* Lam. from Chile (Perez & Kogan, 2003). Studies of the genetics and inheritance of glyphosate resistance in a *L. rigidum* biotype from Australia have indicated that resistance to field applied rates of glyphosate is conferred by a single, nuclear-encoded gene that is inherited in a semi-dominant fashion (Lorraine-Colwill *et al.*, 2001).

In the short to medium term, it is expected that glyphosate-resistant oilseed rape and perhaps other glyphosate-resistant crops will be commercialized in Australia. These crops will increase the current heavy reliance on glyphosate and potentially exacerbate the emerging threat of glyphosate resistance in *L. rigidum* and in other crop weeds. If these technologies are to be successfully incorporated into Australian cropping systems, glyphosate use strategies must be carefully considered to reduce the risks of evolution of glyphosate resistance in weed species.

The only effective alternative herbicide for broad spectrum pre-sowing weed control in Australian cropping is paraquat (often mixed with diquat). Currently, however, the lower price and perceived greater efficacy and spectrum of weed control of glyphosate mean that it accounts for 90% of total sales of non-selective herbicides in the Australian market. Resistance to paraquat and/or diquat has evolved in 21 weed species worldwide in 11 countries (reviewed by Preston, 1994). In Australia, paraquat resistance has evolved in four species, but as yet, no paraquat-resistant biotypes of *L. rigidum* have been reported. Reasons for this lack of paraquat resistance in *L. rigidum* are not clear. In studies of the inheritance of paraquat resistance in other grass weeds resistance is conferred by a single, nuclear-encoded, partially recessive gene (Islam & Powles, 1988; Purba *et al.*, 1993). In the simulations presented we assume a similar mode of inheritance for paraquat resistance should this evolve in *L. rigidum*.

The analyses presented in this paper use a herbicide resistance model (Neve *et al.*, 2003) to explore the implications of past, present and future management practices for the evolution of glyphosate and paraquat resistance in *L. rigidum*.

## Materials and methods

### *Overview of model*

A herbicide resistance model developed in the first of these two papers (Neve *et al.*, 2003) is used to simulate evolution of resistance to glyphosate and paraquat in populations of *L. rigidum*. Resistance to both herbicides is conferred by single genes (locus Y for glyphosate resistance, locus Z for paraquat resistance) that are not linked and segregate independently. Individuals with genotypes yy or zz are susceptible to glyphosate and paraquat, respectively. Genotypes YY and ZZ are homozygous resistant. The phenotype of heterozygotes (Yy or Zz) depends on the relative dominance of the resistance alleles. In the case of both glyphosate (Lorraine-Colwill *et al.*, 2001) and paraquat resistance (Purba *et al.*, 1993), resistance genes are incompletely dominant.

The model is based on a finite *L. rigidum* population (a single population being all *L. rigidum* in a single field or management unit) and the population size is the product of seedbank density and the field area ($\lambda$, see Table 2). In instances where the predicted frequency of resistance alleles is lower than, or close to, the total *L. rigidum* population size, extinction of resistance genes may occur (Diggle *et al.*, 2003). The population is closed (there is no gene flow into the population from surrounding *L. rigidum* populations) and there is completely random mating between individuals within the population. The *L. rigidum* population is 100% outbreeding (allogamous).

### *Crop establishment systems*

Three crop establishment strategies have been defined within the model: early crop sowing, delayed crop sowing and the double knockdown strategy. Delaying crop sowing allows a greater flush of weed emergence, which can be controlled with non-selective herbicides before the crop is established. This strategy has been advocated as a herbicide resistance management strategy where weed populations exhibit widespread resistance to selective herbicides, but has the disadvantage of reducing potential maximum crop yields and increasing selection pressure for resistance to the non-selective herbicides (Neve *et al*., 2003). In practice, crop sowing date in relation to the start of the growing season will depend on the crop sown, the resistance status of weed populations and the date at which the season commences. Historical meteorological records from Wongan Hills, Western Australia (30°52′S, 116°42′E), a typical grain-producing region, have been used to define the probability of early, average and late seasons (Neve *et al*., 2003). The timing of crop sowing, herbicide applications and *L. rigidum* emergence is based on season type and crop establishment system (Table 1).

Under the early sowing strategy in early and average seasons, a non-selective herbicide (glyphosate or paraquat) is applied 7 days after the start of the growing season [7 days of season (DOS)] and the crop is sown 3 days later (10 DOS). In late seasons, no pre-sowing herbicide is applied and crops are sown 2 days after the start of the season (2 DOS). Under the delayed sowing strategy in early and average seasons, a non-selective herbicide is applied 18 DOS and crops are sown 3 days later. In late seasons the non-selective herbicide is applied 7 DOS and crops are sown 3 days later. The ‘double knockdown’ crop establishment strategy is included as a glyphosate resistance management strategy. It requires delayed crop sowing and both glyphosate and paraquat are applied as pre-sowing herbicides. An initial full rate application of glyphosate is followed by a similar full rate application of paraquat. This strategy captures the benefits of pre-sowing applications of glyphosate (efficacy and weed spectrum). The paraquat application increases control of glyphosate-resistant survivors and controls additional *L. rigidum* seedlings that emerge between the two herbicide applications. Because of the requirement for delayed crop sowing, the double knockdown can only be used in early and average seasons (70% of years). In late seasons where the double knockdown is planned but cannot be carried out, the normal strategy for delayed sowing is followed (Table 1). Where the double knockdown is practised, the glyphosate application is 10 DOS and paraquat is applied immediately prior to crop sowing at 21 DOS.

### *Biological and weed management parameters*

Default biological parameters for *L. rigidum* are listed in Table 2 and in Neve *et al.* (2003). These values remain constant for all simulations presented in this analysis, unless otherwise stated in the text. For parameters which may vary according to stochastic demographic events (initial *L. rigidum* seedbank density) or genetic factors (initial allele frequencies and mutation rates), a range of values is specified. During each iteration of the model a random number generator selects a value for these parameters according to a probability distribution (Table 2).

Weed management efficacies for different *L. rigidum* genotypes and cohorts are listed in Table 3. Herbicide rates applied are assumed to be in accordance with label recommendations and achieve 95% control of susceptible individuals in the field. The degree of dominance of

**Table 1** Timing of weed management and crop sowing practices (DOS, days after start of growing season) and relative *Lolium rigidum* cohort proportions for different crop establishment systems in early, average and late season types. *L. rigidum* cohorts are defined in relation to herbicide applications and crop sowing*

| Season type | Crop establishment system | Pre-sow herbicide | Crop sowing | Post-em herbicide | Relative cohort proportions | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Cohort 1 | Cohort 2 | Cohort 3 | Cohort 4 |
| Early | Early sowing | 7 DOS | 10 DOS | 31 DOS | 0.381 | 0.271 | 0.333 | 0.015 |
| | Delayed sowing | 18 DOS | 21 DOS | 42 DOS | 0.921 | 0.029 | 0.044 | 0.006 |
| | Double knockdown† | 10 DOS | 21 DOS | 42 DOS | 0.652 | 0.298 | 0.044 | 0.006 |
| Average | Early sowing | 7 DOS | 10 DOS | 31 DOS | 0.478 | 0.194 | 0.291 | 0.037 |
| | Delayed sowing | 18 DOS | 21 DOS | 42 DOS | 0.885 | 0.031 | 0.065 | 0.019 |
| | Double knockdown | 10 DOS | 21 DOS | 42 DOS | 0.672 | 0.244 | 0.065 | 0.019 |
| Late | Early sowing | None | 2 DOS | 23 DOS | 0.000 | 0.000 | 0.992 | 0.008 |
| | Delayed sowing | 7 DOS | 10 DOS | 31 DOS | 0.561 | 0.346 | 0.085 | 0.019 |

*Cohort 1 is the proportion of *L. rigidum* that has emerged when the pre-sowing herbicide is applied. Cohort 2 is the proportion of *L. rigidum* that emerges between application of the pre-sowing herbicide and crop sowing. Cohort 3 emerges between crop sowing and application of post-emergence herbicides. Cohort 4 emerges after application of post-emergence herbicides. Relative *L. rigidum* cohort sizes are calculated from standard *L. rigidum* emergence curves for early, late and average seasons at Wongan Hills, Western Australia.
†In the double knockdown strategy, the second herbicide is applied immediately prior to crop sowing.

**Table 2** Value ranges for stochastic biological parameters in herbicide resistance simulations. Default values for other parameters are given in Table 3 of Neve *et al.* (2003)

| Parameter | Description | Default |
|---|---|---|
| $Ps_{\text{initial}}$ | The initial *L. rigidum* seedbank density (seeds $m^{-2}$)* | 100–10 000 |
| $f_y$ | The initial frequency of the y allele† | $1 \times 10^{-7}$ to $1 \times 10^{-9}$ |
| $f_z$ | The initial frequency of the z allele | $1 \times 10^{-7}$ to $1 \times 10^{-9}$ |
| $\gamma$ | The mutation rate at y and z loci‡ | $1 \times 10^{-8}$ to $1 \times 10^{-10}$ |

Value ranges for stochastic biological parameters (probabilities for each value in brackets).
**Lolium rigidum* initial seedbank density: 100 (0.1), 500 (0.4), 1000 (0.35), 5000 (0.1), 10 000 (0.05).
†Initial frequencies of resistance alleles: $1 \times 10^{-9}$ (0.1), $5 \times 10^{-8}$ (0.25), $1 \times 10^{-8}$ (0.3), $5 \times 10^{-7}$ (0.25), $1 \times 10^{-7}$ (0.1).
‡Mutation rate: $1 \times 10^{-10}$ (0.1), $1 \times 10^{-9}$ (0.4), $1 \times 10^{-8}$ (0.5).

**Table 3** Default weed control efficacies used in herbicide resistance simulations. Weed control efficacies are set to these values in all simulations unless stated otherwise in the text

| Parameter | Genotype (i) | Cohort 1 | Cohort 2 | Cohort 3 | Cohort 4 |
|---|---|---|---|---|---|
| $P_i\mu_{\text{glyphosate}}$* | yyzz yyZz yyZZ | 0.95 | 0.95 | 0.00 | 0.00 |
| | Yyzz YyZz YyZZ | 0.34 | 0.34 | 0.00 | 0.00 |
| | YYzz YYZz YYZZ | 0.00 | 0.00 | 0.00 | 0.00 |
| $P_i\mu_{\text{paraquat}}$† | yyzz Yyzz YYzz | 0.95 | 0.95 | 0.00 | 0.00 |
| | yyZz YyZz YYZz | 0.50 | 0.50 | 0.00 | 0.00 |
| | yyZZ YyZZ YYZZ | 0.00 | 0.00 | 0.00 | 0.00 |
| $P\mu_{\text{min-till}}$ | ALL | 0.90 | 0.90 | 0.00 | 0.00 |
| $P\mu_{\text{no-till}}$ | ALL | 0.10 | 0.10 | 0.00 | 0.00 |
| $P\mu_{\text{pre-em}}$ | ALL | 0.00 | 0.00 | 0.75 | 0.50 |
| $P\mu_{\text{post-em}}$ | ALL | 0.95 | 0.95 | 0.95 | 0.00 |
| $P\mu_{\text{seed capture}}$ | ALL | 0.60 | 0.60 | 0.60 | 0.60 |

*Lorraine-Colwill *et al.* (2001).
†Purba *et al.* (1993).

resistance alleles and hence the level of survival of heterozygotes is inferred from inheritance studies that established dose responses for heterozygotes from a known glyphosate-resistant *L. rigidum* population (Lorraine-Colwill *et al.*, 2001) and a known paraquat-resistant *Hordeum leporinum* Link biotype (Purba *et al.*, 1993).

Weed management efficacies for other herbicide and cultural practices are not genotype specific. In minimum-tillage systems with 100% soil disturbance, 90% of emerged *L. rigidum* seedlings are assumed to be controlled. In no-tillage systems that have far less soil disturbance, 10% of emerged seedlings are assumed to be controlled. Residual pre-emergence herbicides control 75% of *L. rigidum* emerging as cohort 3 and 50% of cohort 4. Selective post-emergence herbicides applied at standard field rates control 95% of all surviving *L. rigidum* individuals and genotypes but have no residual activity against seedlings that emerge after herbicide application (cohort 4). The average figures used in the model are based on mean control efficacies achieved with pre- and post-emergence herbicides in the field, applied at recommended rates in Australian conditions.

As a response to the widespread evolution of resistance to post-emergence herbicides, farmers in Australia have increasingly adopted weed seed collection systems. These systems collect weed seed during the harvest operation and this seed is subsequently removed from the field or destroyed *in situ* rather than being returned to the soil seedbank. These systems are capable of removing and destroying between 40% and 80% of *L. rigidum* seed produced (Gill, 1996; Matthews & Powles, 1996; Walsh, 1996). Where practised in the simulations presented, weed seed collection removes 60% of *L. rigidum* seed produced.

Increasing crop sowing rates has been shown to increase overall crop competitiveness and reduce *L. rigidum* seed production and may also be included in integrated weed management strategies to maintain low weed densities.

### *Simulated glyphosate and paraquat use patterns*

The model is used to simulate a number of cropping systems and glyphosate and paraquat use patterns. All simulations are based on a 30-year *Triticum aestivum* (L.) (wheat, W), *Lupinus angustifolius* (L.) (lupin, L), wheat, *Brassica napus* (L.) (oilseed rape, R) (WLWR) cropping rotation. Standard crop sowing rates are 100 plants $m^{-2}$ for wheat, 40 plants $m^{-2}$ for lupin and 80 plants $m^{-2}$ for oilseed rape. All crops are conventional, non-transgenic varieties unless stated. Unspecified pre-emergence and selective post-emergence herbicides are applied every year where these are available (in some runs and in some crops it is assumed that no effective post-emergence herbicides are available due to resistance). Pre-sowing herbicide (glyphosate and paraquat) use patterns and crop establishment systems are varied (see below) for each model simulation to explore the effects of these practices on the predicted rate and probability of evolution of resistance to glyphosate and paraquat. For each of the simulations presented, the model is run 1000 times (equivalent to simulating evolution of resistance in 1000 populations of

*L. rigidum*). Data are summarized to give the probability of evolution of resistance (number of runs or populations in which resistance is predicted) and predicted rates at which resistance evolves. A population is defined as resistant to a herbicide when 20% of individuals in the seedbank are heterozygous (Yy or Zz) or homozygous (YY or ZZ) for resistance.

### *Scenario 1. Glyphosate resistance in minimum-tillage crop establishment systems*

The evolution of glyphosate resistance was simulated in a continuous cropping rotation where glyphosate was used every year for pre-sowing *L. rigidum* control. Crop sowing was with minimum tillage (100% soil disturbance) and early and late crop sowing strategies were compared.

### *Scenario 2. Glyphosate resistance in no-tillage crop establishment systems*

Glyphosate was used every year for pre-sowing *L. rigidum* control. Crop sowing was with no-tillage and early and late crop sowing strategies were compared.

### *Scenario 3. Glyphosate resistance with diversified crop establishment systems*

Glyphosate was used every year for pre-sowing *L. rigidum* control. Early and delayed crop sowing strategies were alternated. Rates of predicted evolution of glyphosate resistance were compared in systems with annual use of no-tillage at crop sowing, with annual alternation of no-tillage and minimum tillage, and with a rotation of 2 years no-tillage and 1 year minimum tillage.

### *Scenario 4. Glyphosate and paraquat resistance with diversified pre-sowing herbicide applications*

Early and delayed crop sowing strategies were alternated in a system with annual use of no-tillage at crop sowing. Rates of predicted evolution of glyphosate and paraquat resistance were compared when use of the two herbicides for pre-sowing control of *L. rigidum* was rotated annually, where 2 years of glyphosate use was followed by a single year of paraquat and where the double knockdown strategy was employed.

### *Scenario 5. Glyphosate resistance with a wheat, lupin, wheat, Roundup Ready® oilseed rape ($WLWR_{RR}$) rotation in minimum tillage and no-tillage establishment systems*

Glyphosate was used every year for pre-sowing *L. rigidum* control in a $WLWR_{RR}$ rotation. Glyphosate was used as the only in-crop herbicide in the oilseed rape phase of the rotation. Rates of predicted evolution of glyphosate resistance were compared in minimum and no-tillage crop establishment systems.

### *Scenario 6. Glyphosate and paraquat resistance in a no-tillage $WLWR_{RR}$ rotation with diversified glyphosate and paraquat use for pre-sowing* L. rigidum *control*

The evolution of glyphosate and paraquat resistance was predicted in no-tillage systems where (i) glyphosate and paraquat were rotated annually, (ii) glyphosate was used for pre-sowing *L. rigidum* control following wheat and lupin crops and paraquat was used following glyphosate-resistant oilseed rape and (iii) the double knockdown was practised in early and average starts to the growing season.

### *Scenario 7. Glyphosate and paraquat resistance in a no-tillage $WLWR_{RR}$ rotation using the double knockdown and additional cultural weed control*

The double knockdown crop establishment system was combined with high crop sowing rates (wheat 160 plants $m^{-2}$, lupin 66 plants $m^{-2}$, oilseed rape 120 plants $m^{-2}$) and annual use of weed seed collection at harvest.

### *Scenario 8. Glyphosate and paraquat resistance in a no-tillage $WLWR_{RR}$ rotation with no in-crop selective herbicides available in wheat*

Evolution of resistance to glyphosate and paraquat was simulated in a system identical to scenario 7, but where no options were available for in-crop herbicidal control of *L. rigidum* in wheat crops.

## Results and discussion

### *Glyphosate resistance in minimum-tillage crop establishment systems*

Despite annual glyphosate use in a 30-year WLWR rotation, evolution of resistance to glyphosate in *L. rigidum* is never predicted in a minimum-tillage cropping system with early crop sowing. Under this system selection pressure for glyphosate resistance is low as the herbicide is applied shortly after the start of the season when only a small proportion of annual *L. rigidum* emergence has occurred (Neve *et al.*, 2003). Where initially rare glyphosate-resistant individuals emerge as part of the first cohort of *L. rigidum* emergence and survive glyphosate application, they are controlled by the soil disturbance associated with crop sowing in a minimum-tillage system. At the start of simulations, glyphosate-resistant genotypes are very rare [in a population with an initial glyphosate resistance allele frequency of $1 \times 10^{-8}$ and an *L. rigidum* seedbank density of 1000 seeds $m^{-2}$ there will be 10 heterozygous-resistant (Zz) individuals] and may easily be driven to extinction within the population. Where all other weed control practices are effective and the *L. rigidum* population is declining, the potential for *de novo* mutation to resistance is small.

As resistance to in-crop selective herbicides has proliferated and options for in-crop *L. rigidum* control have declined, delayed crop sowing has become a strategy in integrated weed management systems (Powles & Matthews, 1996; Powles & Bowran, 2000). Simulations were run which incorporated annual delayed sowing into the analysis presented above. Despite the increased selection pressure resulting from a larger proportion of *L. rigidum* population being treated with glyphosate, resistance was predicted in only one of the 1000 model runs (0.1% of populations). These initial analyses demonstrate that continuous glyphosate use in a crop establishment system that incorporates soil tillage can be sustainable. They also provide a baseline against which current and projected future changes in glyphosate use can be compared, enabling future risks of glyphosate resistance to be quantified.

### Glyphosate resistance in no-tillage crop establishment systems

No-tillage systems that use glyphosate every year greatly increase risks of evolution of glyphosate resistance (Fig. 1). Where sowing is early, resistance first becomes apparent after 24 years in a small number of simulations (Fig. 1). The probability of resistance evolving after 30 years is *c.* 20%. When sowing is delayed, the rate and probability of evolution of glyphosate resistance increases (Fig. 1). A delayed sowing strategy with no-tillage and annual glyphosate use results in a 50% probability of phenotypic resistance after 14 years and glyphosate resistance in 90% of populations after 30 years.

The importance of tillage for killing weeds at crop sowing has been demonstrated and results strongly suggest that a continuing move towards no-tillage systems will considerably increase risks of glyphosate resistance, particularly where late sowing is practised. These predicted results concur with observations in the field in Australia, where glyphosate-resistant *L. rigidum* biotypes have always evolved in no-tillage crop establishment systems.

### Glyphosate resistance with diversified crop establishment systems

While delayed crop sowing is acknowledged and advocated as a potential weed management strategy, especially where resistance to selective herbicides reduces options for in-crop weed control, it is unlikely that any field would be sown late in every year. More realistically, sowing time will vary from year to year depending on the crop sown and on other agronomic considerations. The results presented in Fig. 2 are from simulations where early and delayed sowing strategies are alternated and where annual no-tillage is compared with strategies that rotate between no-tillage and minimum-tillage crop establishment systems.

When early and delayed sowing dates are rotated, rates of evolution of glyphosate resistance are lower, with resistance predicted in 50% of populations after 20 years (Fig. 2). Annual rotation between no-tillage and minimum-tillage establishment systems significantly reduces the rate and probability of glyphosate resistance

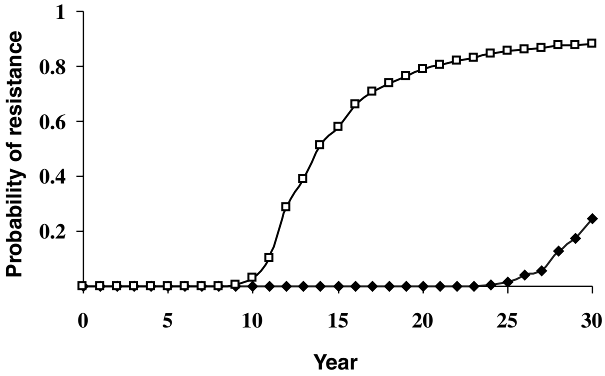

**Fig. 1** Cumulative probability distributions for predicted evolution of glyphosate resistance in a 30-year simulation of a WLWR cropping rotation with early (—◆—) and late (—□—) crop sowing and no-tillage at sowing. Glyphosate is used annually for pre-sowing weed control. Pre- and post-emergence herbicides are used every year.

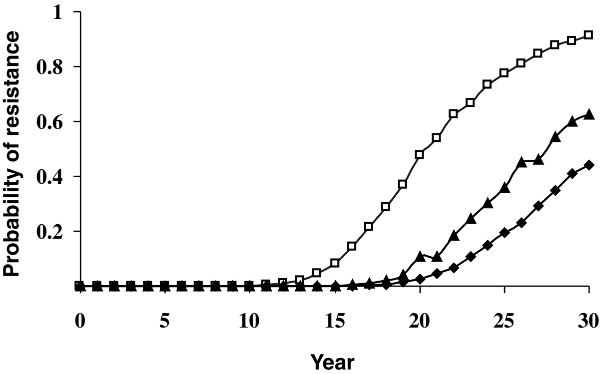

**Fig. 2** Cumulative probability distributions for predicted evolution of glyphosate resistance in a 30-year simulation of a WLWR cropping rotation, with alternating early and delayed crop sowing in systems with no-tillage at sowing every year (—□—), with alternating no-tillage and minimum tillage (—◆—) and with a rotation of 2 years no-tillage and 1 year minimum tillage (—▲—). Glyphosate is used annually for pre-sowing weed control. Pre- and post-emergence herbicides are used every year.

(44% resistance after 30 years). Incorporating minimum tillage in 1 of 3 years in a predominantly no-tillage system results in resistance in 50% of populations after 28 years.

Risks of glyphosate resistance are substantially increased in no-tillage systems. Re-introducing minimum tillage reduces the probability of glyphosate resistance. However, most growers who practise no-tillage are committed to this crop establishment system for its longer-term soil structure and nutritional benefits and will be reluctant to sacrifice these benefits solely for glyphosate resistance management. Clearly, those growers fully committed to no-tillage must consider alternative use strategies for pre-sowing herbicidal weed control.

### *Increasing diversity in pre-sowing chemical weed control: the role of paraquat*

Simulations in which paraquat and glyphosate were alternated for pre-sowing control in a no-tillage WLWR rotation are presented in Fig. 3. Compared with a similar strategy which used glyphosate annually (Fig. 2), risks of glyphosate resistance are considerably reduced when paraquat is used intermittently for pre-sowing control. An annual rotation of glyphosate and paraquat reduced the predicted risk of glyphosate resistance to 17% after 30 years with no resistance predicted before year 23. Paraquat use in 1 of 3 years resulted in glyphosate resistance in 46% of populations by year 30, with no resistance predicted before year 19 of the rotation. Resistance to paraquat did not evolve in these simulations. These analyses demonstrate that risks of evolution of glyphosate resistance in no-tillage systems can be reduced, but not completely eliminated by rotating herbicide modes of action.

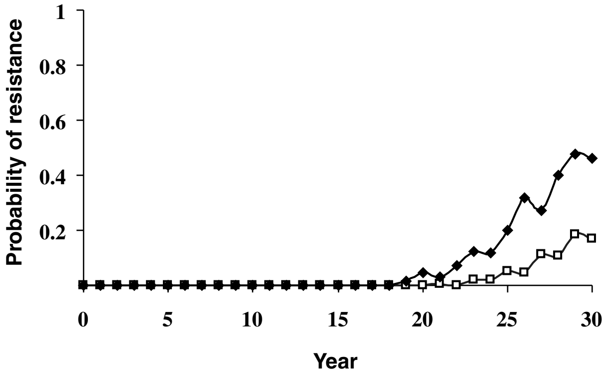

**Fig. 3** Cumulative probability distributions for predicted evolution of glyphosate resistance in a 30-year simulation of a WLWR cropping rotation, with alternating early and delayed crop sowing, no-tillage at sowing and annual rotation of glyphosate and paraquat for pre-sowing weed control (—□—) or 2 years glyphosate and 1 year paraquat (—◆—). Pre- and post-emergence herbicides are used every year.

An alternative to a single pre-sowing herbicide application followed by minimum tillage at sowing is the 'double knockdown', in which glyphosate and paraquat are applied sequentially prior to sowing. The effectiveness of this strategy is dependent on both herbicides being applied at full lethal rates (Diggle *et al.*, 2003). The double knockdown requires application of glyphosate after a substantial *L. rigidum* germination followed by paraquat application 5–10 days later. This is only possible in years with an early or average start to the season and is not practical in years with a late season. Under the double knockdown strategy glyphosate resistance was predicted in only 17 of the 1000 simulation runs, despite the fact that the double knockdown could only be practised in 60% of years. Resistance to paraquat was never predicted. The double knockdown which effectively replaces the physical weed control associated with tillage with a second pre-sowing herbicide application greatly reduces the risks of glyphosate resistance evolution in no-tillage cropping systems.

### *The introduction of glyphosate-resistant oilseed rape to Australian agriculture*

The introduction of glyphosate-resistant oilseed rape to southern Australian cropping systems is expected in the short to medium term. Widespread adoption of glyphosate-resistant crops will undoubtedly increase selection pressure for weed resistance to glyphosate, especially if the current very heavy reliance on glyphosate for pre-sowing weed control is maintained. In a no-tillage system with annual use of glyphosate for pre-sowing *L. rigidum* control, introduction of glyphosate-resistant oilseed rape results in very rapid predicted evolution of resistance (Fig. 4). Resistance is predicted in a small

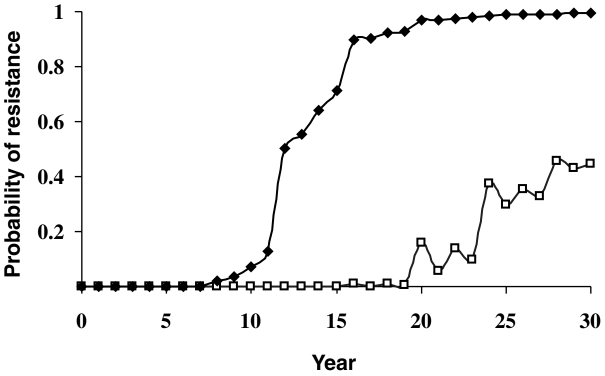

**Fig. 4** Cumulative probability distributions for predicted evolution of glyphosate resistance in a 30-year simulation of a WLWR cropping rotation with glyphosate-tolerant oilseed rape (WLWR$_{RR}$) and alternating early and delayed crop sowing in no-tillage (—◆—) and minimum-tillage (—□—) crop establishment systems. Pre- and post-emergence herbicides are used every year.

number of populations after as few as 7 years and in almost 100% of populations by year 20 (resistance in an identical system without glyphosate-resistant oilseed rape is predicted in 50% of populations after 20 years, Fig. 2). Similarly, risks in a minimum-tillage system were increased from zero to *c*. 40% when glyphosate-resistant oilseed rape was introduced (Fig. 4). These results clearly demonstrate the additional selection pressure for resistance resulting from the introduction of a glyphosate-resistant crop in 1 of a 4-year cropping rotation.

### *Glyphosate-resistant oilseed rape: mitigating risks of evolved glyphosate resistance in* L. rigidum

The results in Fig. 5 are from a series of simulations that predict rates and probabilities of evolution of glyphosate and paraquat resistance in rotations with glyphosate-resistant oilseed rape and no-tillage. Replacing glyphosate with paraquat for pre-sowing weed control in years following glyphosate-resistant oilseed rape has been advocated as a means of reducing the additional selection pressure resulting from the introduction of the glyphosate-resistant crop. However, while the total number of glyphosate applications is the same as when glyphosate is used every year without glyphosate-resistant oilseed rape, selection pressure is not and glyphosate resistance is predicted in 50% of populations after 13 (Fig. 5A) as opposed to 20 years (Fig. 2). Clearly, applications of glyphosate later in the growing season that select for glyphosate resistance in later as well as earlier emerging *L. rigidum* cohorts greatly increase relative selection pressure.

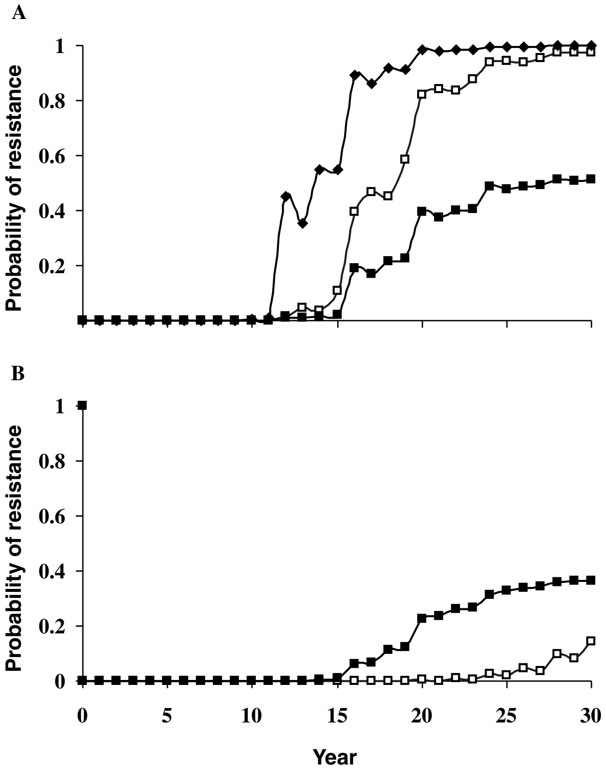

**Fig. 5** Cumulative probability distributions for predicted evolution of (A) glyphosate resistance and (B) paraquat resistance in a 30-year simulation of a WLWR$_{RR}$ rotation with no-tillage at crop sowing. Pre-sowing herbicide use strategies were glyphosate in seasons following wheat and lupin crops and paraquat in seasons following glyphosate-resistant oilseed rape (—◆—), annual alternation of glyphosate and paraquat (—□—) and the double knockdown in early and average starts to the season and glyphosate alone in late seasons (—■—). Pre- and post-emergence herbicides are used every year.

As expected, alternating pre-sowing applications of glyphosate and paraquat, or using the double knockdown, reduces risks and rates of predicted evolution of glyphosate resistance (Fig. 5A). Once again, however, these risks are considerably greater than in systems without glyphosate-resistant oilseed rape. At the same time, increased use of paraquat results in the prediction of resistance in a small number of populations (Fig. 5B). When glyphosate and paraquat are alternated, paraquat resistance is predicted in 14% of populations at year 30. However, this result may be misleading and insignificant from a practical management perspective. Paraquat-resistant individuals arise in the populations as a result of *de novo* mutations when population sizes rapidly increase due to widespread resistance to glyphosate. It is likely that the rotation described would become uneconomical before the appearance of paraquat resistance and hence would not be continued to the point where paraquat resistance evolved. The double knockdown is the most effective pre-sowing strategy for reducing risks of glyphosate resistance. However, where this is implemented in rotations containing glyphosate-resistant oilseed rape, risks of resistance remain significant (Fig. 5A). Glyphosate resistance is predicted in 50% of simulations at year 30, but perhaps more significantly, there is simultaneous selection for resistance to paraquat in 37% of *L. rigidum* populations (Fig. 5B), resulting in the eventual loss of both herbicides for control of some *L. rigidum* populations.

Up to this point, strategies for glyphosate resistance management have focused on pre-sowing herbicidal weed control, tillage at sowing and crop rotations. High crop sowing rates and weed seed collection reduce the total amount of *L. rigidum* seed that is returned to the seedbank and by maintaining weed populations at low densities, increase the probability that rare resistance genes can be driven to extinction, thus preventing selection for resistance. In analyses with high crop sowing rates and weed seed collection at harvest with the double knockdown strategy as described above there was no resistance predicted to either glyphosate or paraquat at year 30 (data not shown).

### *Resistance to in-crop post-emergence herbicide options*

The ever increasing incidence of *L. rigidum* resistant to selective post-emergence herbicides (particularly ACCase- and ALS-inhibiting modes of action) in Australia (Llewellyn & Powles, 2001) means that many farmers now have few or no options remaining for in-crop *L. rigidum* control in some crops. Up to now, all simulations have assumed a full suite of in-crop herbicides are available in all crops. The results in Fig. 6 are from a $WLWR_{RR}$ rotation, with no-tillage, a double knockdown establishment system, high crop sowing rates and weed seed collection at harvest where no herbicides are available for in-crop *L. rigidum* control in wheat.

The loss of in-crop selective weed control from wheat results in large increases in predicted risks of glyphosate and paraquat evolution (Fig. 6). Resistance to both herbicides becomes apparent after 12 years and by year 30 is predicted in almost 90% of populations. These very high resistance risks can be overcome by returning to a system with minimum-tillage system with full soil disturbance at sowing. When this is done, risks of paraquat resistance are eliminated and glyphosate resistance is predicted in only 0.3% of populations (data not shown). This result once again demonstrates the importance of diverse, integrated and efficacious strategies that enable initially rare resistant survivors to be subsequently controlled by additional, non-selective (for the trait in question) management.

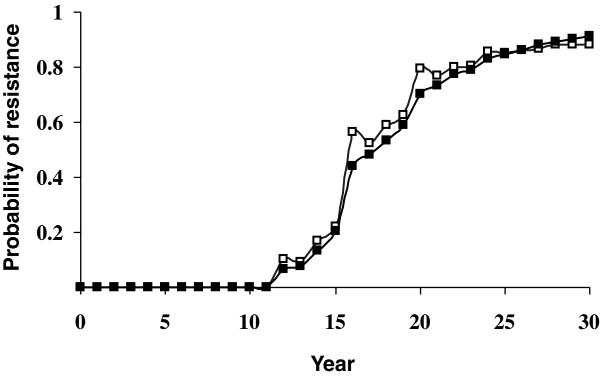

**Fig. 6** Cumulative probability distributions for predicted evolution of glyphosate (—□—) and paraquat (—■—) resistance in a 30-year simulation of a $WLWR_{RR}$ cropping rotation. Crop establishment system is the double knockdown followed by no-tillage sowing. Pre-emergence herbicides are used every year and no in-crop selective herbicides are available in wheat crops. High crop sowing rates and weed seed collection at harvest are practised every year.

### *Bringing it all together: glyphosate and paraquat resistance in the past, present and future*

Following its introduction to Australia in 1974, glyphosate has become widely adopted for broad-spectrum weed control. Simulations presented in this paper have demonstrated that with annual soil disturbance and a full suite of effective pre- and post-emergence herbicides, annual use of glyphosate for pre-sowing weed control is likely to be sustainable for in excess of 30 years (30 applications) from the time when it is first used. However, over the past decade Australian farmers have increasingly adopted no-tillage crop establishment systems that considerably increase risks of glyphosate resistance (Fig. 1). These risks can be reduced by alternating between minimum and no-tillage crop establishment systems (Fig. 2) or by alternating between glyphosate and paraquat for pre-sowing weed control (Fig. 3). However, the most effective strategy for conserving glyphosate susceptibility in no-tillage systems is the 'double knockdown'.

Rotation of glyphosate and paraquat and even the double knockdown cannot fully accommodate the introduction of glyphosate-resistant oilseed rape (Fig. 4). The use of glyphosate as an in-crop herbicide requires other integrated management strategies that reduce the competitiveness and seed production capacity of *L. rigidum* and minimize the amount of mature seed that is returned to the soil seedbank. This can be achieved with high crop sowing rates and weed seed collection at harvest, strategies which are capable of significantly reducing weed population sizes so that despite intense selection the probability of driving initially rare resistance alleles to extinction is increased.

Increasingly, options for in-crop selective *L. rigidum* control are being exhausted as resistance to the ACCase-inhibiting ALS-inhibiting and other herbicides proliferates. In simulations where no options remained for in-crop *L. rigidum* control in wheat crops with glyphosate-resistant oilseed rape included in the rotation, resistance to glyphosate and paraquat could only be avoided by a return to annual minimum tillage at sowing (Fig. 5).

Together, the simulations presented have demonstrated that continued susceptibility of *L. rigidum* populations to glyphosate is considerably more likely in diverse and highly effective herbicidal and cultural weed management systems. The analyses presented in this paper are only a subset of possible management combinations that could be used to enhance the long-term sustainability of glyphosate and paraquat use in the presence and absence of glyphosate-resistant crop varieties. Nevertheless, they neatly demonstrate a number of important principles. It is clear that changing cropping practices in Australia and worldwide, particularly the move towards no-tillage and the introduction of glyphosate-resistant crop varieties, are increasing risks of glyphosate resistance. These risks can be offset by judicious use of available non-selective herbicide chemistries, with sequences of pre-sowing, pre- and post-emergence herbicides with discrete modes of action, and with cultural weed management practices that reduce overall weed burdens.

## Acknowledgements

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