Research Article

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Why was resistance to shorter-acting pre-emergence herbicides slower to evolve?

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Abstract

BACKGROUND: Across several agricultural systems the evolution of herbicide resistance has occurred more rapidly to post-emergence than pre-emergence herbicides; however, the reasons for this are not clear. We used a new simulation model to investigate whether interactions between differences in order of application and weed cohorts affected could explain this historically observed difference between the herbicide groups.

RESULTS: A 10 year delay in resistance evolution was predicted for a shorter-acting residual pre-emergence (cf. post-emergence), when all other parameters were identical. Differences in order of application between pre- and post-emergence herbicides had minimal effect on rates of resistance evolution when similar weed cohorts were affected.

CONCLUSION: This modelling suggested that the historically observed lower levels of resistance to pre-emergence herbicides are most likely to be due to the smaller number of weed cohorts affected by many pre-emergence herbicides. The lower number of weed cohorts affected by pre-emergence herbicides necessitated the use of additional, effective control measures, thereby reducing resistance evolution. This study highlights the advantages of applying multiple control measures to each weed cohort.

Keywords: *Lolium rigidum*; ryegrass; population modelling; mode of action; herbicide resistance

1 INTRODUCTION

High reliance on herbicides in global field crop production has exerted strong selection pressure for the evolution of herbicide-resistant weeds. Here, in global field situations, we are concerned with relative rates of resistance evolution to pre- versus post-emergence herbicides. Pre-emergence herbicides are usually applied before crop emergence and affect germinating weeds, with some length of soil residual activity. Crop-selective post-emergence herbicides are applied after crop and weed emergence and aim to control small, emerged weed seedlings. For example, soil-applied pre-emergence herbicide (e.g. trifluralin) use commenced in Australia in the 1960s for selective control of weeds in cereal crops.[1] Post-emergence herbicides, such as the ACCase-inhibiting herbicides (e.g. diclofop), were introduced from the late 1970s onwards. Post-emergence herbicides helped to facilitate the adoption of reduced-tillage systems, and the greater number of weed cohorts controlled led to a reduction in the use of pre-emergence herbicides. However, since the first report of an Australian weed population resistant to diclofop,[2] ACCase post-emergence herbicide resistance has increased widely across the Australian grainbelt. In contrast, the evolution of resistance to pre-emergence herbicides has been slower, and some pre-emergence herbicides have remained largely effective, despite, in some cases, decades of frequent use across extensive areas.[3–6]

Identification of factors contributing to the more rapid evolution of resistance to certain herbicides has important implications for the use and stewardship of both current and new herbicides. However, trying to analyse and differentiate causal factors through empirical studies is challenging.[7] Computer modelling simulations (*in silico* studies) are a useful tool for rapid investigation of individual factors influencing resistance evolution, while also allowing investigations of the stability of the predictions under various parameterisations.[8,9] Factors that are difficult to investigate in empirical studies and that have received little focus in modelling work are herbicide sequence and weed cohorts affected, and the interactions between them. Moreover, the evolutionary implications of sequential applications of pre- and post-emergence herbicides (applying one after the other in the same year) have not previously been modelled.

The first aim of this study was to investigate whether simulations incorporating different application timings and lengths of herbicide residual effect would mimic the field experience that resistance evolves at different rates to different groups of herbicides. We developed an individual-based evolutionary model capable of simulating differences in the number of weed cohorts controlled, the order of herbicide application and the rate of resistance evolution. This model was used to predict evolution of resistance to pre- versus post-emergence herbicides, when just one of these herbicides was applied each year. The second aim of this study was to use the model to investigate the potential benefits and implications of sequential applications of pre- and post-emergence herbicides, or more specifically, how the order

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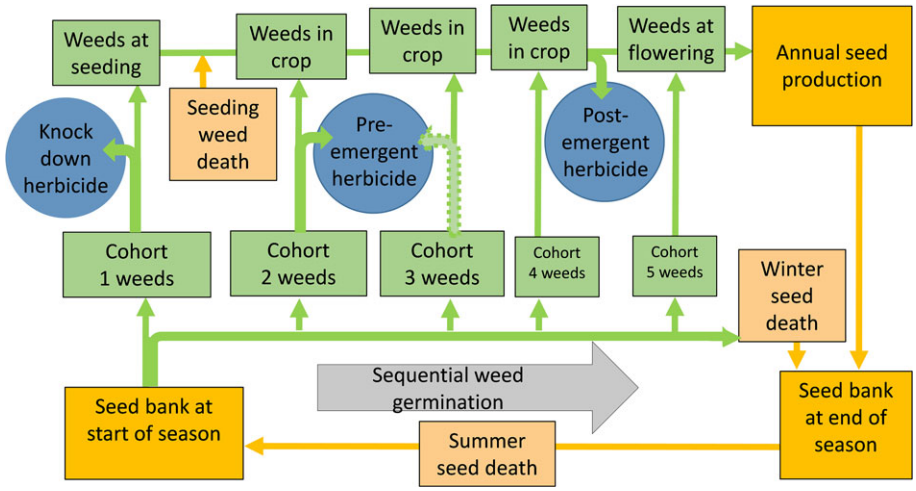

Figure 1. Representation of the life cycle of a managed annual weed infestation growing in a single season cropping system. The dotted arrow from cohort 3 indicates that the pre-emergence herbicide was simulated as either short acting (only cohort 2) or longer acting (cohorts 2 and 3). Each year's weed plants could be divided into cohorts owing to their sequential weed germination. The use of cohorts enabled the simulations to be conducted with alternate herbicide kill rates for weed plants with different germination and emergence dates.

of application and the weed cohorts affected affected resistance evolution when both herbicides were applied each year.

2 METHODS

2.1 Model overview

This study simulated the evolution of herbicide resistance within an annual weed population, using a new individual-based stochastic model implemented in the R programming language.[10] Features of the existing Ryegrass Integrated Model (RIM)[11,12] and the Polygenic Evolution of Resistance To Herbicides (PERTH) model,[13] such as multiple weed cohorts and resistance genetics respectively, were combined and developed in new ways to create a model fit for addressing our specific research questions. By cohort, we mean a group of weeds that germinate at similar times, and are thus of similar age. Each year's germinating weeds were divided into cohorts based on when they germinated in relation to management events (for example, crop sowing date is the division between cohorts 1 and 2) (Fig. 1). Resistance to each herbicide was assumed to be endowed by a single semi-dominant gene conferring resistance to one herbicide mode of action. There were therefore three possible genotypes for each resistance gene: the homozygous susceptible (SS) weeds were killed, whereas the heterozygous resistant (RS) and homozygous resistant (RR) weeds survived herbicide application (Table 1).

2.2 Situation represented

The two herbicides included in this investigation represent a pre-emergence herbicide with soil residual activity (such as trifluralin) and a post-emergence herbicide with contact activity and no residual activity (such as diclofop). Herbicides were applied each year to a population of an annual outcrossing grass weed in a simulated crop field with one annual crop per year. Parameterisation of the model (Table 1) was based on representations of annual ryegrass (*Lolium rigidum*) in a typical spring wheat cropping programme in a Mediterranean-type climate in southern Australia, used in previous studies and models, including RIM,[11,12] PERTH[13] and other simulations.[14] For simplicity, we assumed no rotation of crops or herbicides. Later in the growing season there were few weeds from the early cohorts surviving, owing to the earlier-applied pre-emergence herbicide and increasing plant competition. The post-emergence herbicide in this simulation was capable of killing weeds across a wider spread of cohorts, and was the more effective herbicide, despite the fact that there were a small number of weeds from earlier cohorts still alive when it was applied.

2.3 Annual weed population dynamics

The model represents the yearly life cycle of annual weed germination, growth, reproduction and mortality (Fig. 1). We assume an average initial seedbank density of 125 seeds m^{-2} with an initial resistance probability of 1×10^{-6} in year 1 of the simulations, or around one resistant seed per 8000 m^2. All processes within the annual weed population dynamics were applied stochastically to each weed genotype in each sequential cohort. The annual weed germination and emergence was divided into five distinct growing season weed cohorts, with seasonally adjusted crop competition (Table 2). The first weed cohort comprised seedlings that germinated prior to crop seeding. Weed cohorts 2 and 3 comprised weed seeds that germinated relatively soon after the crop was seeded, while smaller numbers of late-season germinating weed seeds were assigned to cohorts 4 and 5.

We considered a number of herbicide application scenarios (details below). Herbicide treatments were identical each year within a scenario, but varied between scenarios. In all scenarios, an early-season application of a preseeding knockdown herbicide for which we assumed nil resistance was applied annually to remove the weed cohort that emerged prior to crop seeding (cohort 1 in Fig. 1). Mortality due to the pre- and the post-emergence herbicide applications depended on resistance status. Resistant plants that were homozygous for the resistance gene suffered a 5% probability of death during herbicide application owing to physical and/or biological damage, while heterozygous plants were weakened by the herbicide application, equated to a stochastic kill rate of 30%. The susceptible plants suffered varying degrees of higher mortality, dependent on their cohort (Table 3). Pre- versus post-emergence herbicides affected different weed cohorts (Table 2). All plants that survived to the end of the growing season were assumed to produce viable seed. However, the seed

Table 1. Parameter values used in these computer simulations

Reference(s)	Parameter	Value
13	Simulated population area	500 ha
13,33	Initial weed seed density	125 m^{-2}
34	Initial resistance frequency	1×10^{-6}
15	Probability of growing season death of ungerminated seeds	0.10
15	Probability of between-season seed death	0.25
35	Probability of annual seed germination from seedbank	0.80
12	Probability of death from knockdown on weed cohort 1	0.99
12	Wheat density	150 m^{-2}
14	Crop size/competitiveness parameter	0.09
14	Annual ryegrass size/competitiveness	0.0333
36	Maximum annual ryegrass seed per metre	35 000 m^{-2}
13	Probability of seed immigration into the seedbank	0.1 seeds m^{-2} $year^{-1}$
37	Probability of seed loss from the field	0.001 $year^{-1}$
38	Degree of dominance (heterozygous survival, when compared with homozygous survival)	0.7
39	Probability of spontaneous mutations (all alleles)	1×10^{-8}

production of later-germinating plants was reduced owing to competition from both the standing crop and surviving weeds (Table 2). The density of the annual weed population influenced both wheat yield and weed seed production, with an additional reduction in seed and pollen quantity based on relative cohort fitness.[13,15] Seed production for both the crop ($i = 1$) and each of the five weed cohorts ($i = 2{:}6$) was calculated on a per square metre basis following an adaption of a commonly used hyperbolic model of weed competition.[16]

$$\text{Production}_{(i)} = \frac{\text{maximum possible production}_{(i)} \times \text{plant density}_{(i)} \times \text{fitness}_{(i)}}{1 + \sum_{i=1}^{6} \left(\text{plant density}_{(i)} \times \text{fitness}_{(i)}\right)} \quad (1)$$

The weed seedbank was adjusted at the end of each growing season, with gains from seed production and winter losses due to seed decay and predation. Seed death over summer (summer mortality) was then subtracted from the new seedbank. A very small amount of seed emigration (as a proportion of the current seedbank) and immigration (constant rate, using the unselected population genetic ratios) was then applied, and the resultant weed seedbank provided the initial conditions for the next year of the simulation (Table 1).

2.4 Genetics

One and two gene hereditary transition matrices[17] were used for the stochastic modelling of matings between and within each cohort, with enforced self-incompatibility. Stochastic genetic mutations for each gene, *i*, were incorporated in a similar manner, using a probability transition matrix with the following formulation:

$$V_i = \begin{pmatrix} (1-u_i)^2 & 2u_i(1-u_i) & u_i^2 \\ v_i(1-u_i) & (1-u_i)(1-v_i) + u_i v_i & u_i(1-v_i) \\ v_i^2 & 2v_i(1-v_i) & (1-v_i)^2 \end{pmatrix}$$

where the population is expressed as a vector in the form pop_i = (number of S_iS_i, number of R_iS_i, number of R_iR_i) u_i is the probability of the susceptible gene (S_i) mutating into a resistant gene (R_i) and v_i is the probability of the resistant gene (R_i) mutating into the susceptible gene (S_i), and the expected number of each genotype after mutation is given by premultiplying V_i by pop_i. In our model, the numbers of each genotype after mutation were generated stochastically using the rmultinom function in the R programming language.[10] For two independently assorting resistance genes (R1 and R2) a two-gene probability transition matrix was obtained using the Kronecker product of the two probability transition matrices, $V_1 \otimes V_2$. The resultant 9×9 probability transition matrix was used to generate simultaneous mutation in two genes, where the population is expressed as a vector in the form ($S_1S_1S_2S_2$, $R_1S_1S_2S_2$, $R_1R_1S_2S_2$, $S_1S_1R_2S_2$, $R_1R_1S_2S_2$, $R_1R_1R_2S_2$, $S_1S_1R_2R_2$, $R_1S_1R_2R_2$, $R_1R_1R_2R_2$). This procedure was used in each year of the simulation to generate stochastic mutations within each years' new seeds.

2.5 Model calibration

Adjustments to the unverified parameters of weed seed death, weed cohort size and relative cohort fitness within previously established limits[12] were used to give a realistic weed population size at harvest when the two herbicides were used individually in the absence of resistance. During the preliminary simulations, summer death rates from 25 to 50% and the relative fitness of later weed cohorts from 0.02 to 0.75 were trialled. Variation in these parameter values in combination (at levels that maintained a realistic yearly weed population) was found to have no significant effect on the results (data not shown). Consequently, we selected a set of mid-range values (Table 1) coupled with four weed cohort fitness levels (within the range used in RIM) for the five weed cohorts (Table 2).

2.6 Scenarios investigated

Five different scenarios were simulated (Table 3). Scenarios 1a, 1b and 1c represented either a pre-emergence or a post-emergence herbicide applied each year (but not both), while scenarios 2 and 3 represented both pre- and post-emergence herbicides applied sequentially each year. Scenarios 2 and 3 examined a weed population that contained two unlinked semi-dominant genes for herbicide resistance. Allele R_1 at gene 1 conferred resistance to the pre-emergence herbicide (phenotype R_1) and comprised the genotypes R_1S_1 and R_1R_1. Allele R_2 at gene 2 (phenotype R_2) conferred resistance to the post-emergence herbicide and was composed of the genotypes R_2S_2 and R_2R_2. In scenarios 2 and 3, multiple resistance (phenotype R_1R_2) comprised four genotypes: $R_1S_1R_2S_2$, $R_1S_1R_2R_2$, $R_1R_1R_2S_2$, and $R_1R_1R_2R_2$. In scenarios 1a, 1b and 1c, only the gene that conferred resistance to the investigated herbicide was modelled, as the alleles at the other gene site would have made no difference. In scenarios 1a and 2, a shorter-acting residual pre-emergence herbicide successfully contacted only

Table 2. Weed emergence through the season was divided into five weed cohorts,[35] with most of the seeds germinating into the first three cohorts. Later-germinating plants were given a fitness penalty that lowered both their effect on the crop yield and their pollen and seed production

Weed cohort	Proportion of annual seedbank germinating in each weed cohort	Herbicides applied	Relative fitness levels
1	0.40	Knockdown, post-emergence	1
2	0.20	Pre-emergence, post-emergence	0.80
3	0.18	Longer-acting pre-emergence, post-emergence	0.5
4	0.01	Post-emergence	0.02
5	0.01	–	0.02

Table 3. Stochastic-probability-based mortality rates for susceptible weeds in cohorts 2 to 4 that were treated with the herbicides, and initial gene frequencies for resistance in the five different scenarios. Total kill indicates the probability that a susceptible weed that germinated after crop seeding is killed. Total kill is expressed as standard plant equivalents (explained in Section 2.7)

Scenario number	Description	Cohort 2 weeds (%)	Cohort 3 weeds (%)	Cohort 4 weeds (%)	Average kill of all weeds, cohorts 2–5. (%)	Initial frequency, pre-emergence resistance	Initial frequency, post-emergence resistance
1a	Shorter-acting pre-emergence herbicide only	97	–	–	74.8	1×10^{-6}	–
1b	Longer-acting pre-emergence only	97	97	–	95.3	1×10^{-6}	–
1c	Post-emergence only	97	97	97	96.9	–	1×10^{-6}
2	Shorter-acting pre-emergence and post-emergence	97 (twice[a])	97	97	99.0	1×10^{-6}	1×10^{-6}
3	Longer-acting pre-emergence and post-emergence	97 (twice[a])	97 (twice[a])	97	99.8	1×10^{-6}	1×10^{-6}

[a] Weeds in these cohorts are hit twice, giving an effective control rate in susceptible weeds of $0.97 + 0.03 \times 0.97 = 0.9991$.

weed cohort 2; this meant that the kill rate (probability of death) for all susceptible weeds that emerged post-seeding was reduced from 95 to 75% (Table 3). This reduction reflected the lower control levels sometimes reported for pre-emergence herbicides.[18,19] The stated kill rates included an allowance for biological damage and subsequent reduction in fitness of any surviving heterozygous and susceptible plants.

For all scenarios, the simulated field area was 500 ha, containing an initial population of approximately 625 million susceptible weed seeds and 625 seeds resistant to each herbicide. Each scenario ran for 15–20 years, and was replicated 100 times, from which mean annual values were calculated. This was repeated several times with a high level of consistency, indicating that 100 replications over an area of 500 ha was generally sufficient to produce repeatable results.

2.7 Summarised simulation results

For each year, for each of the 100 replications of each scenario, the number and genotype of live weeds present at harvest was recorded. In these records (and within the simulations), the lower competitiveness of later-germinating weed cohorts was accounted for by multiplying the number of plants in each cohort by its relative fitness level (Table 2). This means that numbers presented in Section 3 represented 'standard plant equivalents', where a standard plant was from weed cohort 1, that is, a plant that emerged concurrently with the wheat crop. For example, plants in weed cohort 4 have a relative fitness of 0.02; therefore, 50 plants in weed cohort 4 were required to make up one 'standard plant equivalent'. The relative yield of the annual crop and the genotypic composition of the seedbank at the end of each season was also recorded. The graphs in Figs 2 to 5 were compiled in R,[10] with the polygon function (code donated by Kevin Buhr: buhr@stat.wisc.edu) and the packages 'abind',[20] 'calibrate'[21] and 'MASS'.[22]

3 RESULTS

3.1 Single annual herbicide applications

A shorter-acting pre-emergence herbicide, when used as the sole herbicide treatment, failed adequately to limit weed numbers as its shorter length of soil residual activity meant that it could not control later-emerging weed cohorts (Fig. 2A). It is universally accepted that a shorter-acting pre-emergence herbicide when used alone does not provide a sufficient level of weed control. In contrast, either a longer-acting pre-emergence herbicide used alone or a post-emergence herbicide used alone killed sufficient susceptible weeds to maintain good weed control (low weed numbers) for 5 years, before failure was evident.

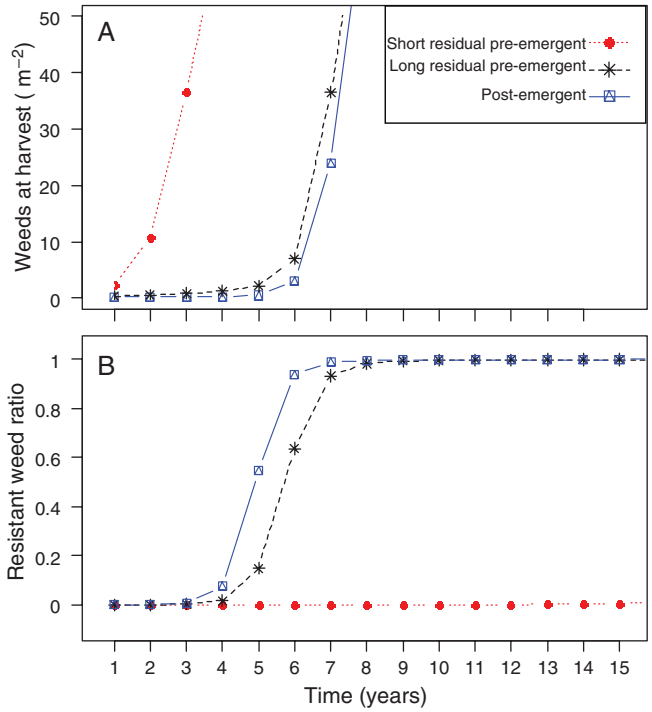

Figure 2. Differing rates of weed population growth at harvest as a response to three different herbicides applied individually at/after seeding (scenario 1). Results were calculated from 100 replications of 500 ha, and expressed as full-size equivalent weeds: (A) the average number of weeds at harvest m^{-2}; (B) the average resistance weed ratio, or the proportion of the weeds present at harvest that are resistant.

The failure of weed control when the longer-acting pre-emergence herbicide was used alone, evident at 5 years (Fig. 2A), was because high levels of herbicide resistance had evolved (Fig. 2B). Similarly, when the post-emergence herbicide was used alone, weed control failure also occurred at 5 years (Fig. 2A) and was again because high levels of herbicide resistance had evolved to the annually applied post-emergence herbicide used alone (Fig. 2B). Closer examination of the genotypes in these simulations of an obligate cross-pollinated weed species (Fig. 3) reveals that the longer-acting pre-emergence herbicide used alone (Fig. 3A) was unable to fully control susceptible weed numbers, whereas the post-emergence herbicide used alone (Fig. 3B) maintained low numbers of susceptible weeds, at least until the sixth year. The lower number of susceptible weeds in the post-emergence herbicide scenario (Fig. 3B) resulted in a lower amount of susceptible pollen and a greater number of homozygous (RR) resistant weeds. The growth in susceptible weed numbers when the longer-acting pre-emergence herbicide was used alone (Fig. 3A) was primarily due to a poor kill rate, and was greater than the seed immigration rate of 0.1 seeds m^{-2} $year^{-1}$. Therefore, the longer-acting pre-emergence herbicide was not fully curtailing the growth in susceptible weed numbers, even if no seed immigration occurred.

3.2 Sequential applications of a shorter-acting pre-emergence herbicide followed by a post-emergence herbicide

It is normal commercial practice in annual field crop production to use sequential applications of a pre-emergence herbicide before or at crop seeding, and a post-emergence herbicide applied to the young crop. Simulations showed that the combination of a shorter-acting pre-emergence herbicide (controlling weed cohort 2) sequentially followed by a post-emergence herbicide (controlling survivors of cohorts 1 and 2, and later-emerging weeds in cohorts 3 and 4) achieved good weed control for 10 years of continuous cropping (Fig. 4A). However, an increase in the ratio of resistant weeds was evident by year 7, at which time the weed population at harvest comprised almost entirely (0.95) resistant genotypes (Fig. 4B). The population ratio demonstrates that resistance evolved to the post-emergence herbicide, while resistance evolution remained very low to the shorter acting pre-emergence herbicide. The rapid speed of resistance evolution to the post-emergence herbicide occurred because the resistant survivors faced no other control and went on to produce viable resistant seed, which entered the seedbank.

3.3 Sequential applications of a longer-acting pre-emergence herbicide followed by a post-emergence herbicide

Certain pre-emergence herbicides have a long soil residual period, controlling sequentially germinating weed seedlings for many weeks. Thus, simulations included a longer-acting pre-emergence herbicide, giving longer seasonal weed control (controlling weed cohorts 2 and 3). The combination of a longer-acting residual pre-emergence herbicide (controlling weed cohorts 2 and 3) sequentially followed by a post-emergence herbicide (controlling cohorts 1 to 4) achieved good weed control for 11 years (Fig. 5A), primarily because the high level of weed control meant that weed numbers were less than 0.1 weeds m^{-2} for the first 10 years. However, once again, although there was good weed control, the average population was evolving to contain more resistant genotypes, and by year 8 the weed survivors were almost entirely (0.95) resistant (Fig. 5B), and by year 11 the population was predominantly (0.9) composed of multiple-resistant genotypes. Thus, sequential applications of two herbicides offering broad-reaching weed control (cohorts 2 and 3 and cohorts 1 to 4 respectively) provided lengthy weed control, but eventually led to the simultaneous evolution of multiple resistance to both the pre- and the post-emergence herbicides. Thus, multiple resistance (to both the pre- and the post-emergence herbicides) evolved in populations selected with a longer-acting pre-emergence herbicide sequentially followed by a post-emergence herbicide.

4 DISCUSSION AND CONCLUSIONS

The aims of this study were to examine to what extent differences in order of application and weed cohorts affected could explain some of the historically observed differences in the speed of resistance evolution to a pre- versus a post-emergence herbicide.

4.1 Single annual herbicide applications

Simulations of a pre-emergence herbicide used alone showed that weed control was insufficient (Fig. 2A and Fig. 3A). This is in accordance with decades of commercial experience. The use of a pre-emergence herbicide alone results in increasing weed numbers, necessitating the use of a post-emergence herbicide and/or an alternative weed control strategy such as harvest weed seed control[23] or rotation with alternative crops or pasture phases, as simulated by Gorddard *et al.*[24] Thus, pre-emergence herbicide failure in southern Australia was typically due to insufficient weed control, rather than resistance evolution.

In contrast, continuous use of a post-emergence herbicide, used alone, provided good weed control until resistance evolution to the post-emergence herbicide occurred.

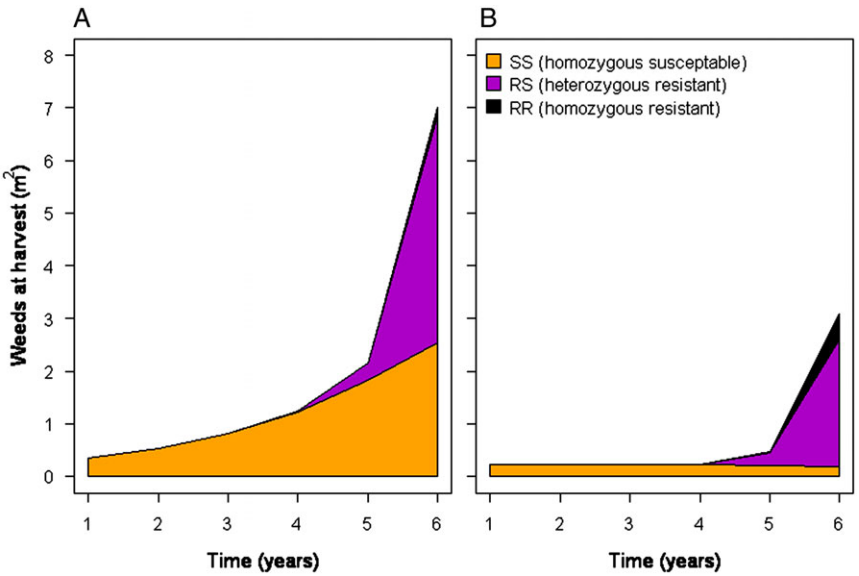

Figure 3. Differing genotypes in the weeds present at harvest as a response to herbicide application for 6 years: (A) the longer-acting pre-emergence herbicide treatment (scenario 1b), resulting in 7 weeds m^{-2} in the sixth year, only 2.3% with the RR (homozygous resistant) genotype; (B) the post-emergence herbicide treatment (scenario 1c), resulting in 3 weeds m^{-2} in the sixth year, 16.2% with the RR (homozygous resistant) genotype.

4.2 Sequential applications of a shorter-acting pre-emergence herbicide followed by a post-emergence herbicide

The annual sequential use of a pre-emergence herbicide followed by a post-emergence herbicide is normal commercial practice in many cropping systems. This combination led to high weed kill rates and thus good weed control for several years, until resistance evolved to the post-emergence herbicide. A lengthy period of good weed control was obtained here primarily because using a shorter acting pre-emergence herbicide lowered the number of weeds being treated with the post-emergence herbicide, thereby slowing (but not halting) resistance evolution to the post-emergence herbicide. High weed population numbers occurred 3–4 years after the evolution of resistance to the post-emergence herbicide, without a corresponding growth in pre-emergence herbicide resistance evolution (although resistant alleles were never eliminated from these 500 ha weed populations). These results show that lower numbers of weed cohorts affected, necessitating follow-up weed control treatment, could explain differences in evolutionary resistance rates to pre- versus post-emergence herbicides. Neve *et al.*[15] identified similar protection for early-season herbicide applications that affected fewer weed cohorts. In this study, the shorter-acting pre-emergence herbicide affected only a small number of weed cohorts, while the post-emergence herbicide affected more cohorts and was primarily responsible for restricting seed set.

4.3 Sequential applications of a longer-acting pre-emergence herbicide followed by a post-emergence herbicide

In commercial practice there can often be the sequential applications of a longer-acting pre-emergence herbicide followed by a post-emergence herbicide. This scenario enabled weed numbers to be kept low for at least 11 years. However, resistance evolution was occurring, such that resistant weeds comprised 95% of the population by year 8, although it took another 4 years before weed control failure was evident. It is important to note that gene flow among initially heterozygous individuals produced homozygous and ultimately multiple-resistant individuals (Fig. 5). The continued use of the same herbicides, both attacking a large number of cohorts, increased the level of multiple resistance, a similar situation to that frequently found in field surveys of resistance levels in cross-pollinated *L. rigidum* and *Raphanus raphanistrum*.[3,25] Notwithstanding low weed numbers in the early years, and the use of two unrelated herbicides each year, multiple herbicide resistance still evolved in this scenario (scenario 3).

This can be explained by referring back to the behaviour of these two herbicides when they were used alone, in scenarios 1b and 1c. The first evidence of resistance evolution, in scenario 3 (in years 6 and 7), was to the post-emergence herbicide only (Fig 5). Post-emergence resistance initially evolved because, even though the longer-acting pre-emergence herbicide controlled weed cohorts 2 and 3, this was insufficient (when used alone in scenario 1b) to stop an overall increase in susceptible weed numbers from year to year (Fig. 3A). In the same way, once weeds were resistant to the post-emergence herbicide in scenario 3, and thus the longer-acting pre-emergence was effectively acting alone, it was incapable of adequately controlling the weeds (Fig. 5). In contrast, the post-emergence herbicide adequately controlled weed cohorts 2, 3 and 4, which was sufficient to limit susceptible weed numbers when used alone (scenario 1c) (Fig. 3B), and the post-emergence herbicide was therefore capable of adequately controlling weeds resistant to the pre-emergence herbicide when both herbicides were used in tandem (scenario 3) (Fig. 5). Even though the pre-emergence resistance gene was maintained in the population, pre-emergence resistance did not evolve in scenario 3 until it could 'piggy-back' on the post-emergence resistance. The pre-emergence resistance alleles could only increase in number when they were in plants also containing post-emergence resistant alleles. Owing to the combined effects of the pre- and post-emergence herbicides, the survival probability of plants with multiple resistance was far superior, and this rapidly became the most frequent form of resistance.

Single gene resistance to pre-emergence herbicides (such as trifluralin), affecting even slightly fewer weed cohorts, was predicted

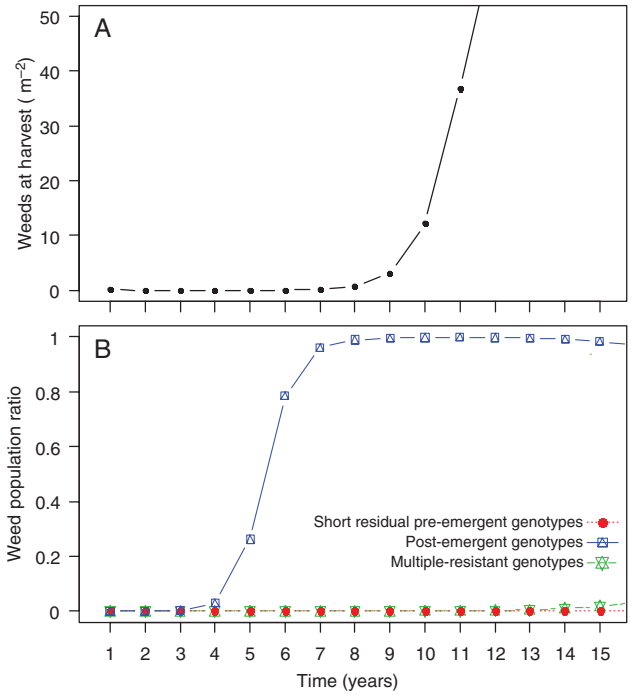

Figure 4. The average weed population (at harvest) that developed in response to annual sequential applications of a shorter-acting pre-emergence herbicide and then a post-emergence herbicide (scenario 2). The symbols used in Fig. 2 are repeated here, illustrating resistance to the same herbicides used in scenario 1: (A) the weeds present at harvest; (B) the weed population was classified utilising their resistant genes – by year 7 the average population was over 95% resistant to the post-emergence herbicide, other forms of herbicide resistance did not evolve. Small numbers of pre-emergence-herbicide-resistant plants were retained within the populations; however, numbers of multiple-resistant plants did not significantly increase until well after year 11.

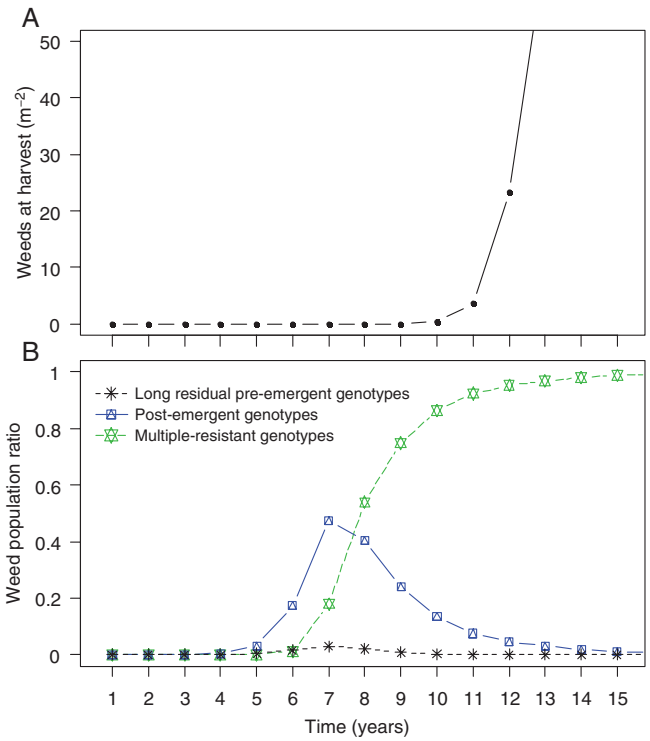

Figure 5. The average weed population (at harvest) that developed in response to annual sequential applications of a longer-acting pre-emergence herbicide and then a post-emergence herbicide (scenario 3). The symbols used in Fig. 2 are repeated here, illustrating resistance to the same herbicides used in scenario 1: (A) the density of weeds present at harvest; (B) the proportions of the weed population resistant to the longer-acting pre-emergent herbicide, to the post-emergent herbicide or to both; in year 8 most weeds were multiple resistant, and the remainder were primarily resist to the post-emergence herbicide. By year 12 the average population was 95% multiple resistant.

to be slower to evolve, and to occur primarily as multiple resistance, in all our multiple herbicide scenarios, which mirrors previous field survey results.[26] Single gene resistance to pre-emergence herbicides is still relatively rare in the field,[3,25] although recurrent selection using a single herbicide at lower rates can lead to the rapid evolution of multigene resistance.[27–30]

4.4 Limitations and future directions

We acknowledge limitations in the extent to which our results can be generalised. The model was parameterised specifically to study the evolution of resistance to a pre-emergence herbicide such as trifluralin and a post-emergence herbicide such as diclofop in a cross-pollinated annual crop weed *L. rigidum*. We investigated differences in the number of cohorts affected, and assumed that resistance to both herbicides was conferred by equally rare, single semi-dominant genes with no fitness cost. The level of similarity between resistance genes may differ, particularly between different-mode-of-action herbicides. There are many possible reasons why, in some cases, one herbicide has lasted longer than another, despite similar levels of use. Annual use of both herbicides was assumed, as was no seasonal variability in cropping system or environment. Annual ryegrass is outcrossing, and care would have to be taken in extrapolating the results of this study to self-pollinated annual weed species. In addition, these results depended on the sequential emergence pattern of the weed species[31,32] and the greater number of these emerging weed cohorts that are typically affected by post-emergence herbicide use. Future work addressing the limitations described above would improve the extent to which our results could be generalised to different species, herbicides and genetics. Furthermore, developing a spatially explicit model that can represent spatial structure in density and genetics by dividing the annual weed population into smaller spatially related subpopulations would potentially give more accurate predictions and further insights. This kind of model is needed to gain a better understanding of how the usage patterns and weed cohorts affected by different herbicides may interact with spatial factors, such as spatial heterogeneity and localised pollen and seed spread, to influence the evolution of multiple herbicide resistance. There is scope for future work to test whether these spatial factors affect the results we found here to an important degree, and thus whether more realistic, spatially explicit modelling is necessary.

5 CONCLUSIONS

These results confirmed that the slower evolution of resistance to pre-emergence herbicides in Australia can be explained by their lower seasonal kill rate. Pre-emergence herbicides typically have a lower seasonal kill rate, influenced by their early-season application timing, and the gradual emergence pattern of many weeds. In contrast, Australian post-emergence herbicides kill more weed cohorts each season. Because of their high kill rate, post-emergence herbicides are good at protecting other

herbicides applied in the same year, and keeping susceptible weed numbers very low. A strategy placing too much emphasis on one herbicide (here, the post-emergence herbicide), which successfully affects a significantly higher percentage of the annual weed cohorts, resulted in a tremendous selection pressure for resistance. In contrast, any additional method (that is, in addition to the post-emergence herbicide) that successfully contacted 95% of the same or similar weed cohorts would both reduce overall weed numbers each year and reduce the total numbers of weeds resistant to the post-emergence herbicide. Herbicides that affect a smaller percentage of the year's annual weed cohorts (such as trifluralin) were ineffective at protecting herbicides that affect a high percentage of the annual weed cohorts (such as diclofop) when used repetitively year after year, without additional integrated weed management strategies.

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