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# The weeds fight back: Individual-based simulation of evolution of polygenic resistance to herbicides

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**Abstract:** The management of weeds is often described in military terms, such as 'winning the war against weeds' or 'fighting the green invaders', with weeds described as a 'green menace' or 'alien invaders' and those trying to manage them as 'weed warriors'. Is this a war that the humans can win? Weeds are evolving to be resistant to the herbicides on which world agricultural production relies. The problem appears to be being exacerbated by the wide spread adoption of genetically modified herbicide resistant crops. Weeds are even becoming resistant to herbicides that were previously thought to be difficult to evolve resistance to, such as the world's most important herbicide: glyphosate.

It is difficult to fully understand how weeds evolve resistance in field situations and thus determine optimal strategies for avoiding, minimising or delaying the development of resistance in these situations. To investigate these questions through realistic field trials would require huge experimental areas and long time frames – by the time the answers had been found, it is likely they might no longer be useful as the weeds could already be resistant. Simulation modelling provides a tool for predicting how resistance evolves in field situations and thus for finding optimal control strategies.

In this paper, a modelling approach for predicting the possibility and rate of weeds developing polygenic resistance is presented. The approach taken is a stochastic individual-based approach that explicitly takes into account the fact that weed populations consist of individual organisms each of a particular genotype, and that some genotypes may be very rare or absent in a particular population. The overall model dynamics (illustrated in Figure 1) are discussed, followed by explanations of the way the model represents genotype and resistance status, and how it determines death or survival of individual plants, total seed set, and the genotype of new seeds.

Two alternative approaches for representing populations and implementing simulation processes are described. One approach represents the population as a list of individuals' genotypes, and the other represents the population as a list of total numbers of individuals of each genotype. These approaches are then compared in terms of efficiency and usefulness.

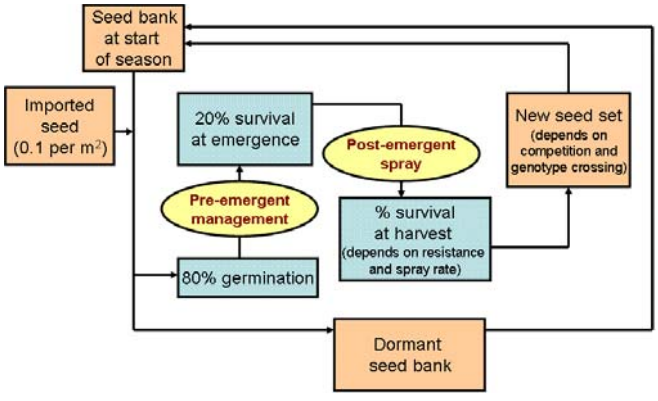


**Figure 1.** The process flow represented in the model.

An example related to polygenic herbicide resistance is presented that deals with the question: does using lower herbicide doses in cropping systems leads to faster or slower rates of development of herbicide resistance in weeds? A description of how the polygenic herbicide resistance model was used to analyse this problem is provided, together with some preliminary results.

The paper finishes with some concluding remarks including a list of possible future extensions and additions to the model that would increase the range of questions and situations to which the model could be applied. A list of interesting and important issues and questions that this model of polygenic herbicide resistance could be used to address in future is also provided.

***Keywords:*** *population dynamics, management, herbicide resistance, simulation model, low rates*

## 1. INTRODUCTION

Herbicide resistant weeds are a growing problem in Australian cropping systems and across the globe (Heap 2009 Owen et al. 2007). The wide-spread use of herbicide resistant crops appears to be exacerbating this problem (Owen and Zelaya 2005, Service 2007, Powles 2008). Management strategies for avoiding, delaying or minimising herbicide resistance are needed. There is some uncertainty about which strategies are really effective and it is difficult or impossible to test many strategies experimentally (Friesen et al. 2000). Simulation modelling provides a useful tool for testing different management strategies.

Simulation modelling of weed population dynamics has previously been used to predict how different management strategies will influence the rate at which herbicide resistance evolves in the field (eg Maxwell et al. 1990, Diggle et al. 2003, Neve et al. 2003, Roux and Reboud 2007). However, this modelling has mostly focused on monogenic resistance, where a high degree of resistance is conferred by the presence of a single allele of a single gene. It is likely that herbicide resistance is often actually polygenic, where resistance is the result of the additive or multiplicative effect of a number of minor alleles that each individually have relatively minor effects, but in combination can confer effective resistance (Neve and Powles 2005).

In this paper a modelling approach for predicting the possibility and rate of development of polygenic resistance in weeds is presented. Firstly, the overall model dynamics are discussed followed by explanations of how the model represents genotype and resistance status, and how it determines death or survival of individual plants, total seed set, and the genotype of new seeds. Two alternative approaches for representing populations and implementing simulation processes are described and compared. An example related to polygenic herbicide resistance is presented with a description of how the polygenic herbicide resistance model was used to analyse this problem. The concluding remarks include a list of possible future extensions to the model and applications it could be used to explore and analyse. Validation of the model is not discussed in this paper but the issue of validating models such as this is discussed in detail in another paper in these proceedings (Thornby et al. 2009).

## 2. MODELLING APPROACH

The model is implemented in the Python programming language (http://www.python.org). It is an individual-based simulation model where the current state is two lists, one representing the population of weed seeds and one representing the population of weed plants. The model runs on an annual time step, and the dynamics of the system are represented using a series of functions that act upon these two lists in a set sequence each year. Most of these functions (such as the functions for germination or death due to herbicide) simply iterate across the list of individual seeds and determine the fate of each individual seed stochastically. Other functions also extract global information from the whole list; for example the competition function calculates the total plant population size and returns the total number of seed set. There is also a function that creates the initial weed seed population. This section aims to present a general overview of the modelling approach and structure, rather than a particular parameterisation of the model.

The overall model dynamics are illustrated in Figure 1. At the beginning of a model run an initial population of weed seeds is created using specified initial gene frequencies, while in subsequent years the weed seed population carries over from the previous year. In either case, there is a list representing a collection of weed seeds existing in a dormant weed seedbank at the start of each season. A small number of extra weed seeds are then added to the seedbank. Each seed in the weed seedbank then has a certain chance of germinating and becoming established, otherwise it remains dormant. Each of these established weed seedlings then has a certain chance of surviving pre-emergent management. A post-emergent herbicide is then applied. The chance of a weed seedling surviving this spray depends on the spray rate and on the resistance status of the seedling, which in turn depends on its genotype. Seedlings that survive are assumed to reach maturity and set seed. The number of seeds set depends on the density of both the weeds and the crop. The genotype of each of the seeds set depends on the relative proportions of weed genotypes setting seed. Set seed is added to the pool of dormant seeds to create the seedbank for the start of the next season.

Genotype and resistance status are represented individually for each weed seed or plant. There are several options for specifying the relationship between genotype and resistance status. The number of genes involved in resistance can be set as one (monogenic) or any number greater than one (polygenic). A particular individual can then have zero, one or two ‘resistance’ alleles present at each gene (locus). The gene effect at each locus can be set as dominant (one allele has the same effect as two), recessive (one allele has no effect) or intermediate (one allele has a partial effect). The gene effects from each locus can combine either additively (linearly) or multiplicatively (non-linearly), to give *R*, a number that represents the resistance

status of that genotype. The maximum strength of resistance (the *R* value for individuals with all possible resistance alleles) must also be specified.

The chance of a weed surviving the post-emergent herbicide application depends on the spray rate and the resistance status (*R*) of the individual weed, according to a family of logistic dose response curves, as illustrated in Figure 2. Note that the *R* value thus corresponds to the ratio between the LD50 (dose needed for 50% kill rate) for the individual's genotype and the LD50 (a commonly used measure of resistance status) for the completely susceptible genotype.

The amount of seed set is calculated using the hyperbolic competition function (Firbank and Watkinson 1985) commonly used in weed population models (eg. Diggle et al. 2003), and depends on crop and weed densities. This function also gives the crop yield as a percent of the maximum possible. The genotype of each of these seeds is chosen by randomly selecting a father and a mother from the weed population, and randomly choosing one allele from the mother and one allele from the father at each relevant locus.

The area to be simulated and the initial seedbank density must be set at the beginning of a model run. The size of the initial seedbank population is then the product of these two numbers. The initial resistance allele frequency for each gene (locus) related to resistance must also be specified at the start of each run and the initial seedbank is then set up according to these initial frequencies.

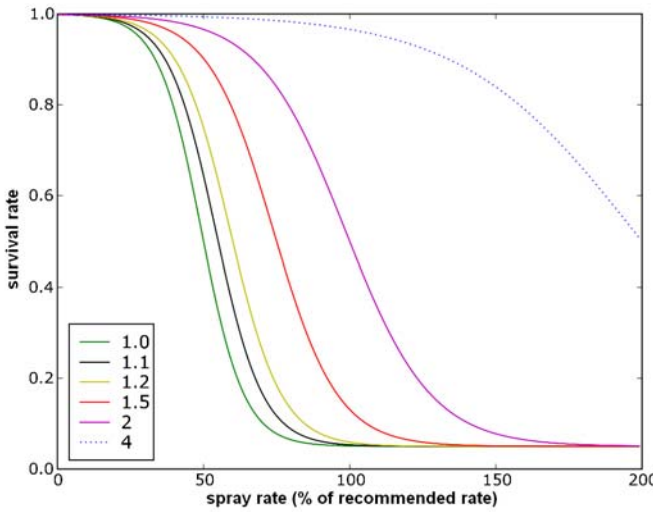


**Figure 2.** Chance of weeds surviving the post-emergent herbicide application for different spray rates and different resistance statuses (R=1 ,1.1 ,1.2 ,1.5 ,2 ,4).

## 3. ALTERNATIVE SIMULATION APPROACHES

Taking an individual-based approach of representing the genotype of every individual weed and seed with its genetics resulted in significant computational demands. For this reason, two alternative simulation approaches were implemented for the model: the PopSum approach and the PopList approach. These two approaches involve different representations of the weed populations and different implementations of the model processes described above. The PopList approach represents the population as a list of individuals' genotypes, with one list element for every individual in the population. The PopSum approach represents the population as a list of total numbers of individuals with each genotype, with one list element for each genotype.

### 3.1. Comparison of approaches

For larger simulated areas, as resistance starts to develop and weed populations increase, the number of individual weeds becomes very large. Implementations of model processes developed for the PopList approach, such as generating the population of new seeds from an existing population of plants, require multiple iterations across the list of individual plant genotypes, as well as large numbers of random selections from these lists. This means that simulations using the PopList approach became time-consuming when large areas were simulated. The PopList approach is also necessarily stochastic – giving slightly different results each time the model is run. The algorithms involved are generally conceptually simpler than the PopSum approach, as they involve iterating over the list of individual plants or seeds and applying a simple rule to each.

The PopSum approach was developed to help address this problem. Implementations of model processes developed for the PopSum approach are based on calculating expected proportions. For example, when generating the population of new seeds from an existing population of plants, large pre-calculated 'mating table' matrices are used to determine the expected proportion of new seeds of each possible genotype based on the lists of totals of each genotype of parent plants. This means that the PopSum approach can be used deterministically, when the expected proportions are used as the actual proportions, or stochastically, when

actual numbers are generated as random binomial variables based on the expected proportions and the total number of seeds. When the number of genes is relatively small (less than 7 or 8) and thus the number of possible genotypes is relatively small (less than about 5000) it is feasible to pre-calculate these mating table matrices. In this case, simulating even huge areas is relatively fast. For example, with a typical simulation of resistance evolution involving three genes, the PopList approach was found to take approximately 0.5 seconds while the PopSum approach took approximately 25 seconds. For large virtual experiments involving hundreds or thousands of individual runs, these differences become very significant. However, when the number of genes is greater than 7 or 8 then it becomes impossible to calculate these mating table matrices due to computer memory constraints. In this case, the PopList approach must be used and simulations are very time-consuming.

When used stochastically, the two approaches are exactly equivalent and produce the same results (apart from stochastic variation). To illustrate this, consider the example of the death due to herbicide function. In the PopList approach, this function iterates across the list of individual plants, calculates the probability of death for a plant of that genotype, and then determines stochastically whether that individual survives or not. The function is thus applied once for each individual in the population. In the PopSum approach, the equivalent function takes the total number of individuals of a given genotype, calculates the probability of death for a plant of that genotype, and then uses an in-built random binomial function to determine the total number of plants of that genotype surviving. The function is thus applied once for each genotype in the population. In this simple example, it is clear that the results of the two functions representing the same process in the two different approaches will give the same probability distribution of results.

A second illustration of the points mentioned above is provided by the function that determines the new seed produced by the weeds at the end of the year. After the hyperbolic function has been used to determine *nt*, the total number of weed seeds produced, this seed set function actually determines what genotypes these seeds will be. In the PopList approach, this function is a loop repeated *nt* times. Each iteration randomly selects a mother and a father from the population of all possible parent plants, and then at each locus (gene) randomly selects an allele from the mother and another from the mother to determine the genotype of the seed. The result is the required *nt* seed genotypes. In the PopSum approach, the equivalent function uses an array *A*, known as the Probability Baby Array, which is pre-calculated once at the beginning of the model run. This array *A* is a three-dimensional array where the element $a_{1jk}$ is the probability of choosing getting offspring of genotype *k* if the mother is of genotype *i* and the father of genotype *j*. The function first calculates a two-dimensional array *B*, where the element $b_{ij} = p_i m_j$, where in turn $p_i$ is the proportion of the paternal population that is of genotype *i* and $m_j$ is the proportion of the maternal population that is of genotype *j*. For each genotype *gt*, the two-dimensional matrix *C* that is the slice of *A* where *i=gt* is extracted, another two-dimensional matrix *D* is calculated as *D=CB* and then all the elements of *D* are summed to give the expected proportion of seeds of genotype *gt*. This can then be multiplied to by the total number of seeds, to give an exact number of seeds of this genotype, or passed to a random binomial function if the stochastic version of the model is required. This is a good example of how the PopList approach results in algorithms that are conceptually much simpler than the PopSum approach. In this example, it is probably much less clear that the results of the two functions representing the same process in the two different approaches will give the same probability distribution of results, but careful testing confirms that they do. In fact, it has been tested that this is true for all the functions used in the model.

## 4. AN EXAMPLE ISSUE: LOW HERBICIDE RATES

An example problem involving herbicide rates will now be presented, together with a description of how the polygenic herbicide resistance model described above was used to analyse it.

### 4.1. The Issue

Using lower herbicide doses in cropping systems has been suggested as an attractive economic option and it has been suggested that lower herbicide rates could lead to slower rates of development of herbicide resistance, due to decreased selection pressure (Blackshaw et al. 2006, Doyle and Stypa 2004). However, glasshouse experiments have shown that using lower-than-recommended rates of diclofop-methyl can actually result in development of resistance to that herbicide in annual ryegrass (*Lolium rigidum* Gaudin) while higher rates lead to extinction in these small experimental populations (Neve and Powles 2005). The resistance that evolved in the studies of Neve and Powles (2005) is almost certainly polygenic resistance, where significant resistance is the result of the additive or multiplicative effects of a number of minor alleles that individually have relatively minor effects. Is it possible that low rates of herbicide application in the field could also increase the chance of developing herbicide resistance and the rate at which it is developed?

### 4.2. Parameterisation

Before the model could be used to address this question regarding low rates, it had to be parameterised. It was decided to parameterise the model to represent the evolution of resistance in the weed annual ryegrass in a Southern Australian farming system. This weed is probably the most significant weed in these systems, and is certainly the most prone to developing resistance (Owen et al. 2007). The herbicide was assumed to be a grass-selective 'in-crop' herbicide, such as diclofop-methyl, that affects grass weeds like ryegrass but not the crop, and can thus be used after the crop has been sown. For simplicity, it was assumed that wheat was the crop grown every year and that the same management was applied every year. This management included pre-sowing management, which was assumed to kill 80% of emerging ryegrass, and post-emergent application of the grass-selective herbicide. The possibility of evolving resistance to the pre-emergent management was ignored for the purpose of this study

Values of parameters describing the competitiveness of the weed and crop, the maximum seed set and dormancy of the weed were based on parameters taken from other models of ryegrass population dynamics (Neve et al. 2003, Pannell et al. 2004). For example, the chance of a seed germinating in a given year was 80%. Other model parameters were assigned realistic values after consultation with weed scientists and experts. For example, the chance of a weed surviving pre-emergent herbicide treatment was set to be 20%. It was decided to simulate an area of $10000m^2$ and the initial ryegrass seedbank density was assumed to be $100/m^2$, so the initial seedbank population was always one million seeds. The initial resistance allele frequency for each gene (locus) related to resistance and other genetic parameters were varied as described below.

### 4.3. Analysis

The model shows that in the absence of herbicide resistance post-emergent kill rates greater than 88% lead to a sustainable system where ryegrass numbers decline each year. This kill rate can be achieved by spray rates as low as 70% of the recommended rate. Even spray rates as low as 65% of the recommended rate control weeds adequately for 20 years. So why would a farm manager spend more money to spray the full recommended rate, when these lower rates appear to be effective? Does the possibility of herbicide resistance make a difference?

To consider this question a series of 18 virtual experiments using the model described above were run. For each experiment a particular relationship between genotype and resistance status and a particular initial allele frequency were assumed. For each experiment five spray rates (70, 80, 90, 100, 110 and 120% of the recommended rate) were considered with each rate replicated four times. With each of these repetitions the simulation was run over twenty years, and a number of output variables were recorded for each year. These included the weed density at harvest, the crop yield, and the proportion of resistant weeds in the population.

**Table 1.** Summary of the 18 virtual experiments including experiment number, number of genes assumed to be involved in resistance, the maximum level of resistance, gene effect (dominant, co-dominant or recessive), gene combination (combine linearly or multiplicatively) and initial allele frequency (IAF).

| Exp. | Genes | Max*R* | Dominance | Combination | IAF |
|---|---|---|---|---|---|
| 1 | 1 | 20 | Dominant | - | $10^{-5}$ |
| 2 | 1 | 21.5 | Dominant | - | $10^{-5}$ |
| 3 | 2 | 20 | Dominant | Linear | $10^{-5}$ |
| 4 | 2 | 20 | Dominant | Multiplicative | $10^{-4}$ |
| 5 | 2 | 20 | Co | Multiplicative | $10^{-2}$ |
| 6 | 2 | 1.5 | Co | Linear | $10^{-2}$ |
| 7 | 2 | 20 | Recessive | Multiplicative | $10^{-2}$ |
| 8 | 3 | 20 | Dominant | Multiplicative | $10^{-3}$ |
| 9 | 3 | 20 | Co | Multiplicative | $10^{-2}$ |
| 10 | 3 | 1.5 | Co | Linear | 0.015 |
| 11 | 3 | 2 | Co | Linear | $10^{-3}$ |
| 12 | 3 | 2 | Co | Linear | 0.05 |
| 13 | 3 | 20 | Recessive | Linear | 0.1 |
| 14 | 5 | 20 | Recessive | Linear | 0.2 |
| 15 | 5* | 4 | Dom/ Co | Linear | $10^{-5}/10^{-1}$ |
| 16 | 5* | 4 | Dom/ Co | Linear | $10^{-5}/10^{-2}$ |
| 17 | 5* | 4 | Dom/ Co | Linear | $10^{-5}/10^{-3}$ |
| 18 | 4* | 4 | Dom/ Co | Linear | $10^{-5}/10^{-1}$ |

*One major dominant gene that gives full resistance and three or four co-dominant genes where alleles can combine additively to give full resistance when all are present. IAF is for major/minor genes.

The genetics considered in the 18 experiments included weak and strong dominant monogenic resistance. Weak and strong polygenic (two, three or five genes) resistance, including recessive, co-dominant and dominant effects, and both linear and multiplicative gene interactions were considered. Finally, cases where full resistance could be conferred by either a single major gene *or* a combination of minor genes were also considered. A summary of the 18 experiments is given in Table 1.

In each experiment results were considered to determine whether there was a clear distinction between lower and higher spray rates in terms of the number of years before significant levels of resistance developed. This was defined to be when weed densities at harvest were above 200/m$^2$.

### 4.4. Results of Analysis

Figure 3 shows results from Virtual Experiment 16, where resistance depends on one very rare dominant major gene and four relatively common co-dominant minor genes. It is clear in this example that the lower the spray rate, the faster the weed density at harvest rises above 200/m$^2$. The result that lower rates lead to less sustainable systems in terms of herbicide resistance was found clearly in 13 of the 18 experiments. In another two, Experiments 7 and 9, there was some complexity or variability, but on average higher rates were still significantly better. In another, Experiment 8, results were not clear, but indicated higher rates could be better. The only experiments where weed populations did not reach uneconomic levels faster for lower application rates were Experiment 1 (strong monogenic resistance) and Experiment 5 (two dominant genes combining linearly to give strong resistance), where there was no difference.

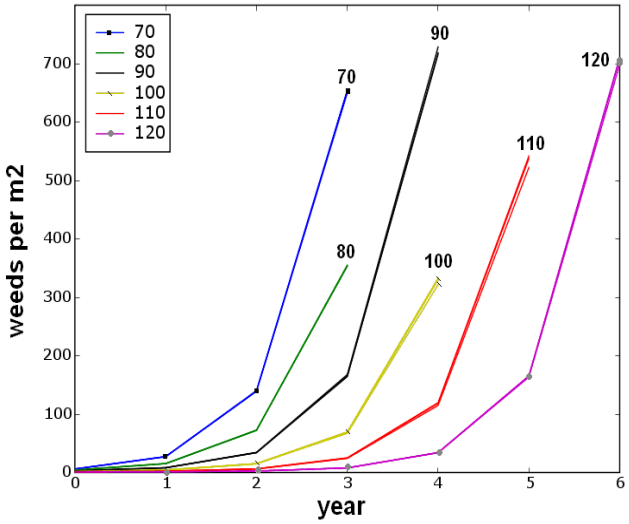


**Figure 3.** Experiment 16 results: weed densities increasing over time for different spray rates (as percentage of recommended rate: 70, 80, 90, 100, 110, 120% - repetitions are barely distinguishable).

The results from the virtual experiments indicate that low rates tend to cause faster development of herbicide resistance. However, further modelling work needs to be done to clearly identify the range of situations where this is true.

## 5. CONCLUDING REMARKS

This paper presented a modelling approach for predicting the development of polygenic herbicide resistance in weeds in the field. This stochastic individual-based approach explicitly takes into account the fact that weed populations consist of individual organisms each of a particular genotype, and that some genotypes may be very rare or absent in a particular population.

This model describing the evolution of polygenic herbicide resistance evolution has been designed in a modular way to facilitate future developments, extensions and applications. There are a range of possible extensions that would increase the range of questions and situations to which the model could be applied. These include developing the model to account for

- possible fitness penalties when resistance genes are present;
- death of seeds in the seedbank;
- ongoing mutation rates;
- different weed emergence times;
- phytotoxicity effects of herbicides on crops;
- herbicides causing reduced seed set in weeds instead of or in addition to weed death;
- resistance to multiple herbicides, including pre-, with- and post-emergent herbicides, which include cross-resistance (where genes conferring resistance to one herbicide also confer resistance to another herbicide) or specific-resistance (where genes confer resistance to one specific herbicide);
- spatial variability and spread of pollen and seeds; and
- temporal variability across different years.

There are many questions and issues related to evolution of herbicide resistance that would require a model that accounts for polygenic herbicide resistance. Planned future applications of the model include:

- simulating polygenic resistance to pre-emergent herbicides, including glyphosate, which is probably the most important herbicide in the world (Powles 2008);
- finding optimal strategies and farming systems for avoiding, minimising, or delaying the development of polygenic herbicide resistance;
- investigating whether strategies for minimising the development of polygenic herbicide resistance are also likely to minimise the development of monogenic herbicide resistance;
- predicting whether weed biology factors such as breeding system (cross-pollinated or self-pollinated), dormancy, competitiveness, longevity, fecundity or dispersal mechanisms will make some species develop resistance faster or more often than other species, and determine if this depends on whether the resistance is monogenic or polygenic;
- simulating controlled experiments that have selected for herbicide resistance in weeds at relatively low rates over several generations, with the aim of determining what resistance genetics could possibly have lead to the observed results.

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