Simulating the evolution of glyphosate resistance in grains farming in northern Australia

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Received: 16 April 2009 Returned for revision: 7 May 2009 Accepted: 12 May 2009 Published electronically: 30 June 2009

INTRODUCTION

Resistance to glyphosate \([N\text{-}(\text{phosphonomethyl})\text{glycine}]\) in weed populations is a worldwide and increasingly common phenomenon (Heap, 2008, 2009). While there are now biotypes over a range of species that are resistant to one or more of many herbicide modes of action, resistance to glyphosate poses an especially significant threat in most agricultural systems. Reasons for this include the effectiveness of glyphosate on a broad range of species, its relatively low cost, and relatively high safety for operators and the environment (Baylis, 2000; Powles, 2008). Grains farming systems in Australia have moved substantially towards reduced tillage practices, and thus have come to rely heavily on glyphosate for weed control.

The cropping systems of the sub-tropical north-eastern Australian grain region (‘the northern grains region’), which stretches from northern New South Wales to central Queensland, are subject to considerable yearly and regional diversity in growing conditions (Webb et al., 1997; Freebairn et al., 2006). Cropping historically has been dominated by wheat grown in winter, but there is a recent trend towards increasing frequency of sorghum in summer (Osten et al., 2007). Weed control in summer fallows is particularly important in sub-tropical grains cropping, because winter crops rely largely on stored summer rainfall (Webb et al., 1997). Summer fallow weeds reduce stored soil moisture in paddocks, with heavy summer fallow weed burdens having the potential to reduce yields in following winter crops. Glyphosate application is the most common method of controlling summer fallow weeds, particularly summer grasses (Osten et al., 2007), and is also commonly applied for pre-sowing burndown in summer cropping.

Awnless barnyard grass \((Echinochloa colona);\) hereafter simply referred to as barnyard grass) is a major weed of cropping systems in north-eastern Australia, and was identified as being at high risk of evolving glyphosate resistance (Walker et al., 2002; Osten et al., 2007). Since 2006, several glyphosate-resistant barnyard grass populations have indeed been identified (Heap, 2009). Glyphosate-resistant barnyard grass has the potential to reduce farming profitability significantly across the sub-tropical Australian grains region, given the prevalence of barnyard grass, its high seed production and its propensity to compete with increasingly important summer grains crops.

Two other species of interest have been identified as being resistant to glyphosate in the region. Glyphosate-resistant annual ryegrass (Lolium rigidum) populations have been found in the northern grains region, though confirmed resistant populations are currently limited to the southern end of the region (Preston, 2008). The most recently confirmed resistant species in the region is liverseed grass (Urochloa panicoides), with resistance to glyphosate identified in 2008 (Heap, 2009).

Modelling weed populations

Computer models have been used to identify key risk and/or mitigating factors in farming systems for weed control issues generally, and for herbicide resistance issues specifically, with varying levels of success in the past (Maxwell et al., 1990; Holst et al., 2007). The development of herbicide resistance in a weed population is a result of interacting genetic, ecological and agronomic factors occurring over several years, and this complexity benefits from a systems or modelling approach. The particularly diverse range of crops, weed species, management practices and climatic conditions in the northern grains region add further complexity. An effective model of the development of herbicide resistance in this region must take this variability into account in order to provide useful predictions. Existing models predicting herbicide resistance in other cropping systems have not been designed to deal with cropping processes and/or population dynamics processes that vary substantially (or qualitatively) from year to year (Richter et al., 2002; Diggle et al., 2003; Pannell et al., 2004; Werth et al., 2008). While they produce robust predictions of the rate of herbicide resistance under typical conditions for the regions in which they were developed, they are not designed to compare rates of resistance evolution under the set of agronomic tactics and cropping regimes used in the sub-tropical northern grain region.

To account for this variability and complexity, and to produce a set of more detailed recommendations for reducing the rate of glyphosate resistance evolution, we elected to couple the APSIM crop model (Agricultural Production system SIMulator, Agricultural Production Systems Research Unit; Keating et al. (2003)) with extensions allowing for aspects of population dynamics that crop models do not normally consider. Using a crop model allows us to simulate the weed population’s responses to highly variable growing conditions (including climatic and agronomic factors) over a number of years or decades, without needing to produce a detailed model of competition and resource-limited growth from scratch.

Here we describe the construction of our herbicide resistance model, and its validation for use in predicting the evolution of glyphosate resistance in barnyard grass populations in the northern grains region.

MATERIALS AND METHODS

Crop models in general consist of a set of equations that model growth- and yield-related biological processes, giving daily or end-of-season predictions of factors such as yield and biomass, from a set of inputs including seeding rates, rainfall, soil type and fertilizer applications. The crop model APSIM is a modular set of models that have been shown to produce realistic yield predictions for a wide variety of crops (Keating et al., 2003).

APSIM does not simulate processes that occur outside of the crop growth cycle where farmers control seeding rate and the timing of sowing. Population dynamics such as entry of seeds into the seed bank, mortality in the seed bank and seed persistence are not modelled in APSIM. Nor are genetics factors such as gene flow from outside sources, gene frequencies (such as for single-allele resistance genes) and results of mating between resistant and susceptible biotypes. However, APSIM is able to create dynamic links with mathematical process models implemented in Vensim (version 5.5c; Ventana Systems, Inc., Harvard, MA, USA), and this can be used to simulate the ‘missing’ aspects of weed population dynamics (Smith et al., 2005). We devised sub-models in Vensim for mating and seed bank processes, to fill the life cycle gaps in the crop model.

Software environment

The model simulates a population with a single-gene glyphosate resistance mechanism that is assumed to provide strong, partially dominant resistance (Thornby et al., 2006). The software environment consists of a set of APSIM modules and a set of control and parameter files for the APSIM simulation, and a Vensim process model split into separate sub-sections for seed bank processes, mating processes and communication between APSIM and Vensim (see Supplementary Data, available online). This approach is used to implement a combination of age-class population dynamics and genetics broadly similar to that given in Maxwell et al. (1990), which has formed the basis of most herbicide resistance modelling. The APSIM modules used in the model simulate management, weather, soil moisture and nutrient balance, output of variables, growth of crops and weed species, and competition between them. Vensim is called by APSIM and runs without a user interface, as a dynamic linked library. Variables are passed between the APSIM and Vensim sub-models using APSIM’s daily time step. All user interaction is with APSIM, so variables for reporting are passed from Vensim where appropriate. The model can, through APSIM, return a range of variables concerning crop and weed growth stage, biomass and stress level, seed production (or yield), seed bank density, soil moisture and nutrient status, and proportion of resistance in the weed population.

As the model consists of a set of general population dynamics factors linked to APSIM, it is able to simulate a range of weed species given adequate parameter estimation; in this sense, it is a generic weed population and herbicide resistance model. Due to its prevalence and relatively high resistance risk (Osten et al., 2007), we chose to use awnless barnyard grass growing in a northern region grains rotation as a test case. A diagrammatic version of the model is shown in Fig. 1. The life cycle of any weed simulated with the model is broken down into several stages: the seed bank, germinants, growing plants, mating and seed production. The age-structured life cycle model is similar to those used in most other process models of weed population in growth stages, following the description by Maxwell et al. (1990). The growth of plants from seedling to adult, between the germination and mating phases, is handled by APSIM.
The weed modules use APSIM’s generic plant module, parameterized to simulate multiple chronologically separate barnyard grass cohorts. The other phases are handled in separate sub-models in Vensim, with varying degrees of data input from APSIM as required. The sections of the model that were specifically created for this study and are therefore not described in previous publications for APSIM are described below.

**Weed modules**

The generic weed module in APSIM was parameterized to model barnyard grass, as summarized in Table 1. Barnyard grass germinates in cohorts throughout spring and summer (Wu et al., 2004; McGillion and Storrie, 2006): flushes may appear as early as September and as late as March. Minimum, optimum and maximum germination temperatures were found to be 15, 30 and 40 °C, respectively, by Uremis and Uygur (1999). Fecundity is high; each plant may produce tens of thousands of seeds, and a dense stand of plants can produce hundreds of thousands of seeds per square metre (Mercado and Talata, 1977). To date, detailed analysis of the mechanism conferring resistance to glyphosate in barnyard grass is not available, though some preliminary work by Preston and co-workers at the University of Adelaide (pers. comm., 2008) suggests a single mutation conferring relatively weak resistance to glyphosate. Early

<table>
<thead>
<tr>
<th>Parameter description</th>
<th>Value</th>
<th>Unit</th>
<th>Data source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weed seed bank density</td>
<td>40</td>
<td>Seeds m⁻²</td>
<td>Estimate for a light initial infestation</td>
</tr>
<tr>
<td>Frequency of resistance alleles in unselected population</td>
<td>1 × 10⁻⁸</td>
<td></td>
<td>Assumption based on other species (Neve et al., 2003)</td>
</tr>
<tr>
<td>Maximum seed production per plant</td>
<td>12 000</td>
<td>Seeds plant⁻¹</td>
<td>Assumption from Mercado and Talata (1977)</td>
</tr>
<tr>
<td>Annual mortality of up to 1-year-old seed</td>
<td>0-1</td>
<td></td>
<td>Walker et al. (2006)</td>
</tr>
<tr>
<td>Annual mortality of 1- to 2-year-old seed</td>
<td>0-4</td>
<td></td>
<td>Walker et al. (2006)</td>
</tr>
<tr>
<td>Annual mortality of seed &gt;2 years old</td>
<td>0-95</td>
<td></td>
<td>Walker et al. (2006)</td>
</tr>
<tr>
<td>Fraction of seed rain entering the seed bank</td>
<td>0-7</td>
<td></td>
<td>Assumption based on observations of seed predation and viability Madsen et al. (2002)</td>
</tr>
<tr>
<td>Proportion of self-pollination</td>
<td>0-95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum density of weeds reaching reproduction</td>
<td>2000</td>
<td>Plants m⁻²</td>
<td>Assumption from expert opinion</td>
</tr>
<tr>
<td>Sorghum planting density</td>
<td>7</td>
<td>Plants m⁻²</td>
<td>APSIM standard value</td>
</tr>
<tr>
<td>Maximum germination from one cohort as a proportion of current seed bank</td>
<td>0-85</td>
<td></td>
<td>Wu et al. (2004), Walker et al. (unpublished research)</td>
</tr>
<tr>
<td>Optimal accumulated temperature since start of season or previous cohort germination for maximum germination</td>
<td>300</td>
<td>Degree days (base 15 °C)</td>
<td>Estimated from unpublished data</td>
</tr>
<tr>
<td>Minimum accumulated temperature since start of season or previous cohort germination for appearance of new cohort</td>
<td>120</td>
<td>Degree days (base 15 °C)</td>
<td>Estimated from unpublished data</td>
</tr>
<tr>
<td>Optimal accumulated rainfall for maximum germination of a cohort</td>
<td>100</td>
<td>mm rainfall accumulated over 4 d</td>
<td>Estimated from unpublished data</td>
</tr>
<tr>
<td>Minimum accumulated rainfall for germination of a cohort</td>
<td>40</td>
<td>mm rainfall accumulated over 4 d</td>
<td>Estimated from unpublished data</td>
</tr>
</tbody>
</table>
experimental results also suggest that the glyphosate resistance mechanism in barnyard grass is not particularly strong (Cook et al., 2008; Storrie et al., 2008).

The appearance of 3–4 cohorts in a summer season is normal in the northern region, though ≥6 may occur in wetter parts of the region in some years (Wu et al., 2004). One or two early cohorts are often larger than subsequent ones. Because of this tendency in our test species to grow in distinct cohorts, we simulate cohorts in the model, with a maximum of four cohorts permissible in any one season. APSIM forces a limit to the maximum number of cohorts possible in a season, since they must be specified before run time with separate modules.

Cohorts are germinated as a whole, each on a single day in the model, with contributions from each age fraction of the seed bank for each genotype. The size of a cohort fraction \(C_{gi}\) of genotype \(g\) emerging from age fraction \(i\) is determined thus:

\[
C_{gi} = C_{gmax}D_{rain}D_{therm}D_{seed-i}
\]

where \(C_{gmax}\) is the maximum number of seeds that could germinate in a cohort (a fixed proportion of the current seed bank), \(D_{rain}\) is a discount factor for rainfall amounts accumulated in APSIM over 5 d that falls between the minimum (40 mm) and the optimum (100 mm), \(D_{therm}\) is a discount factor for thermal time in degree days (base 12 °C) accumulated since the beginning of the season or the time of the last cohort, and \(D_{seed-i}\) is a discount factor that reduces potential germination in older seed bank age fractions.

Thus, the size of a cohort is determined by environmental factors and the size of the current seed bank. Early-germinating cohorts are usually the largest in terms of plant density, because the available seed bank is largest at the beginning of the season. Seed bank depletion towards the end of the season tends to result in smaller cohorts, even though temperature requirements in particular are likely to be fully met in January and February.

**Mating**

In the model, the whole barnyard grass population is made up of sub-populations of three biotypes: homozygous susceptible (SS) and resistant (RR), and heterozygous (SR) for a single partially dominant gene conferring glyphosate resistance. As a simplification, and because APSIM is not spatially explicit, mating occurs freely within and between sub-populations. The results of the mating sub-model are evaluated on a single maturity date, determined in APSIM, for each weed cohort. Ova in the model may be pollinated by ‘free’ pollen or ‘selfed’ pollen, in proportions determined for the weed species in question. Barnyard grass is largely self-pollinating (Madsen et al., 2002), so our simulations assume 95% selfed fertilization and 5% free fertilization. Haploid susceptible (S) or resistant (R) ova are produced in proportion to the number of surviving adult plants in each genotype. Sufficient pollen is assumed to be produced to fertilize all ova in the population (or a predetermined proportion of all ova), and is tracked as R and S proportions of the selfing and outcrossing pollen ‘populations’. R and S gametes of either type are assumed to be equally likely to survive and produce progeny, since there are currently no data suggesting that gamete survival differs between genotypes.

**Weed seed banks**

The weed seed bank is programmed in the Vensim module as a set of state variables with rates of input and output affected by relationships with static and dynamic variables (see Table 1). The progeny from the mating sub-model fall as seed rain, which is reduced by a predation factor before it enters the seed bank. Seed may not enter the seed bank until the end of the season, to simulate the short dormancy requirement displayed (in the main) by barnyard grass seed (Mercado and Talatala, 1977; Wu et al. 2004). Species in which there is no dormancy or after-ripening would require the model to be adjusted so that seed rain may enter the seed bank immediately. There are three age-class seed bank fractions (new seed up to 1 year old, 1- to 2-year old seed and seed older than 2 years) for each of the three biotypes that make up the population. Each of the seed bank fractions contributes to the pool of germinants when a germination event is determined to occur (through APSIM’s ability to track rainfall over several days). The numbers of germinants are determined as a proportion of the current seed bank size, where the proportions differ according to seed bank age. An annual summary of the size of the seed bank age fraction \(i\) for genotype \(g\) in any year \(n\) \([S_{ig}(n)]\) is determined as:

\[
S_{ig(n)} = S_{ig(n-1)} + E_{ig(n-1)} - [G_{ig(n)} + M_{ig(n)} + T_{ig(n)}]
\]

where \(E_{ig(n-1)}\) is the amount of seed entering the seed bank fraction, either from seed rain for the first fraction or as transport from younger fractions for fractions two and three; \(G_{ig(n)}\) is the number of germinants from this fraction this year, \(M_{ig(n)}\) is the seed bank mortality for this fraction, and \(T_{ig(n)}\) is the transport out from this fraction to the next oldest fraction, if applicable, at the end of the season. Seed bank mortalities are applied as a simple multiplier (see Table 1) and simplified to ignore considerations of variation from year to year due to changing climate, soil microbial activity or any possible effects from agricultural activity in the field. Individual simulations could be constructed to take these variables into account if needed.

**Crops**

As noted above, both summer and winter cropping occur in the northern grains region. Our model uses wheat as the winter crop and sorghum as the summer crop. Code permits the simulation of systems with 0–5 summer crops per 5 years, and with traditional tilled and minimum-till planting techniques. Winter cropping is programmed to fit around the summer cropping regime used: where there is a summer crop, the following winter is fallow. Alternative instructions allow continuous winter cropping regardless of summer cropping regime, or winter cropping can be left out altogether.
Control tactics

Herbicide and non-herbicide control options in the model are processed as scripting statements within APSIM and are processed by APSIM’s manager module (Keating et al., 2003). Tactics available for barnyard grass control in the model cover a range of tactics typically used to control summer grasses in the northern grains region, and include: glyphosate knockdown in fallow, pre-planting and for spray-out at sorghum harvest; non-glyphosate knockdown in fallow and pre-planting; residual herbicide used pre-planting or at planting; tillage at planting or used strategically in fallow; double knock [here assumed to be glyphosate followed by an alternative knockdown herbicide (Werth et al., 2008)]; and hand-hoeing. These can be triggered in the model by rules considering a combination of date, days since last application, time since (or at) crop sowing, weed emergence and weed density. A multiyear herbicide rotation can affect the types of herbicides used in any one application. Several tactics can be used in a single simulation, including the use of multiple control tactics in any one year. User-defined variables are used to count applications made within phases of arbitrary length, and rules using ceilings of application numbers can be applied. APSIM rules also define and control crop rotations in simulations where summer cropping is included.

Glyphosate is typically very effective on susceptible barnyard grass plants while they are small and growing vigorously (Adkins et al., 1998). In practice, efficacy can be reduced due to plant stress and increased size when actually sprayed – in some cases, seedlings that emerge after relatively small rainfall events can become moisture stressed within a few days and remain stressed at the time the herbicide is applied (D. Penbarth, pers. comm., 2008), or may not actually be sprayed until beyond the recommended size for spraying. Accordingly the model allows for a wide range of efficacies. The model can also be adjusted to simulate an efficacy range stochastically, by changing the efficacy from a single value per cohort to a random variable that can be determined by Vensim according to a normal or uniform distribution during runtime, with upper and lower bounds specified by the user in the simulation’s initialization files. APSIM calculates a value for plant stress according to plant size and environmental conditions, and this value can be used to adjust herbicide efficacy rates in the model. A range of estimated efficacies for the simulated weed control treatments were generated through discussion with a panel of the region’s weed scientists (Table 2).

Cohorts can be affected by the same or different control methods in any simulation. Cohorts that germinate in years of summer cropping may be affected by a residual herbicide application and/or tillage or a knockdown herbicide, or (if germinating later in the crop phase) may not be affected by any controls at all. Cohorts that germinate in fallow summers may be affected by one or more knockdown herbicide applications, and by follow-up control of survivors. Some cohorts may be so small that they fall under an application threshold if one is specified, and thus are not controlled.

Weed control tactics are applied based on several rules. Residual herbicides are applied in years where summer cropping is planned, either on the planting date or at a date a given number of days prior to a predicted planting window.

The residual herbicide is not specified in terms of product or mode of action, but is assumed to be effective at the kill rate specified for the weed species being simulated. Residual efficacy in the model declines linearly from a maximum figure on the day of application to zero, over 8 weeks; this is a simplification of available data for atrazine efficacy over time, which declines exponentially at a rate depending on soil type and moisture content (Walker et al., 1997).

Knockdown herbicides are applied at a predetermined number of days after weed emergence, providing no crop is present. Glyphosate and non-glyphosate knockdown applications are separated in the model, but there are no differences specified between different non-glyphosate knockdown herbicides. Planting operations, if a cohort is present at the time, have an associated kill rate which may be varied to represent different levels of soil disturbance. Non-planting tillage for weed control can also be applied under the same circumstances as knockdown herbicides. While efficacy for all weed control applications is specified for each genotype separately, the values applied vary only for applications of or including glyphosate. That is, we assume that glyphosate resistance confers no resistance to any other method of weed control. Double knock efficacies combine efficacies from glyphosate and non-glyphosate herbicides, and there is no assumption of reduced efficacy of the second knock following application of the glyphosate first knock.

### Table 2. Default efficacy values of weed control tactics on barnyard grass used in the model

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Proportion of SS plants killed</th>
<th>Proportion of SR plants killed</th>
<th>Proportion of RR plants killed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyphosate knockdown in fallow or pre-planting</td>
<td>0.95–1.0</td>
<td>0.2–0.6</td>
<td>0.01–0.4</td>
</tr>
<tr>
<td>Residual herbicide (initial efficacy declining to zero after 8 weeks)</td>
<td>0.85</td>
<td>0.85</td>
<td>0.85</td>
</tr>
<tr>
<td>Full disturbance tillage at planting</td>
<td>0.95–1.0</td>
<td>0.95–1.0</td>
<td>0.95–1.0</td>
</tr>
<tr>
<td>Full disturbance tillage applied strategically in fallow</td>
<td>0.95–1.0</td>
<td>0.95–1.0</td>
<td>0.95–1.0</td>
</tr>
<tr>
<td>Non-glyphosate knockdown herbicide in fallow or pre-planting</td>
<td>0.95–0.999</td>
<td>0.95–0.999</td>
<td>0.95–0.999</td>
</tr>
<tr>
<td>Double knock</td>
<td>0.99–1.0</td>
<td>0.99–1.0</td>
<td>0.99–0.999</td>
</tr>
</tbody>
</table>

RR homozygous resistant; SS homozygous susceptible; SR, heterozygous.

*Upper values in each range refer to kill rates on pre-tillering unstressed plants. Larger and/or stressed plants are affected towards the lower value in each range. Stochastic variation within the range can also be used.*

Validation

In 2007, a population of barnyard grass from Bellata, New South Wales, was confirmed to be resistant to glyphosate (Cook et al., 2008). We elected to use the evolution of this population to validate our model. We gathered available information about the weed population and the agronomic history of the paddock through discussions with local agronomists.
and weed specialists from the NSW Department of Primary Industries. The population is from a paddock that had historically been used for winter cereals, with some summer cropping only since 2001. First use of glyphosate was estimated to be in 1985, and the paddocks have been zero-tilled in most summers since 1990 excepting up to one year in five where strategic tillage for weed control occurred, or tillage for terrain management coincided with weeds being present. Between 1985 and 1989 one large weed cohort (>20 plants m$^{-2}$) per summer was assumed to be controlled with strategic tillage. Glyphosate efficacy was reported as being occasionally as low as 80% due to frequent poor conditions for application. Residual herbicides have been applied for grass control as part of recent summer cropping, but no significant rotation of knockdown herbicides away from glyphosate has been made. There has been little or no use of non-glyphosate knockdown options, or attempts to control glyphosate survivors with non-glyphosate options.

We set up the model to simulate the conditions experienced by the Bellata resistant populations, using climate and soil data for Bellata between 1985 and 2006, plus 9 years of synthetic climate records for 2007–2015 which consisted of re-labelled randomly selected years from the period 1990–2006. There was no summer cropping except for sorghum sown in 2001 and 2006. In those years, a residual herbicide was applied at planting, and glyphosate was used to desiccate sorghum at harvest, with activity on barnyard grass cohorts present at the time of sprayout.

Due to the unavailability of data on glyphosate efficacy throughout the evolution of resistance in the real population, and the expectation that glyphosate efficacy was often not extremely high, we ran 100 simulations with a stochastic level of glyphosate efficacy between 80 and 100% on susceptible plants for every application made. A plant density application threshold of 5 plants m$^{-2}$ can trigger a second application of glyphosate after a user-specified reaplication gap (default value 14 d), on dense cohorts where initial control is poor.

High selection pressure and regional variation simulations

We expect that maximum selection pressure for glyphosate resistance is exerted in a system where glyphosate is the only weed control method used, is used every time weeds appear and where there is no competition from crops. In the case of barnyard grass, this equates to a winter grains rotation with continuous zero-till summer fallows in which all barnyard grass flushes are sprayed with glyphosate. We simulated this in the model, to confirm that this is indeed the worst case scenario, and to attempt to predict how quickly herbicide resistance problems might evolve under maximum practical selection pressure. These simulations start with default model parameter values; glyphosate is applied to all flushes of barnyard grass, and, where weed density after kill remains above the application threshold, follow-up applications of glyphosate on the same cohort may be made. We ran the simulation for three separate locations in the northern grains region – Moree (northern New South Wales), Emerald (central Queensland) and Dalby (southern Queensland) – using specific soil and climate data for each, to determine how much impact variation due to location in climate and soil may have on the predicted rate of resistance evolution.

Critical variables

Several variables are thought to be critical to model results. We identified initial resistance allele frequency, strength of resistance conferred by the allele, glyphosate efficacy on susceptible plants, level of self-fertility and persistence (measured as annual mortality of the oldest fraction of the seed bank) as being possible sources of significant variation in the model’s predictions, and ran simulations to test the resulting variability. Each test was performed with the model set to the default conditions except for the variable being tested.

While the model may also be sensitive to a variety of plant biology and ontogeny parameters, these are handled by APSIM. Variable and output testing in APSIM has been well documented (Keating et al., 2003).

RESULTS

Validation

The mean of 100 runs of the stochastic model predicts that 49% of the population is resistant after 15 years, and that this proportion is >97% after 18 years (Fig. 2). There are only partial quantitative estimates available as to the allele frequency in the population at the time resistance was confirmed: the results of herbicide efficacy studies on site and in glasshouses suggest that the resistance proportion across the whole paddock was in the range of 40–60% (Cook et al., 2008). The mean of the stochastic model runs for 40–60% resistance proportion falls between 14 and 17 years after first use of glyphosate. If we assume that the first use of glyphosate was (in line with the estimate available) 1985, 21 years elapsed between first use of glyphosate and confirmation of resistance in the real population. Under these assumptions, the model predicts somewhat more rapid evolution than actually occurred in the field.

Fig. 2. Evolution of glyphosate resistance: annual proportion of barnyard grass population resistant to glyphosate for historical simulation of Bellata glyphosate-resistant barnyard grass. Filled circles represent predicted data from 20 random runs of the model; the dotted line represents mean of 100 runs; error bars represent the s.e.m.
High selection pressure simulation and variation between locations

Continuous summer fallows relying solely on glyphosate for barnyard grass control are predicted to result in a largely resistant population after 14–15 years in three locations in the northern region (Fig. 3). Further, Fig. 2 shows that the model does not predict substantial differences in the rate of evolution across the region.

Critical variables

The frequency \( f \) of resistance alleles being present in the population prior to any selection with glyphosate is a sensitive variable; substantial variation in this parameter between \( f = 1 \times 10^{-4} \) and \( f = 1 \times 10^{-10} \) caused, as expected, large differences in predicted rate of resistance evolution (Fig. 4).

The effects of varying efficacy, resistance strength, level of self-fertility and mortality of seeds in the oldest fraction of the seed bank are compared in Table 3. Glyphosate efficacy is varied concurrently for both susceptible and resistant plants, i.e. as efficacy on susceptible plants increases, efficacy on resistant plants also increases. Strength of resistance is tested by varying efficacy on resistant plants while efficacy on susceptible plants remains the same.

Interestingly, a median glyphosate efficacy value (95%) resulted in slightly slower evolution of glyphosate resistance than values on either end of the scale tested. Changing the strength of resistance resulted in 2 years’ difference between the strongest tested resistance (0% kill of resistant plants) and the weakest tested resistance (40% kill of resistant plants), i.e. the weakest putative resistance mechanism takes some 15% longer to result in a resistant population than the strongest mechanism. Self-fertilization is predicted to result in very slightly higher rates of evolution than out-crossing, and changes in the rate of decay of the oldest seed bank fraction had very little effect on the model’s predictions, although low levels of mortality did result in very large seed banks (data not shown).

DISCUSSION

The model described in this report predicts that the rate of evolution of glyphosate resistance in barnyard grass populations results in substantially resistant populations within 15–18 years from the introduction of intensive glyphosate use (Figs 2 and 3). The results from the validation simulations show that the model was able to simulate the evolution of glyphosate resistance with reasonable accuracy: the time to resistance is predicted to within 3–4 years of the real population from which parameters were estimated.

The simulations predict, in general terms, a long ‘latent phase’ of low level, hard to detect and so effectively ‘invisible’ glyphosate resistance in the population (Fig. 3). This is followed by a ‘rapid change phase’ of rapid increase in resistance

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**Table 3. Effects of variation in key model parameters, measured as number of years required before the predicted proportion of resistant plants exceeds 50% of the population**

| Parameter variation | Time to exceed 50% resistant plants as a proportion of whole population (years) | Resistance proportion in year first exceeding 50% (%)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Maximum glyphosate efficacy on susceptible plants</td>
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<tr>
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<td>13</td>
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<td>99.9%</td>
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<td>88.5</td>
</tr>
<tr>
<td>Maximum glyphosate efficacy on resistant plants</td>
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<td></td>
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<tr>
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<td>40%</td>
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<td>Proportion of ovules fertilised by self pollen</td>
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<tr>
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<td>71.6</td>
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<td>Annual mortality rate in oldest fraction of seed bank</td>
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<tr>
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<td>70%</td>
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<td>90%</td>
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<td>89.9</td>
</tr>
<tr>
<td>99%</td>
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**Fig. 3.** Predicted evolution of glyphosate resistance in barnyard grass under continuous zero-till summer fallows relying solely on glyphosate for weed control, applied in three locations in the northern region

**Fig. 4.** Varying rate of evolution of glyphosate resistance in barnyard grass according to initial frequency of glyphosate resistance alleles in the weed population

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**Table 3. Effects of variation in key model parameters, measured as number of years required before the predicted proportion of resistant plants exceeds 50% of the population**

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**Fig. 3.** Predicted evolution of glyphosate resistance in barnyard grass under continuous zero-till summer fallows relying solely on glyphosate for weed control, applied in three locations in the northern region

**Fig. 4.** Varying rate of evolution of glyphosate resistance in barnyard grass according to initial frequency of glyphosate resistance alleles in the weed population
proportion. Under continuous high selection pressure, the rapid change phase, where resistance changes from $<10$% to $\geq 80$%, can occur over as little as 2 years. This is followed by the ‘resistant phase’ where a high level of resistance is maintained in the population.

The model developed here is able to simulate the variability of northern region grains farming systems, in that it allows for a range of cropping options, farming decisions can be varied from year to year, and the effects of soil and climate differences across the region and from year to year can be accounted for. The model simulates weeds generically, and therefore can be altered to suit different weed species of interest in the future. Similarly, a range of weed control options are included, can be applied flexibly from year to year and could be added to as required. Because the model allows for variation in weed species biology, climate and agronomic factors, the results of complex interactions between them can be examined.

As the model is flexible enough to simulate a wide range of situations in the region, it promises to be useful for generating and refining recommendations for preventing glyphosate resistance in northern region weeds. It is also an effective way to examine the risk levels of a range of different farming systems and practices (Thornby et al., 2006); the results of these risk assessments can then be communicated to farmers and other stakeholders, allowing them to make better, lower risk decisions in the future.

### Predictions, validation and limitations

Given the complexity of interactions between climate, weed ecology and farming system in the field and in the model, the selection pressure that weed cohorts are subjected to can vary substantially from one cohort to another. Thus, overall annual glyphosate resistance selection pressure can vary significantly, depending on the operations undertaken during any given year, and these annual differences can compound to produce substantially different rates of resistance evolution. While our model is sufficiently detailed and flexible to deal with this variability, its complexity introduces a trade-off between the quality of the predictions made and the level of detail required for accurate parameter estimation.

A major reason for modelling the evolution of resistance to herbicides in weed populations is that it takes so long to occur in the field. This is particularly the case for glyphosate resistance, which generally has required 15 years or more of use in the field before being confirmed, even given relatively consistent cropping programmes and climate over time. Thus, it would be very difficult to run real-world experiments to validate model results at a large number of sites and using different management situations. The alternative is to attempt to validate models of herbicide resistance using historical records from real-world resistance cases.

Much of the information we were able to gather for the validation simulation set was, as noted, in the form of estimates and approximations. No recorded data were available for parameters other than recent cropping rotations. Key parameters that were not available in the form of collected data include the initial number of resistant plants in the population before the first use of glyphosate, and the identity and efficacy of each weed control operation over the time since glyphosate was first used – in particular, it is not known how many tillage operations were used to control barnyard grass between the introduction of glyphosate and the switch to zero-till summer fallows, and therefore how many effective chemical summer fallows occurred between 1985 and 2006. The model assumes that all tillage events applied in summer are timed to control a large flush of barnyard grass. Critically, the year of first use of glyphosate, and hence the period over which resistance evolved, was estimated rather than recorded. If we were to assume that either the first year of glyphosate use was some years later than 1985, and/or that the summer tillage operations between the first use of glyphosate and the introduction of zero-till summer fallows were not timed to be effective against barnyard grass in particular, the model’s predicted rate of evolution of glyphosate resistance closely matches the observed response of the real population.

The proportion of the whole seed bank that consisted of resistant individuals when the resistance problem was identified is estimated to be between 40 and 60%, based on glyphosate efficacy studies on the population following the confirmation of resistance, but no statistically testable estimate sampled across the whole population was available. Clearly, no quantitative assessments were ever made of resistance levels in the population prior to its identification as a suspected resistance case in 2006, so the rate of increase in the frequency of resistance-conferring alleles in the real population cannot be tracked over time. These variables are unlikely to be available as statistically tested, planned collations of observations for any normally occurring resistance evolution event over a time scale of more than a decade. This makes straightforward data-fit validation problematic.

Because the model is spatially homogenous, there are two weaknesses in this trend in predictions. First, in practice, noticeably large patches of herbicide failure may constitute as little as 5–10% of a large paddock and, in cases where farm management is diligent, the patch or patches may be given remedial treatment. Nevertheless, the resistance issue in that patch would not disappear given a single season of remedial action. Secondly, as noted by Diggle et al. (2003), models that allow free mating throughout the population, with no spatial component for gene flow through pollen movement, risk overestimating the rate of evolution of glyphosate resistance. Since our model is spatially homogenous, it may fall into the trap of overestimating resistance evolution rates. The model also does not consider a fitness penalty for plants carrying the resistance gene, since no data on this issue were available for barnyard grass. A significant fitness penalty is likely to reduce the rate of evolution of resistance even under glyphosate-only selection, and may also cause non-glyphosate weed control options to have a greater effect on reducing the rate of selection.

These factors notwithstanding, the model’s predictions do fall within the range expected for the evolution of glyphosate resistance. The resistance evolution rate predictions made by the model are somewhat slower than those made by other models (Diggle et al., 2003; Werth et al., 2008), but fit reasonably with observations that resistance to glyphosate generally occurs after something like 15 years of relatively intensive use. The validation exercise predicts approximately this rate of
evolution. The Bellata data used to compare with the model are in some ways less than ideal for our purposes. In future, more complete background data may be available for other newly confirmed glyphosate-resistant barnyard grass populations, which may allow a more comprehensive validation process.

Despite the existence of hidden parameter values and outcome variables, the model consists of individual components that are in some cases well validated (such as the growth and competition models in APSIM) and in others the result of expert assessment (such as the efficacy values for different weed control tactics). Over the whole length of a 30 year simulation, relatively simple accounting procedures are used to track the development of resistance given these reasonably well understood factors.

Key variables and sensitivity

Variation in initial frequency of resistant individuals in unselected populations caused substantial variation in the model’s predictions. This is well established in previous model studies, as well as being observed to be the case in real-world resistance cases (Diggle et al., 2003; Neve et al., 2003; Werth et al., 2008). However, in this case, the parameter variations themselves are substantial: while there is a large difference in the predicted time to a resistance problem between initial frequencies of $1 \times 10^{-4}$ and $1 \times 10^{-10}$, there are several orders of magnitude difference between the parameter values themselves. Thus while the model is sensitive to changes in this parameter, the changes must be one or more orders of magnitude for the effect to be critical.

Other tested parameters were not found to induce great change in the model’s predictions or qualitative behaviour. It is particularly surprising that changes in efficacy on susceptible plants, and strength of resistance (as measured by glyphosate efficacy against resistant plants), do not result in large differences in the model’s predictions. One possible cause of this is the fact that the model allows repeated applications of glyphosate on cohorts as long as density remains over an application threshold. That is, where efficacy on the population as a whole is relatively low, more applications are made, increasing the selection pressure over the season. In terms of representing real-world resistance evolution, then, in this way the model’s accuracy depends on how closely the assumed behaviour of the model ‘grower’ (in applying herbicide multiple times following partial failures of initial control attempts) mirrors the real behaviour of growers.

In the case of both selfing proportion and mortality rate of the oldest seeds, varying parameter values had little effect on the time to 50% resistance, but the length of the rapid change phase increased slightly (data not shown). The selfing population sorts rapidly into homozygous resistant and susceptible individuals which produce only identical progeny, so the rapid change phase under significant selection pressure is shorter than in outcrossing species, even though the rate of initial selection is slower.

Conclusions and further work

Model predictions both in this model and in previous herbicide resistance models for other species and other regions rely on assumptions for a range of parameter values that in real systems are hidden; this is true both for initial construction of the model and for verification and validation exercises. New and continued validation work will be needed for this model, using validation data sets from newly confirmed glyphosate-resistant barnyard grass populations as they are identified. The model will now be used to investigate glyphosate resistance evolution rates and risk levels for a range of regional farming systems and weed control practices, including strategies designed to prevent the development of glyphosate resistance problems. We expect the model to be useful in identifying and quantifying risk factors for glyphosate resistance, and the interaction between ecological and agronomic influences on the rate of evolution of glyphosate resistance.

As the model uses a generic representation of weed biology and population dynamics, we intend to use it to simulate the likely evolution of glyphosate resistance of other important northern region weed species. These include liversedge grass (Urochloa panicoides), flaxleaf fleabane (Conyza bonariensis), sweet summer grass (Brachiaria eruciformis) and common sowthistle (Sonchus oleraceus). As the model continues to improve we expect it to be a useful tool for generating and refining resistance prevention and management strategies and recommendations, and ultimately to have an impact on the sustainability of glyphosate use in northern region farming systems.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following five text files. (1) Vensim seed bank and mating module. (2) Vensim initialization file. (3) APSIM control file. (4) APSIM parameters file. (5) APSIM barnyard grass module initialization file.

ACKNOWLEDGEMENTS

We thank the Northern Herbicide Resistance project team, Jeremy Whish and Patrick Smith from CSIRO/APSRU, Drew Penbarthy, Gary Onus, and our consultative committee of northern region agronomists. This work was supported by the Grains Research and Development Corporation (DAQ00079).

LITERATURE CITED


Agronomy, Crop Science Society of America, Soil Science Society of America.


