

## Patch management solves early infestations of glyphosate resistant awnless barnyard grass

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**Summary** Glyphosate-resistant awnless barnyard grass (*Echinochloa colona* (L.) Link) is an increasing problem on farms of subtropical north-eastern Australia. Current management strategies are effective, but more costly than the glyphosate-centric status quo. However, since barnyard grass is not normally highly mobile, land managers may have an opportunity to contain or eradicate patches of resistance while small, reducing costs by treating patches differently from the surrounding field.

In order to investigate opportunities for patch eradication in Australian cotton cropping, we constructed a computer model, the Spatial Herbicide Resistance Analyser (SHeRA), which assesses spatial population dynamics of glyphosate resistant barnyard grass. SHeRA consists of a grid of cells, with a population sub-model running in each cell. Cells communicate with each other for seed and pollen movement. SHeRA simulates crop competition, a range of cotton-specific weed control measures, and natural and machine-mediated seed movement.

A range of scenarios demonstrate that eradication of awnless barnyard grass in small patches is feasible. Eradication usually occurs in four years of intensive treatment. Eradication is only predicted when:

- small late-season emergences are not ignored, at least in the intensively treated zone,
  - the intensive treatment zone extends 3 m beyond the patch boundary, and 6 m in the direction of travel of machinery, and
  - there are no years where glyphosate is used alone.
- In failed strategies, patches can expand from 4 m<sup>2</sup> to over 500 m<sup>2</sup> in a few years. Periodic re-establishment of zone boundaries is useful for slowing patch expansion in non-robust systems, but does not consistently lead to eradication in those cases.

**Keywords** Cotton, glyphosate resistance, awnless barnyard grass, modelling, eradication, patch management.

### INTRODUCTION

Across the world, wherever industrialised production of food and fibre plants occurs, weeds have evolved to become resistant to commonly used herbicides (Heap 2014). At first, weeds became resistant to selective high-risk herbicides such as ACCase and ALS inhibitors, but changes in usage patterns over the last 30 years have seen the widespread development of populations resistant to even lower risk herbicides like glyphosate.

In Australian agriculture, the emphasis on dealing with resistance (to glyphosate in particular) is shifting from prevention to management. Enough farmers and land managers are now confronted with a population of resistant weeds that it makes sense to investigate and promote strategies for dealing with resistant biotypes that are present, rather than resistance as it evolves. Resistance management recommendations to date include useful tactics, but package them in non-quantified, generic ways, and are not aimed at eradication.

Through modelling, biochemistry and molecular biology, science has developed a good understanding of how, why, and when glyphosate resistance occurs, including substantial genome-level understanding of resistance mechanisms and some knowledge about their heritability (Sammons and Gaines 2014). This knowledge led to, underpins and validates current management recommendations. However, questions remain about the epidemiology of resistance: where it occurs in space, how patches grow, move, and spawn new patches, and at what rate these processes occur for different species. Understanding resistance epidemiology better at a field level could help us decide whether, and under what conditions, local eradication of resistant biotypes is a feasible goal.

In order to examine the spatial dynamics of herbicide resistance in an agricultural situation, we developed SHeRA, the **S**patial **H**erbicide **R**esistance **A**nalyser. SHeRA is a stochastic integer-based model of weed life cycles and gene flow, implemented in Python 3.2.

## MATERIALS AND METHODS

**Model development** Patch dynamics in an agricultural weed are a function of the pressure for expansion and propagation exhibited by the patch, and the manager's pressure for containment and eradication (Cousens and Mortimer 1995). In the case of resistance, patch expansion occurs both through seed dispersal and through pollen flow from resistant patches to the surrounding population. Sub-populations of weeds of 1 m<sup>2</sup> each, arranged in a grid, are subjected to a set of management tactics and, through flowering and seed set, communicate with each other through short- and long-distance movement of pollen and seeds.

The presence of non-resistant plants of the same species causes a complex balance of competing non-resistant pollen (Baker and Preston 2008) and the presence of potential seed parents for the creation of new, relatively distant heterozygous offspring. In the case of species that are self-fertile, like *E. colona*, questions remain over how important rare outcrossing events are in the propagation and expansion of resistance patches. Since successful outcrossing mainly occurs with close neighbours, and less frequently at longer distances that are limited by wind speed and pollen lifespan, SHeRA includes processes for both. Short-distance movement is simulated through sharing of pollen clouds and seeds proportionally with neighbours within a pre-defined distance. Long-distance movement is simplified as a random allocation of a random number of propagules with randomly-chosen distant cells.

SHeRA runs on a yearly timestep. The events in one step are as follows:

1. Germinate weed cohort one.
2. Apply control measures to cohort one.
3. Germinate cohort two.
4. Apply control measures to cohort one survivors and cohort two.
5. Germinate cohort three.
6. Apply control measures to cohort one and two survivors and cohort three.
7. Apply control measures to mature survivors of all cohorts.
8. Determine potential seed production.
9. Produce and move pollen between neighbouring cells and at long distance.
10. Determine progeny genotypes.
11. Move progeny (seed) between neighbouring cells.
12. Process end-of-year mortality of new seeds prior to entering seed bank, and between-seasons mortality of old seeds in seed bank.
13. Seed rain enters seed bank – return to start.

Integer modelling is used in determining the survivorship of plants under self-thinning processes and simulated control tactics. As each cohort germinates,

a number of plants (proportional to the cell's current seed bank density, rounded down) are entered into a Python list either as a 0 (no resistance alleles), 1 (one resistance allele, heterozygous) or 2 (homozygous resistant). Separate lists are maintained for each cohort. SHeRA simulates populations with a single-gene resistance mechanism, though that mechanism may be dominant, recessive, or in-between. For most currently-known glyphosate resistant populations, this is a reasonable simplification.

Cohort lists are tested for survivorship against estimates of herbicide efficacy relative to genotype and plant age. Testing continues until the model has determined the number and genotypes of individuals that survive a whole season of management tactics.

Seed production per plant is affected by plant density according to a hyperbolic yield penalty model (Cousens 1985), potentially reduced to account for fitness penalties due to resistance. We developed parameters and mechanisms to test the patch dynamics of glyphosate-resistant awnless barnyard grass in a glyphosate-resistant cotton farming situation, using a variety of published and unpublished data. Key parameter estimates for the *E. colona* implementation of SHeRA are given in Table 1.

**Table 1.** Parameter values for SHeRA simulating patches of glyphosate resistance in *Echinochloa colona*.

Parameter	Value
Cohort 1 germination proportion (of total seed bank)	0.05
Cohort 2 germination proportion	0.05
Cohort 3 germination proportion	0.01
Carrying capacity	3000 plants m <sup>-2</sup>
Initial seed bank density	400 seeds m <sup>-2</sup>
Initial resistance patch size	4 m <sup>2</sup>
Proportion of pollen shared with nearby cells	0.4
Pollen spread distance	2 cells
Chance of long distance pollination, per cell	0.01
Proportion of pollen spread at long distance	0.001
Mortality of seeds prior to entering seed bank	0.1
Annual seed mortality	0.5
Proportion of seed shared with nearby cells	0.2
Seed spread distance	1 cell
Self-fertilisation proportion	0.95
Maximum seed production	15,000 plant <sup>-1</sup>

**Simulations** We tested the effects of a range of weed control systems used in (and modified from) cotton production in Australia for their propensity to result in either an increase in the size and spread of the resistant population. Several categories of simulations are described here: rate of spread under glyphosate-alone treatment; current best management practice (BMP) treatments; and the effects of small and large containment zones under strong eradication pressure. Each scenario is simulated in the model ten times, and the mean of the ten runs is reported in the Results below. The specific treatments included in each simulation are as follows:

- **Scenario group A: Glyphosate alone.** Up to two glyphosate applications per cohort, with an application threshold of 1 plant  $m^{-2}$ . Tillage is applied prior to emergence of the first cohort either annually or biennially, and either in the same direction each year, or in alternating directions in alternating years.
- **Scenario group B: Best Management Practice (BMP) plus eradication tactics.** In all simulations, an early residual (prior to emergence of the first or second cohort) is applied, followed by up to three applications of glyphosate, and a second residual herbicide that affects the second or third cohort. An eradication tactic may also be applied as follows: early: a very early residual or a grass selective herbicide (in alternating years); mid-season: a mid-season residual with or without shielded paraquat; late: chipping or a grass selective herbicide (in alternating years).
- **Scenario Group C: Containment zone size.** In these two scenarios, we tested glyphosate alone in the background zone, with glyphosate plus paraquat on each cohort emerging in the patch zone and a containment zone extending either 0, 1 or 6 m beyond the edge of the initial patch.

## RESULTS

After six simulated years, resistant sub-populations either dominate the landscape (as in the case with all glyphosate-alone scenarios in group A; Table 2), are eradicated (as in scenarios B4, C1 and most runs of C2), or are constrained somewhat in their expansion by poorly controlled susceptible sub-populations in the background zone. With glyphosate used alone and substantial frequent disturbance by tillage, patches were predicted to reach up to 400–500  $m^2$  in size after six years, from a modest starting size of 4  $m^2$ . In fact, in each scenario in group A, the population almost filled the 50 × 50 grid used for the simulations. A larger grid would show that the patch in fact could grow larger than this in six years.

**Table 2.** Size and population density of resistant (R) patches and background population density of susceptible (S) plants after six years, under a range of simulations with SHErA.

Simulation	# cells infected <sup>A</sup>	Mean R plants $m^{-2}$ <sup>B</sup>	Mean S plants $m^{-2}$
<i>A: Glyphosate alone</i>			
A1: No tillage	263	61032	5
A2: Annual tillage	474	34799	234
A3: Biennial tillage	427	34543	199
A4: Annual tillage, reversing	525	35551	131
A5: Biennial tillage, reversing	465	34352	224
<i>B: Best Management (BMP) plus eradication early, mid, or late season</i>			
B1: BMP	50	4010	990
B2: BMP + early	60	5200	930
B3: BMP + mid	57	1546	18724
B4: BMP + late	0	0	0
<i>C: Containment zone size</i>			
C1: 6 m cont. zone	0	0	2
C2: 1 m cont. zone	23 <sup>C</sup>	337	7
C3: No cont. zone	228	22894	26

<sup>A</sup>As cells are 1  $m^2$  each, this figure also describes the size of patches in  $m^2$ .

<sup>B</sup>This figure describes the mean density of R plants in cells that contain resistance – not the mean over the whole field.

<sup>C</sup>In 80% of simulations of scenario C2, the resistance gene was eradicated; the remaining simulations resulted in patch escapes leading to eradication failure.

## DISCUSSION

There is a range of possible scenarios for eradicating glyphosate resistant patches of awnless barnyard grass, but these occur only under vigorous attempts to eradicate. Frequent use of non-glyphosate knockdowns is required; the residual-centric BMP scenarios (group B, Table 2) were largely not successful. Late-season control using something other than glyphosate is critically important: even if the last cohort is relatively small, selecting its glyphosate-resistant members with glyphosate and then allowing seed set to occur clearly provides good conditions for the resistant patch to increase, and to create satellite patches.

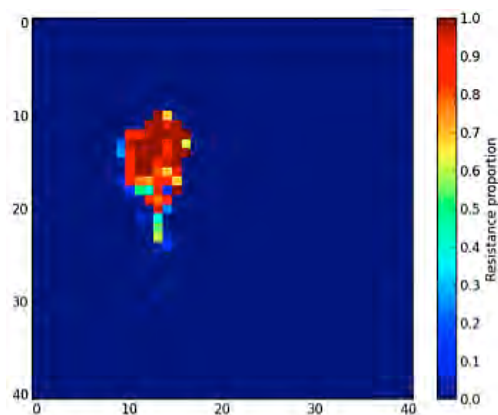
The size of the containment zone is clearly important. If not large enough, eradication may occur,

but not reliably; escapes into the background zone are able subsequently to establish and create a new, uncontrolled patch. Early escapes leading to large resistant patches are all but inevitable if no containment zone is used (scenario C3, Table 2). Short-distance seed movement by gravity is easily counteracted with a containment zone strategy, but longer-distance movement of seed on machinery, such as by soil adhering to tyres, is a substantially more difficult problem even if (as in the case of these simulations) it is relatively rare. Seed movement in overland water flows is potentially an even greater problem.

Spatially aware strategies for weed management are not likely to be of great assistance in controlling sub-populations of very mobile species, such as wind-blown seed producers. In relatively slow-moving coloniser species, such as most grasses, however, this kind of approach offers possibilities for the application of high-intensity, high-cost programs on a fraction of a whole field. To date, uptake of resistance prevention strategies has been sluggish, largely due to the cost and reduced utility of non-glyphosate options. Zonal management, if designed effectively, has the potential to reduce costs while maintaining the benefits of these more expensive strategies. The results of models like SHeRA will be very useful in defining and, in particular, describing zonal management strategies to farmers and other land managers.

SHeRA outputs also include visualisations of the field. A sample is shown in Figure 1. While the numerical data (as in Table 2) is useful in comparing between scenarios, the visual output could equally be persuasive, especially when used as animations. The computing requirements of communicating between large numbers of cells, and of creating visualisations of the field, are substantial, so while very large fields (of tens of thousands of cells or more) could provide better answers to some questions, such as the effects of long-distance pollen drift between disparate susceptible and resistant patches, or a more realistic set of predictions around the creation of satellite patches, these very large fields require much more powerful computing environments (such as clusters of machines) to be processed reliably and efficiently. Future applications of SHeRA to more detailed questions may need this kind of infrastructure.

The results shown here were obtained in modelled cotton systems. Similar scenarios could readily be devised for broadacre grains farming and even non-cropping weed management situations where herbicide resistance has become a problem.



**Figure 1.** SHeRA visual output of one run of scenario B3; BMP strategy plus mid-season eradication tactics.

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