Herbicides used in combination can reduce the probability of herbicide resistance in finite weed populations

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Summary

A simulation study was conducted to examine the effect of pattern of herbicide use on development of resistance to two herbicides with different modes of action in finite weed populations. The effects of the size of the treatment area (analogous to initial weed population), germination fraction and degree of self-pollination in the weed were investigated. The results indicate that the probability of developing resistance to one or both herbicides decreases as the size of the area/initial population decreases. For treatment areas of 100 ha or less with an initial weed seedbank of 100 seeds m^{-2} and initial frequencies of the resistance genes of 10^{-6}, development of resistance to both herbicides (double-resistance) is uncommon within 50 years for all types of weeds if both herbicides are used in all years (used in combination). If herbicides are used in alternate years (rotated) double-resistance almost always occurs in 100 ha areas but is uncommon in areas of 1 ha or less. The results suggest that adoption of practices that limit movement of weeds in conjunction with using herbicides in combination rather than in rotation can substantially delay development of herbicide resistance.

Keywords: simulation, modelling, mixtures, reduction, herbicide resistance.

Introduction

The development of herbicide resistance in weed populations under herbicide selection is an evolutionary phenomenon. Herbicides are very intense selective agents and where genetic variability for herbicide response exists in weed populations, evolution of herbicide resistance can be rapid. The probability and rate of herbicide resistance evolution depends on the interplay between the population dynamics and population genetics of weed populations (Maxwell & Mortimer, 1994; Jasieniuk *et al*., 1996; Diggle & Neve, 2001). Important evolutionary factors include the intensity of selection (degree of discrimination between genotypes); the frequency of resistance traits in natural (unselected) populations; the mode of inheritance of resistance; the relative fitness of susceptible and resistant biotypes in the presence and absence of herbicide; and gene flow within and between populations. The intrinsic population dynamics of weed populations is also important, especially in the area of seedbank dynamics where it is recognized that a persistent seedbank can act as a buffer to evolution (Mortimer *et al*., 1993).

Several simulation models of the population genetics and dynamics of herbicide resistance in weed populations have been developed (Gressel & Segel, 1978; Maxwell *et al*., 1990; Gardner *et al*., 1998; Cavan *et al*., 2000). These models differ in their approaches, particularly in relation to population genetics, and the applications and limitations of the various methodologies have been discussed by Diggle and Neve (2001). In particular, the authors draw attention to the benefits of explicitly accounting for each genotype in the population. The common alternative approach of assuming Hardy–Weinberg equilibrium between successive generations may lead to erroneous conclusions about resistance

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evolution under conditions that violate the assumption of random mating. Such conditions are common and include incomplete germination, immigration and emigration of seed and pollen (gene flow); staggered flowering time; and any bias towards self-pollination. These factors affect the rate of evolution of herbicide resistance because they determine the frequency of the haplotypes that are involved in mating at any given time.

Where more than one resistance gene is being considered, an assumption of genotypic equilibrium between successive generations is inappropriate, even under conditions of completely random mating. While individual genes reach equilibrium in each generation, equilibrium combinations of genes are only achieved after many generations of random mating. For this reason, simulation of the dynamics of more than one gene in a population requires explicit accounting of each genotype. The evolution of herbicide resistance in the field typically involves simultaneous selection by herbicides with more than one mode of action. Consequently, consideration of two (or more) genes is of practical significance.

Population size is also an important factor in the rate of evolution of herbicide resistance. Where gene flow between adjacent populations is zero, the probability of a resistant individual occurring in a population is a function of population size (weed density $\times$ population area) and the initial gene frequency. While mutation rates and hence initial gene frequencies are beyond the control of management, weed population densities are not. Weed control practices that maintain low weed densities can considerably decrease the chance of resistance evolution by reducing the number of resistance alleles in a population (Christoffers, 1999).

A practical consequence of this principle, where more than one rare resistance gene is present in the population, is that individuals with multiple resistances are *extremely* rare prior to herbicide selection (Gressel & Segel, 1990). Hence, within any given local area, multiresistant individuals will, on balance of probabilities, be absent in any one generation.

Rotations and mixtures of herbicides that have discrete modes of action and that are not capable of degradation by a common metabolic pathway (mode of degradation) have long been proposed as a means to prevent or delay resistance evolution (Gressel & Segel, 1990; Wrubel & Gressel, 1994; Powles *et al*., 1997). Powles *et al*. (1997) modelled herbicide resistance evolution in a weed population of infinite size when two herbicides were rotated annually or used each year as a mixture. In the absence of fitness penalties, (which would cause selection against resistant alleles in 'off' years) the rotation strategy did not increase the number of applications before resistance for either herbicide. When the herbicides were used in mixture, resistance was delayed by approximately 4 years.

When an infinite population is assumed, no matter how improbable, extremely rare double-resistant individuals will always be assumed to be present and influencing the rate of evolution of resistance. By explicitly simulating all genotypes in a finite population a more realistic prediction of the dynamics of the evolutionary process can be achieved. This capability is particularly important when examining the implications of patterns of herbicide use and may modify the relative benefits of alternative herbicide use strategies.

The model described in this paper was developed to investigate the influence of weed life history traits (seed dormancy and plant mating system) and management (population size and genetic isolation) on the rate of resistance evolution under three contrasting herbicide management regimes.

Model development

Overview

The model has been developed to simulate the evolution of herbicide resistance at two discrete, unlinked nuclear gene loci in a weed of broad-area farming. In accordance with most cases where the inheritance of herbicide resistance has been documented (Darmency, 1994) resistance is assumed to be conferred by alleles at a single gene locus. Mutant alleles at these two loci confer resistance to different herbicide modes of action so that evolution of resistance to two distinct herbicides or herbicide modes of action can be tracked within a single weed population (multiple resistance). It is assumed that alleles at the two loci segregate independently.

The herbicides to which resistance is being simulated are hypothetical. Both allow selective post-emergence control of the weed and both are effective enough so that the weed is adequately controlled when either herbicide is used on its own, resulting in 'redundant kill' [control of resistant survivors of one herbicide by a second, chemically dissimilar, herbicide as defined by Comins (1986)] when they are used in combination. There is no capacity for resistance at one locus to confer cross-resistance to other herbicide chemistries. The majority of herbicide resistance traits are inherited in a dominant or semi-dominant manner (Darmency, 1994). Resistance to both herbicides in this model is assumed to be inherited in a completely dominant manner at field-applied rates.

The model is constructed from a number of submodels and is based on the life cycle of a

hypothetical annual weed. The life cycle model simulates the population biology and demographics of a single population over successive generations. The reference point for the life cycle is the soil seedbank. Processes of germination, emergence, establishment, growth and reproduction are regulated by intrinsic population dynamics (density dependence and seedbank dynamics) and extrinsic environmental (climate) and management factors. Population dynamics and the efficacy of weed control strategies together govern transition probabilities between life history stages (Fig. 1).

The model was implemented in the Stella simulation language (version 7) produced by High Performance Systems, Inc., Hanover, NH, USA (http://www.hps-inc.com).

A crop (wheat, *Triticum aestivum* L.) is sown during each year of the simulation. Crop seeding and germination rates and crop establishment characteristics are defined. Competition between the crop and the weed is simulated using a reparameterized version of the hyperbolic equation (see below) used by Firbank and Watkinson (1986) to predict crop yields and weed seed production.

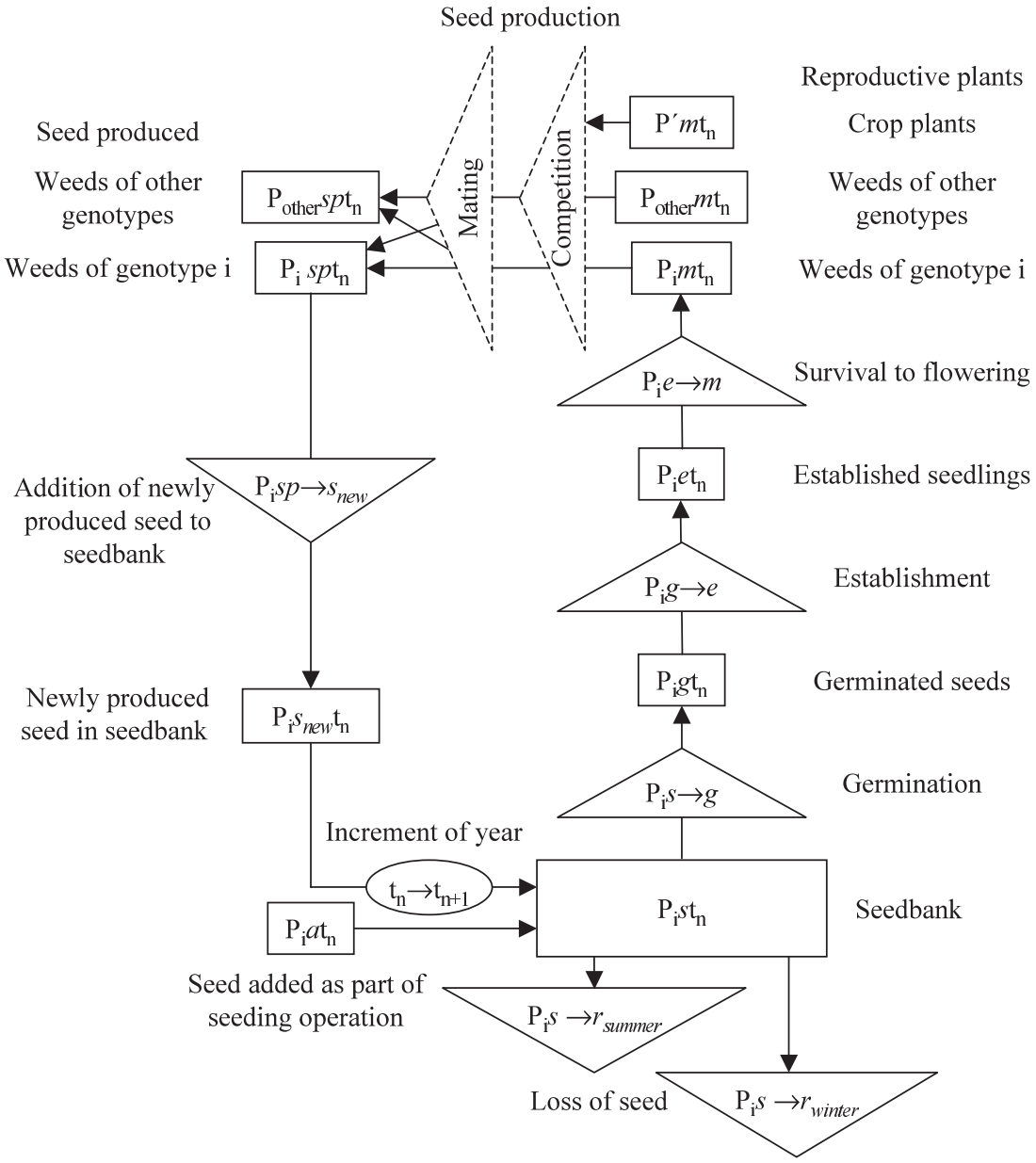

Fig. 1 Life cycle of the weed as represented in the model, illustrated for one genotype (i). Variables representing densities of plants in particular life history stages are enclosed in rectangles. Variables representing transition probabilities between life history stages are enclosed in continuous triangles. More complex relationships between stages are illustrated by broken triangles.

A weed population genetics submodel tracks the fate of herbicide-resistant and susceptible genotypes and alleles within the population. Control efficacies and population dynamics are defined individually for each of the nine possible weed genotypes (see below). A stochastic extinction routine is incorporated into the model to account for the possibility that rare alleles may be totally eradicated from the population.

During the reproductive stage, alleles at the two loci segregate independently into gametes (pollen and ovules) and recombine during completely random mating. The rate of out-crossing may be varied. Following seed-set and maturation, seeds are returned to the soil seedbank.

Weed life cycle

As with previous models of herbicide resistance, the life cycle forms the central element of the model through which changes in population density and the frequency of individual genotypes are accounted. During each generation, individuals of weed population (P) are represented in a number of states; viable seeds in the seedbank (s), germinated seeds (g), established seedlings (e), mature plants (m), seed produced on mature plants (sp), seed removed from the seedbank other than by germination (r), and seed added to the seedbank as a contaminant at crop sowing (a). The starting point for all simulations is the initial weed seedbank (Ps_{initial}) (seeds m^{-2}). The number of mature plants of genotype i in year n (P_imt_n) is defined by equation 1:

$$P_imt_n = (P_ist_n + P_iat_n) \quad Ps \rightarrow g \;\; Pg \rightarrow e \;\; P_ie \rightarrow m, \quad (1)$$

where P_ist_n is the weed seedbank of genotype i in year n, P_iat_n is weed seed of genotype i added to the seedbank as part of the sowing operation in year n, $Ps \rightarrow g$ is the fraction of the weed seedbank which germinates, and $Pg \rightarrow e$ and $P_ie \rightarrow m$ are transition probabilities for individuals becoming established seedlings and mature plants respectively.

Total seed production (P_{sp}) is calculated in a separate competition submodel (see below). The total amount of seed of each genotype that is returned to the soil seedbank at the end of the growing season ($P_is_{\text{new}}t_n$) is the product of total seed production of genotype i in year n ($P_i\text{sp}t_n$) and the fraction of seed produced that reaches the seedbank ($P_{\text{sp}} \rightarrow s_{\text{new}}$).

The weed seedbank at the start of the following year (P_ist_{n+1}) is calculated as

$$P_ist_{n+1} = \{[1 - Ps \rightarrow g(P_ist_n + P_iat_n)](1 - Ps \rightarrow r_{\text{winter}}) + P_is_{\text{new}}t_n\}(1 - Ps \rightarrow r_{\text{summer}}), \quad (2)$$

where $Ps \rightarrow r_{\text{winter}}$ and $Ps \rightarrow r_{\text{summer}}$ are the fractions of ungerminated weed seeds lost from the seedbank during winter and summer respectively.

Crop/weed competition submodel

Competition between crops and weeds is simulated using a modified version of the hyperbolic function used by Firbank and Watkinson (1986):

$$P_{\text{sp}} = \frac{Pm\ P_{\text{sp max}}\ kP}{1 + (Pm\ kP) + (P'mk\ P'\ A)}, \quad (3)$$

where Pm is the number of mature weed plants, $P'm$ is the number of crop plants, $P_{\text{sp max}}$ is the potential maximum seed production of the weed per unit area, kP is the weed plant size coefficient, the inverse of the weed density (Pm) at which seed production is half of the predicted maximum ($P_{\text{sp max}}$), kP' is the crop plant size coefficient and A is the interspecific antagonism of the weed species by the crop.

Population genetics submodel

The model tracks the frequencies of herbicide-susceptible and resistant alleles at two discrete, independently segregating loci. The two loci confer resistance to two hypothetical herbicides, designated Y and Z, with different modes of action. Both loci are diallelic with the susceptible wild type alleles designated by lower case letters (y and z) and the initially rare mutant resistant alleles by upper case (Y and Z). Resistance alleles are specified as dominant. The model accounts for the frequency of nine genotypes within a single weed population (P_{YYZZ}, P_{YYZz}, P_{YYzz}, P_{YyZZ}, P_{YyZz}, P_{Yyzz}, P_{yyZZ}, P_{yyZz} and P_{yyzz}).

The initial weed population size is the product of the initial seedbank density (Ps_{initial}) and the area (m^2). The initial frequencies of resistant alleles Y and Z are defined as f_{Y} and f_{Z}, respectively, and alleles, assumed initially to be in Hardy–Weinberg equilibrium, are explicitly accounted thereafter.

Extinction

To avoid anomalous results resulting from fractional numbers of plants, the model represents small populations as integer values. Where less than 10 individuals of any genotype are calculated to occur at any stage of the life cycle, an integer number of individuals is derived assuming that the calculated weed density is a probability of occurrence in a Poisson process (Vose, 2000). Where 10 or more individuals are expected, the probability that an extinction event would occur in a Poisson process is less than 1 in 20 000. If the populations of all genotypes that contain any particular allele are resolved to 0 at any time then that allele is considered extinct and will remain extinct unless it is reintroduced as a contaminant during sowing. Random numbers conforming

to Poisson distributions were generated using the Stella simulation software with sequences of random numbers seeded using the system clock. Because the extinction process is effectively random, repeated runs of the model with identical parameters will produce different outcomes.

Mating

Resistance alleles segregate independently. The fraction of self-pollination in the weed species can vary between 1 (autogamous) and 0 (allogamous). The frequencies of pollen haplotypes for each plant genotype are the averages of the frequencies for that plant genotype and the frequencies for the total plant population weighted according to the self-pollination fraction. Ovules and pollen are produced in direct proportion to predicted seed yields and all gamete haplotypes have an equal chance of reproductive success (pollination, embryo development and seed maturation).

Pollen and ovule haplotypes recombine at random to produce diploid zygotes that develop into mature seed. The simplifying assumption of random recombination within the weed population is common to existing models that assume infinite populations, but it is inaccurate because of uneven spatial distribution of genes within the population. This assumption of random recombination becomes less realistic as the simulated area becomes greater, and it will tend to result in an overestimation of the rate that resistant alleles multiply after multi-resistant individuals develop. However, once resistance has developed it is reasonable to assume that it will eventually spread throughout the population. The randomness of recombination does not influence the probability that rare alleles will be present in finite populations and hence it should have minimal influence on the probability that resistance will develop initially.

Parameter values

Weed management practices, together with intrinsic population processes (mortality, loss of viability and competition), act in conjunction to regulate weed population dynamics and ultimately, weed densities in the field. In the model, these processes are defined by the parameters that affect the probability of any individual moving from one life history stage to the next (e.g. from germinated seeds to established seedlings). These parameters have been adapted from those presented by Pannell *et al*. (2003) to approximate conditions and management typical of wheat grown in the cropping belt of Western Australia.

All parameter values are constant for all genotypes of the weed except for the probability that established seedlings will become mature plants ($P_i e \rightarrow m$), which is affected by application of post-emergence herbicides. The values for all constant parameters are given in Table 1. The probability that established seedlings will become mature plants ($P_i e \rightarrow m$) is the product of the probabilities that plants will survive application of herbicides Y and Z ($P_i e \rightarrow m_Y$ and $P_i e \rightarrow m_Z$ respectively). The probabilities of genotypes surviving when herbicides are applied are presented in Table 2. Where herbicides are not applied $P_i e \rightarrow m$ is 1.

We have chosen not to include density-dependent mortality of plants because we anticipate that plant densities will typically be low while herbicides are still effective. For this reason, density-dependent mortality is unlikely to be an important factor in the dynamics that affect initial development of herbicide resistance.

Table 1 Descriptions, variable names, values and units for constant parameters

Parameter description	Variable name	Value	Unit
Density of crop plants	$P'm$	100	plants m^{-2}
Initial weed seedbank	$Ps_{initial}$	100	plants m^{-2}
Initial frequency of allele for resistance to herbicide Y	f_Y	10^{-6}	
Initial frequency of allele for resistance to herbicide Z	f_Z	10^{-6}	
Annual import of unselected weed seed	Pat_n	0.1	plants m^{-2}
Fraction of weed seedbank lost in winter	$Ps \rightarrow r_{winter}$	0.1	
Fraction of weed seedbank lost in summer	$Ps \rightarrow r_{summer}$	0.1	
Fraction of germinated seed that establishes as seedlings	$Pg \rightarrow e$	0.2	
Fraction of seed produced on weeds that reaches the seedbank	$P_{sp} \rightarrow s_{new}$	1	
Maximum viable weed seed production	$P_{sp\ max}$	30000	plants m^{-2}
Weed plant size coefficient	kP	0.04	m^2 $plant^{-1}$
Crop plant size coefficient	kP'	0.09	m^2 $plant^{-1}$
Crop/weed antagonism parameter	A	1.3	

Table 2 Probabilities that plants of specified genotypes will survive applications of herbicides Y and Z

Genotype for gene Y	yy	Yy	YY
Value of $P_i e \rightarrow m_Y$ when herbicide Y is applied	0.05	1	1
Genotype for gene Z	zz	Zz	ZZ
Value of $P_i e \rightarrow m_Z$ when herbicide Z is applied	0.05	1	1

Accuracy of the model is less important in circumstances where herbicide resistance has already developed and weeds have reached high densities. Such situations are unlikely to occur in practice as farming would not be profitable and farmers would alter their practices accordingly.

Plant life history strategies

Four contrasting plant types were specified for the weed population. These plant types were combinations of high and low germination fraction ($Ps \rightarrow g = 0.9$ or 0.1) and high and low self-pollination fraction (0.99 or 0). All constant weed related parameters have been chosen to approximate *Lolium rigidum* Gaud. (annual ryegrass), which is cross-pollinated and has a high germination fraction similar to that specified above.

Herbicides and herbicide use patterns

In all simulations two herbicides (Y and Z) were available for post-emergence control of the weed population. Both herbicides achieve 95% control of susceptible individuals (see Table 2), they have distinct modes of action and cannot be degraded by a common metabolic pathway (there is no potential for evolved cross-resistance to both herbicides). In the absence of herbicide application there is no fitness penalty associated with resistance to either herbicide. Fitness penalties associated with target-site resistance to the triazine herbicides have been documented in a number of weed species. However, the results for resistance to all other herbicide modes of action have been more equivocal (Holt & Thill, 1994).

Three patterns of herbicide application were defined. These were:

Rotation strategy: Herbicides Y and Z were applied in alternate years beginning with Y in the first year.

Threshold strategy: Herbicide Y was applied in all years until the frequency of the Y resistance allele (Y) exceeded a threshold value of 0.01, after which point Z was applied until the frequency of the Z resistance allele (Z) exceeded the same threshold, whereafter whichever herbicide had the lowest fraction of resistance was applied. Please note that we are using this term in a non-standard way to refer to thresholds of frequencies of resistance alleles rather than thresholds of weed density.

Combination strategy: Both herbicide Y and Z were applied in all years. There were no antagonistic or synergistic interactions between the two herbicides. It is important to note that this strategy involves application of more herbicide than does either of the other two strategies.

For each plant type in combination with each herbicide application strategy 32 repeated 50-year simulations were conducted for each of 19 treatment areas ranging from 10^2 m^2 to 10^8 m^2 on a log scale.

Results

Each model run results in a time series of populations of the nine genotypes. For simplicity these can be identified as four phenotypes: susceptible to both herbicides (yy zz); resistant to Y only (yY zz and YY zz); resistant to Z only (yy zZ and yy ZZ); resistant to both Y and Z (yY zZ, YY zZ, yY ZZ and YY ZZ).

While each of the runs is unique due to stochastic processes, runs that gave similar results can be grouped according to the characteristic dynamics of the populations (seedbanks). For each of the 32 runs of the model for each combination of plant type by herbicide strategy at each treatment area, three outcomes were possible: no resistance develops; resistance develops to a single herbicide; resistance develops to both herbicides.

For example, the outcrossing high germination fraction plant type produced eight typical patterns of seedbank behaviour (Fig. 2). Where no resistance developed the population steadily declined (Fig. 2A and B). This decline was typically more rapid where the herbicides were used in combination (Fig. 2C).

Where herbicides were used in rotation and resistance developed to either of the two herbicides, the population of resistant plants increased exponentially and exceeded the population of susceptible plants at around year 10 (Fig. 2D). From that time the population of resistant plants increased rapidly until stabilizing at numbers in excess of 10 000 seeds m^{-2}. There was also a transient increase in the population of susceptible plants as a consequence of recombination amongst the progeny of heterozygous resistant plants (i.e. yY or zZ). The population of susceptible plants ultimately fell as the frequency of the susceptible allele (i.e. y or z) declined.

In cases where a single resistance emerged under the threshold strategy, control of the population was maintained by the use of the alternate herbicide, with the population of resistant plants remaining below 10 plants m^{-2} (Fig. 2E).

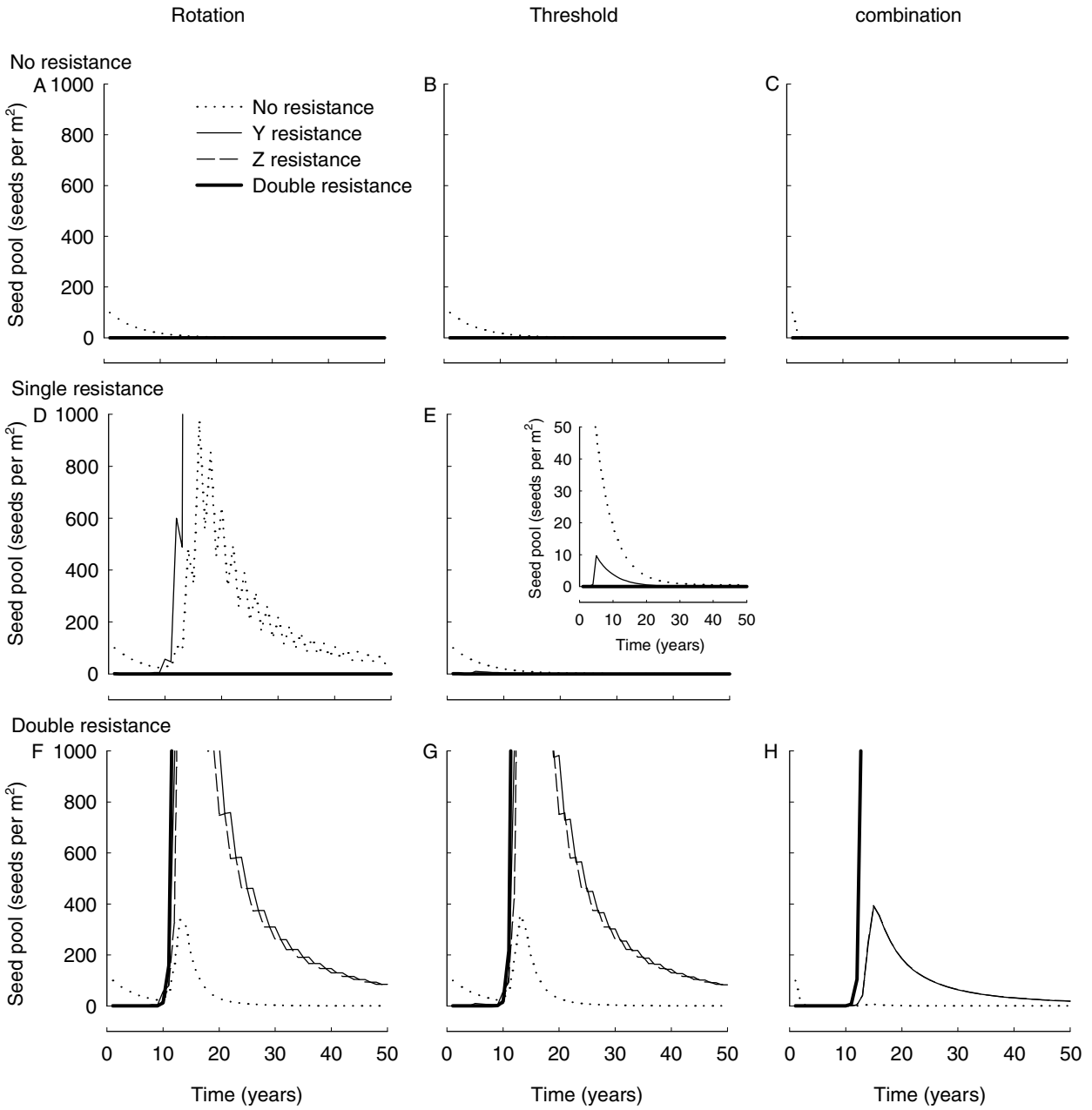

Fig. 2 Selected examples of typical time series of seed pool size for the four phenotypes of the plant type with 0.9 germination fraction and 0 self-pollination fraction for the three classes of developed resistance (rows) for each of the patterns of herbicide use (columns). The probabilities of these typical outcomes vary with treatment area (see Figs 4 and 5).

In all cases where double-resistance developed, the population of double-resistant plants increased rapidly from about year 10 (Fig. 2F–H). Transient increases in the populations of the other three genotypes occurred in response to the recombination effects as described for single resistance (Fig. 2D).

Variations occurred in the timing of development of resistance. In general, resistant populations increased more slowly where the germination fraction was low (0.1) (data not shown). For all types of resistance there were examples where build up of resistance was delayed. An example illustrating development of double-resistance with delayed occurrence of Z resistance is shown in Fig. 3. Delayed resistance of this sort occurred where resistance genes in the initial population became extinct but were reintroduced in contaminant seed.

All model runs were categorized according to the highest resistance status attained by the population during the simulated 50-year period, namely: no resistance; Y resistance only; Z resistance only; and double-resistance. The relative frequencies of these categories

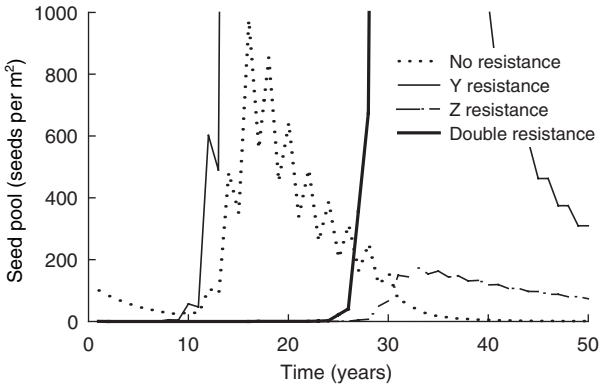

Fig. 3 An example time series of seed pool size for the four phenotypes of the plant type with 0.9 germination fraction and 0 self-pollination fraction illustrating development of double-resistance where Z resistance is delayed. Herbicides were applied in rotation with a 21.4 ha treatment area.

varied in response to the treatment area (≅ initial population size).

For the cross-pollinated plant type with high germination fraction (0.9) for all herbicide use patterns, development of resistance was rare in the smallest treatment areas (Fig. 4). As the area increased, the frequency of single resistances and, subsequently, double-resistance increased. This trend occurred because the probability of extinction of resistance genes decreases as area (≅ initial population size) increases.

The relationship between probability that no resistance would occur and area treated was very similar where herbicides were applied in rotation or according to the threshold strategy (Fig. 4A). In both cases resistance always occurred in some form in treatment areas larger than 20 ha. Where herbicides were used in combination, resistance became frequent only in much larger areas. A treatment area in the order of 100 times larger was required to produce similar probabilities of development of resistance (Fig. 4A).

Where herbicides were applied in combination, resistance to either Y or Z alone did not occur because plants with resistance to only one herbicide were adequately controlled by the other herbicide. Consequently, only populations of double-resistant plants were able to increase. Resistance to herbicide Z alone did not occur where the threshold strategy was used (Fig. 4B). This is because according to this strategy herbicide Z was only applied after resistance to herbicide Y had developed.

Where herbicides were applied in rotation, Y resistance tended to occur more frequently than Z resistance because Y was the first herbicide applied in the rotation (Fig. 4B). Consequently the population initially treated by Z was reduced, increasing the probability of extinction of the Z resistance allele.

The relationship between probability of occurrence of resistance and treatment area was broadly similar for all plant types, but the relative effect of using herbicides in combination was larger for plant types with lower germination fraction or higher fraction of self-pollination. For self-pollinated weeds with a high germination fraction, a treated area in the order of 1000 times larger was required to produce similar probabilities of development of resistance for herbicides used in combination vis-à-vis the other strategies (Fig. 5A). This factor was greater than 10 000 times for weeds with low germination fraction (Fig. 5D and G). In the case of self-pollinated weeds with low germination fraction

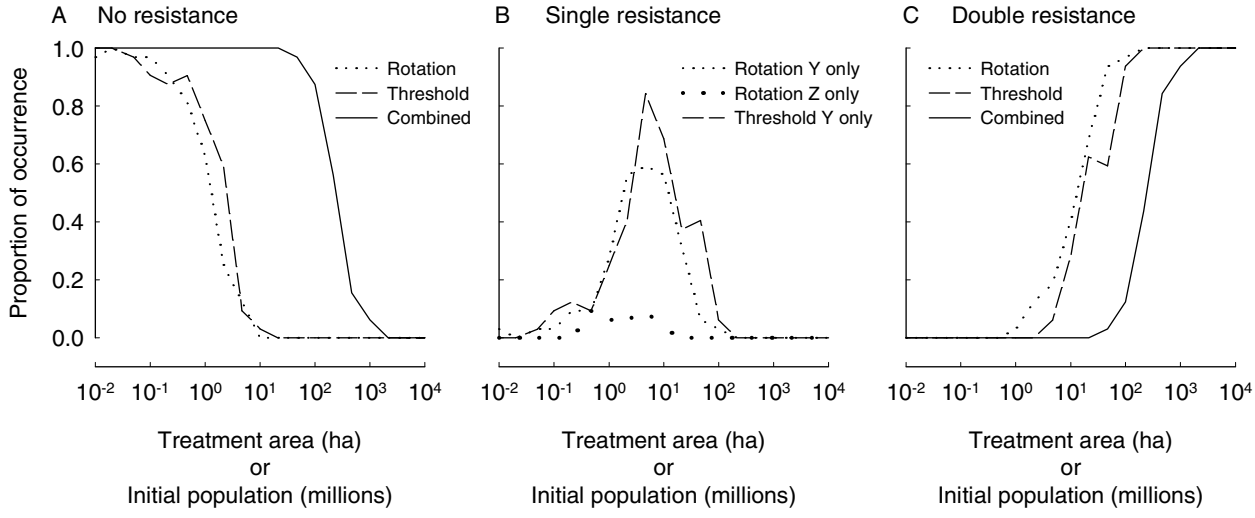

Fig. 4 Proportion of simulation runs where no resistance occurred (A), where resistance to a single herbicide type occurred (B) or where resistance to both herbicide types occurred (C) versus treatment area (≅ initial population size) for the plant type with 0.9 germination fraction and 0 self-pollination fraction.

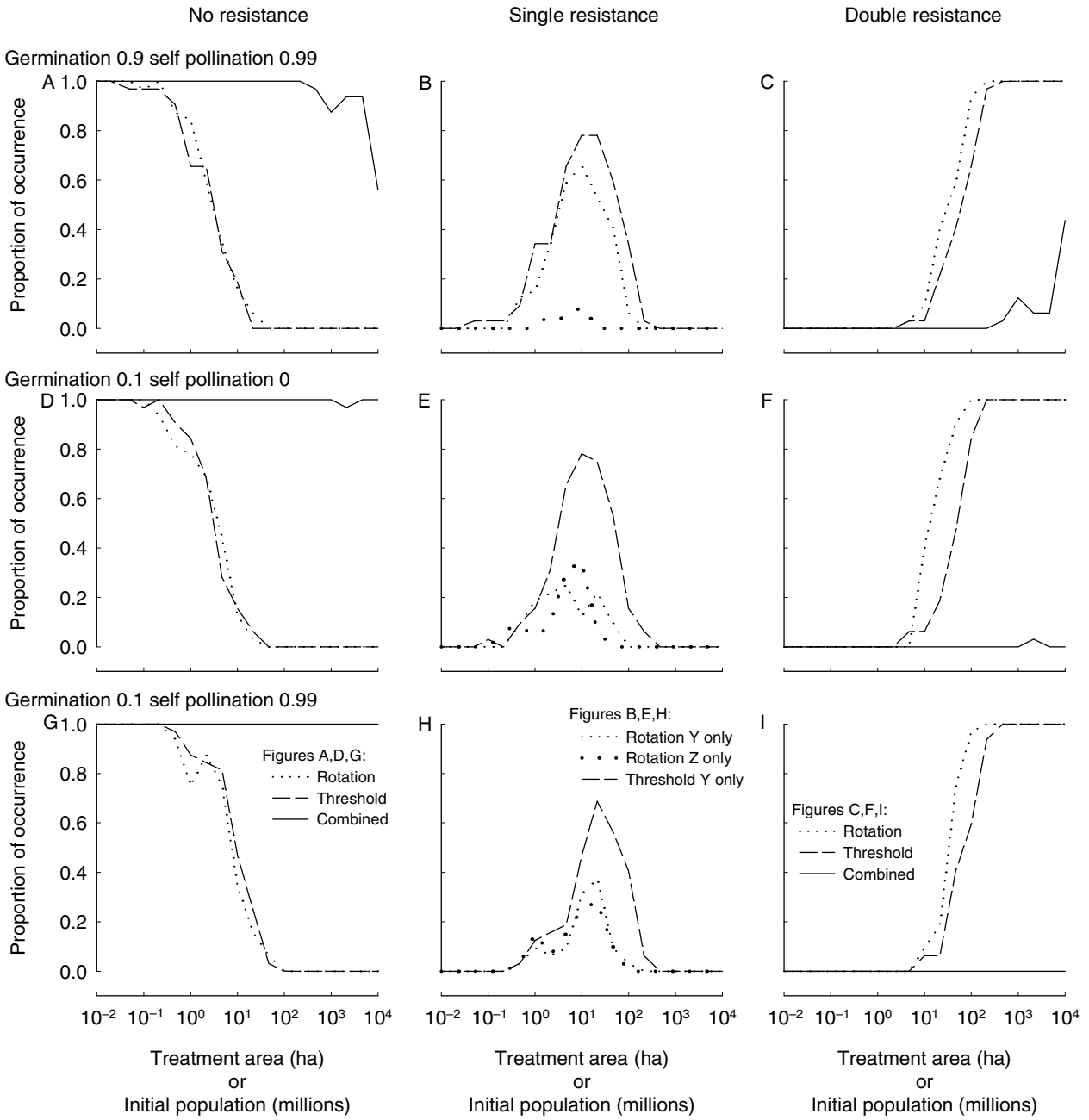

Fig. 5 Proportion of simulation runs where no resistance occurred (A, D and G), where resistance to a single herbicide type occurred (B, E and H) or where resistance to both herbicide types occurred (C, F and I) versus treatment area (≅ initial population size) for three plant types (in rows).

herbicide resistance was never observed where herbicides were used in combination (Fig. 5G).

Where herbicides were applied in rotation, germination fraction had an effect on the relative frequencies of the two types of single resistance. Where germination fraction was low a smaller fraction of the population was exposed to Y in the first year, hence the probability of extinction of the Z resistance allele was reduced and the frequency of Z resistance was higher (Fig. 5E and H versus Fig. 5B).

For all plant types the transition from single resistance to double-resistance occurred at marginally larger treatment area under the threshold strategy than under the rotation strategy (Figs 4C, 5C, F and I).

Under all strategies the transition to increased levels of resistance occurred at marginally larger areas for self-pollinating plant types than for outcrossing plant types (Fig. 4A–C versus 5A–C and 5D–F versus 5G–I). This is due to the decreased incidence of heterozygous-resistant individuals in populations of self-pollinated plants.

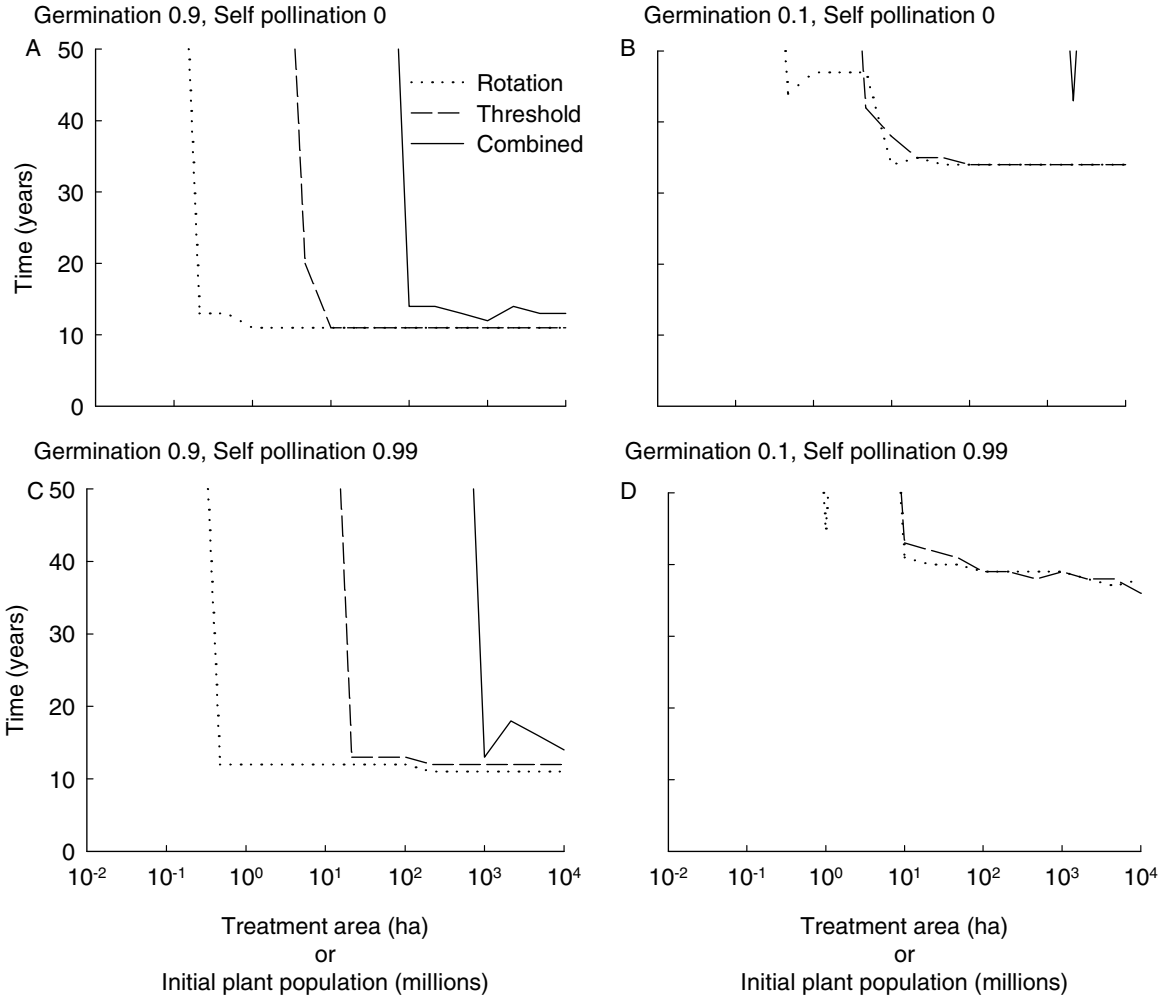

Fig. 6 Year of the time series where the average size of the weed seed pool for all replicate simulation runs exceeded 1000 seeds m^{-2} versus treatment area (≅ initial population size) for the three patterns of herbicide application for the four plant types in separate figures.

The timing of appearance of large populations (indicating the development of resistance) differed markedly between the plant types with high and low germination fractions (Fig. 6). The time taken for the seedbank to exceed 1000 seeds m^{-2} was approximately three times greater in low germination fraction types than in the high germination fraction types (Fig. 6A versus 6B and 6C versus 6D). Where germination fraction is lower a smaller proportion of the population is exposed to herbicides in each year, effectively slowing the rate of resistance evolution in those populations.

At small treatment areas, where resistance generally did not develop, the mean populations never exceeded 1000 seeds m^{-2}. The minimum treatment area where the seed pool did exceed this value was lowest where herbicides were used in rotation and highest where they were used in combination.

For large treatment areas for all plant types the increase in seedbank was equally rapid for the rotation and threshold strategies. In the high germination fraction populations the increase in plant population was only marginally slower where herbicides were used in combination (Fig. 6A and C). In low germination fraction populations the mean seed pool generally did not reach 1000 seeds m^{-2} under the combination strategy (Fig. 6B and D).

Discussion

The importance of population size in relation to rare genes

The first major conclusion from this study is that minimizing the effective weed population size substantially decreases the rate of evolution of herbicide resistance. Clearly, the smaller a population is, the less likely it is that rare resistance genes will be present in the population. Even where resistance genes do occur at low frequencies in small populations, stochastic demographic processes are more likely to result in the extinction

of these genes. When considering two resistance genes, as we have simulated here, the probability of both genes occurring in the same individual is orders of magnitude smaller again. Management practices that effectively segregate weed populations into smaller, genetically isolated units will, therefore, result in a lower incidence of herbicide resistance.

The degree to which weed populations can be segregated and contained will depend in large part on the movement of genes in pollen and seeds or propagules. Gene flow in pollen is something over which land managers have little or no control. However, Maxwell (1992) showed that only 7% of pollination events occurred at distances greater than 1 m in diclopfop-methyl-resistant *Lolium multiflorum* Lam. Rieger *et al.* (2002) have shown that pollen-mediated gene flow from herbicide-resistant to non-herbicide-resistant oilseed rape (*Brassica napus* L.) crops does occur over considerable distances, but only at very low frequencies. Where a weed species has a high degree of outcrossing and viable pollen can travel long distances from source populations, the goal of genetic isolation may be unattainable.

Gene flow by seed movement is a factor that land managers can influence because for many agricultural weeds farm management largely dictates mobility of the seed. Through strict farm hygiene, land managers can limit the importation of weed seed to a farm, the movement of seed between fields on a farm and movement of seed within fields. This may involve improved screening of seed and fodder brought on to the farm to ensure it is free of weeds; the cleaning of machinery between segregated areas; and the catching of weed seeds during the grain harvest so that they are not redistributed. The situation is analogous in many ways to that of a newly invading weed species, except that in this case the 'invading weed' is not visually distinguishable from the existing weed population. Management strategies that reduce rate of movement of the weed would be expected to increase the time until herbicide resistance becomes a problem in areas where resistant individuals were initially absent.

The importance of pattern of herbicide application

A second major conclusion is that rotation of herbicides, commonly recommended as a strategy to delay the development of herbicide resistance in weeds (Powles & Shaner, 2001), is markedly inferior to the use of herbicides in combination, and is not superior to an 'expend and swap' approach typified by the threshold strategy discussed here. This conclusion is contingent on the validity of the first conclusion and on the assumptions made, i.e. that both herbicides achieve efficacy that is high enough to ensure 'redundant kill' (are used at full rates), are not subject to linkage disequilibrium, are not associated with fitness costs and have different modes of action (no cross-resistance).

For large effective areas (population size) there is very little effect of pattern of herbicide application. However, for effective areas less than 100 ha, there was a marked advantage in using the 'combination' strategy in all scenarios tested here. The range of initial populations for which the combination strategy was superior varied markedly with germination fraction and degree of self-pollination in the weed, but it is likely that an effective initial population within this range is achievable for most agricultural weeds, particularly where initial population density is low and where the weed is self-pollinated. The utility of pesticide mixtures as opposed to a threshold approach has been similarly demonstrated for insecticide resistance management (Mani, 1985). However, Comins (1986) qualifies this 'redundant kill' strategy by indicating that it will only be effective where population size is small. The results presented in this paper agree with these conclusions.

It must be noted that the combination strategy has disadvantages that should be considered before deciding to use it in practice. Two of these disadvantages are the cost and the possible ecological implications of using more herbicide. Furthermore, the cost of using more herbicide is immediate, while the returns from delayed occurrence of herbicide resistance will be realized in the future and hence, by classic economic theory, will be reduced in terms of present value.

Another factor that must be considered is that the combination strategy requires farmers to apply two herbicides to control a weed even when the density of that weed is extremely low. Such a strategy could be considered counter intuitive. However, in Western Australia, where many farmers have first hand experience with herbicide resistance in weeds which are difficult to control, a form of the combination strategy is currently recommended to delay the occurrence of glyphosate resistance (Neve *et al.*, 2003).

Previous modelling of herbicide use strategies that have assumed infinite population sizes (which are analogous to the largest population sizes simulated here) have considerably underestimated the benefits of herbicide mixtures and sequences as management tools to prevent or delay herbicide resistance. Such models do not account for the strategically important possibility of local extinction of rare alleles. While the methodology we have used does not explicitly simulate spatial processes, the results do indicate that a quantitative understanding of the spatial dynamics of resistance genes is very important to a fuller understanding

of evolution of herbicide resistance. Improved quantitative understanding of the spatial dynamics could be achieved by embedding a model similar to the one presented here in a spatial framework such as that presented by Richter *et al*. (2002) for the one gene case. With the model presented here it appears highly likely that the rate of development of herbicide resistance can be limited by reducing movement. Furthermore, where movement can be limited, a strategy that uses herbicides in combination would be superior to rotation of herbicides in terms of rate of evolution of resistance.

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