THE ROYAL SOCIETY

Resistance management: the stable zone strategy

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The different strategies of insecticide resistance management that have been formulated so far consist of delaying the appearance and spread of resistance genes. In this paper, we propose a strategy that can be used even if resistance genes are already present. This strategy consists of applying insecticides in an area smaller than a certain critical size, so that gene flow from the untreated area, combined with the fitness cost of the resistance genes, prevents its frequency reaching high equilibrium value. A two-locus model was analysed numerically to determine population densities at equilibrium as a function of selection coefficients (insecticide selection, fitness costs of resistance genes and dominances), gene flow and size of the treated area. This model indicates that there is an optimal size for the treated area where a minimal and stable density reach equilibrium, and where resistance genes cannot invade. This resistance management strategy seems applicable to a large variety of field situations, but eventually it may encounter obstacles due to a modifier which reduces the fitness costs of resistance genes.

Keywords: insecticide; pest; density; resistance gene; control

1. INTRODUCTION

Resistance to xenobiotics is one of the major problems faced by pest control programmes in the last 40 years. Therefore, 'resistance management' has received much attention, and numerous strategies have been proposed to delay the appearance of resistance (see Denholm & Rowland (1992) for a review). These different strategies have mainly been evaluated and compared using mathematical models with few actual field tests.

Modelling resistance management strategies requires the description of both resistance gene frequencies and population densities, through time and space. As inheritance of resistance has usually been found to be controlled by a few major genes (Mallet 1989; McKenzie & Batterham 1994), most models have focused on one or two loci. These models consider several parameters; the fitness of the different genotypes in the presence and absence of insecticides, gene flow, population density regulation and spatial considerations. To our knowledge, no model of resistance management has been published which takes into account all of these factors simultaneously. The most common simplifications are (i) to ignore gene flow or its geographic scale; (ii) to ignore the scale of the treated and untreated areas relative to the scale of gene flow; and (iii) to ignore the cost of resistance, i.e. the lower fitness of resistant genotypes in the absence of insecticides (table 1). However, in some circumstances, omitting one or several of these factors can lead to valid conclusions. The simplest case would be the absence of a large untreated area relative to the scale of gene flow (i.e. the range of gene flow may be larger than the size of the untreated area). Yet, these simplifications give a pessimistic view of resistance evolution, since the end result of resistance dynamics in this case is the fixation of resistance genes. As a consequence, most of the strategies were formulated with the only aim of delaying the spread of resistance genes.

Delaying the increase in frequency of resistance genes does not necessarily achieve the best results when managing resistance. Fitness costs associated with resistance genes in the absence of insecticides may prevent this fixation in some realistic situations. Therefore, a more optimistic goal of management could be to maintain resistance genes at low and stable frequencies, especially when such genes are already present and no alternative pesticides are available.

The entry of susceptible individuals into the treated area (from refuges or artificial releases), or the escape from insecticide exposure within the treated area, have been considered in most models of resistance management. There is a consensus that gene flow slows down the development of resistance in these circumstances. From a population genetics point of view, resistance to xenobiotics is a local adaptation process, whereby a migration–selection balance is established between areas differing in the fitness associated with the different alleles. This model was developed during the mid 1970s (Nagylaki 1975; Slatkin 1973), but has received little attention in resistance modelling. The only exception is the model presented by Comins (1977); however, fitness costs associated with resistance genes were not included and therefore, in this case, a migration–selection balance was not reached.

The critical difference between the model presented here and those currently used for evaluating resistance management strategies is the comparison of the scale of the treated area with the scale of gene flow. In other words, a characteristic length (that depends on selection coefficients and gene flow) can be computed, that

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Table 1. *Existing models ordered chronologically*

(The various possible simplifications of each model are depicted: (1) to ignore gene flow or its geographic scale, (2) to ignore the scale of the treated and untreated area relative to the scale of gene flow, and (3) to ignore the fitness cost of resistance genes in the absence of insecticides.)

References	(1)	(2)	(3)
Comins (1977)	—	—	x
Georghiou & Taylor (1977)	x	x	—
Curtis *et al.* (1978)	x	x	x
Taylor & Georghiou (1979)	x	x	—
Wood (1981)	x	x	x
Tabashnik & Croft (1982)	x	x	—
Taylor & Georghiou (1982)	x	x	x
Curtis (1985)	x	x	x
Mani (1985)	x	x	x
Comins (1986)	x	x	—
Mani (1989)	—	x	—
Roush (1989)	x	x	—
Mallet & Porter (1992)	x	x	x
Caprio & Tabashnik (1992)	—	x	—
Curtis *et al.* (1993)	x	x	—
Tabashnik (1994)	x	x	x
Caprio (1994)	—	x	—
Alstad & Andow (1995)	x	x	x
Birch & Shaw (1997)	x	x	x
Roush (1997)	x	x	—

corresponds with the minimum size of the treated area below which the frequency of a resistance gene cannot increase. In this model, gene flow is defined as the variance of the distribution of parent–offspring distance. This is a more appropriate way to describe this process in natural populations than the more commonly used proportion of migrants.

This model was analysed for one locus and weak selection by Nagylaki (1975) using diffusion approximation. It can be briefly described as follows: let us consider a one-dimensional environment $(-\infty < x < +\infty)$ with a treated area between $-a$ and a and a single locus with a susceptible (S) and a resistant (R) allele. The fitness of the genotypes are

resistant homozygotes: $1+s\times g(x)$,
heterozygotes: $1+d\times s\times g(x)$,
susceptible homozygotes: $1-s\times g(x)$,

with

$$\begin{cases} g(x) = 1 \text{ for } -a < x < a, \\ g(x) = -\alpha^2 \text{ elsewhere,} \end{cases}$$

where d is the level of dominance $(-1 < d < 1)$, x is the position of the individual, s is the intensity of selection, and α^2 the ratio of the selection coefficients between untreated and treated areas. For codominance and $\alpha = 1$, Nagylaki (1975) showed that a frequency cline of allele R may be maintained across treated and untreated areas provided that $k > \pi/4$, where $k^2 = 2sa^2/\sigma^2$ and σ is the standard deviation of parent–offspring distance measured along one dimension. The qualitative conclusion is that if the treated area is too small relative to selection and gene flow, the resistance allele will not be maintained at equilibrium. The question is therefore to evaluate the critical size a^* of the treated area below which resistance cannot occur. In the absence of fitness costs associated with the resistance gene, a^* equals zero and resistance genes will always reach fixation if insecticides are used. In this case the management strategy consists only of delaying the fixation (Comins 1977). However, as soon as a fitness cost is present $(\alpha > 0)$, a^* is positive, allowing for a possible alternative strategy that avoids fixation of resistance genes. The stable zone strategy consists of applying a given insecticide in an area where the size is inferior to the critical size of the adaptive pocket. We investigated the relevance of this strategy for controlling the density of a pest using deterministic simulations. In particular, the influence of the different factors that can be manipulated in a resistance management strategy were considered: the size of the treated area, the dose of insecticide, the proportion of treated sites within the treated area, the quality of the environment in the treated area relative to the untreated one, the number of insecticides and their pattern of application.

2. MODEL

We used deterministic simulations to infer the allelic and density distributions at equilibrium because an analytical study is not tractable with more than one locus and requires low selection coefficients. A one-dimensional circular environment was simulated by a series of demes connected by migration. Distribution of parent–offspring distance (X) along this dimension was assumed to follow a symmetric binomial distribution $B(2t, 1/2)$ (the simplest discrete approximation of a Gaussian distribution).

$$P(X = i) = \binom{2t}{i+t} \times \left(\frac{1}{2}\right)^{t-i} \times \left(\frac{1}{2}\right)^{t+i}.$$

The variance of this distribution σ^2 equals $\epsilon^2 t/2$, where ϵ is the distance between demes. Two unrelated insecticides, acting on two loci separated by r units of recombination and each being polymorphic for a susceptible (S) and a resistance allele (R) were considered. Genotypic fitnesses were defined for locus i as

$$\begin{cases} w_{\mathrm{RR}i} = 1 - c_i \\ w_{\mathrm{RS}i} = 1 - h_{ci} \times c - h_{si} \times s_i \times g_i(x) \\ w_{\mathrm{SS}i} = 1 - s_i \times g_i(x), \end{cases}$$

where c_i and h_{ci} are, respectively, the fitness cost and dominance level associated with locus i. Parameter s_i is the fraction of the population exposed to treatment by insecticide i, and h_{si} is the dominance associated with insecticide selection for locus i (which is positively correlated with the dosage of insecticide i). The function $g_i(x)$ equals one if insecticide i is used in deme x; otherwise it is zero. Fitness values were multiplicatively combined across the two loci (i.e. there was no cross-resistance). Hard-selection was assumed, i.e. densities were reduced according to the selection. The order of processes were assumed to be reproduction–selection–migration. Demes were assumed to be re-saturated to maximal density (K) after reproduction. Densities were computed after selection (d_s), and after migration (d_m).

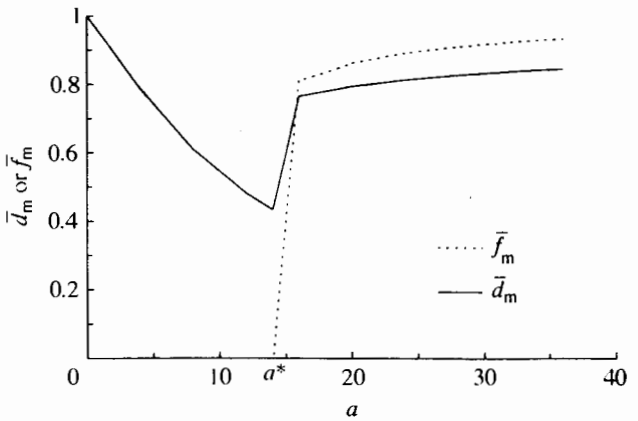

Figure 1. Average density ($\bar{d}_m$) and average resistance gene frequency ($\bar{f}_m$) after migration in a treated area of size a (km), at equilibrium. Above a threshold value a^*, resistance genes become almost fixed in the centre of the treated area and a migration–selection balance is established between treated and untreated areas. This example is with $s=0.9$, $h_s=0.75$, $c=0.1$, $h_c=0.5$ and $\sigma=6.6$ km generation$^{-1/2}$.

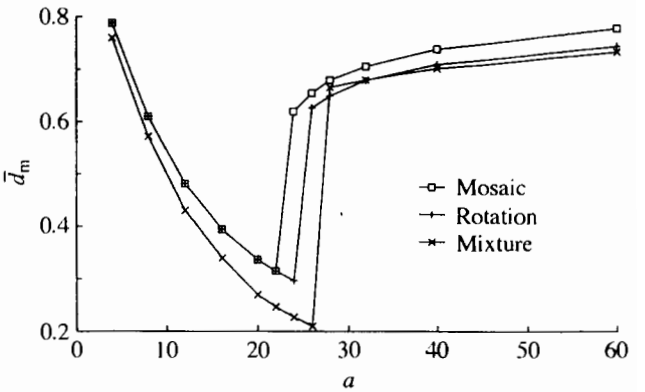

Figure 2. Average density ($\bar{d}_m$) after migration in a treated area of size a (km), at equilibrium. The treated area is subdivided into two equal sub-areas where two different insecticides are applied. In the mosaic, each sub-area always receives the same insecticide, in a rotation each sub-area alternately receives each insecticide, and in a mixture each sub-area simultaneously receives both insecticides each generation. In the example given, $s_1=s_2=0.9$, $h_{s1}=h_{s2}=0.75$, $c_1=c_2=0.1$, $h_{c1}=h_{c2}=0.5$, $r=0.5$ and $\sigma=6.6$ km generation$^{-1/2}$.

Procedures were checked using analytical results for one locus, and weak selection provided by equations 32–33 in Nagylaki (1975). First, a series of simulations were performed to determine the influence of the size of the treated area on the density and frequency of a resistance gene at equilibrium. The influence of the pattern of application of two insecticides was considered by comparing mixture, rotation and mosaic strategies. The significance of the quality of the environment was studied by setting different carrying capacity in the treated and the untreated area. Finally, a series of simulations were performed to determine the effects of insecticide dosage and of the intensity of selection, by varying the parameters h_s and s, respectively.

3. RESULTS

(a) *Single insecticide*

The size of the treated area relative to gene flow is critical for the increase in frequency of resistance genes. When the size of the treated area is less than a critical value a^*, resistance genes cannot invade. There are only susceptible individuals in the populations, therefore, the density after insecticide selection at equilibrium is proportional to the intensity of treatment ($d_s \propto 1-s$) whereas, the density after migration depends on the distance to the untreated populations. When the size of the treated area is above a^*, resistance genes increase in frequency, a selection–migration balance is established at equilibrium and the efficacy of insecticide usage is markedly reduced. Therefore, there are two opposite effects that explain the presence of an optimal treated area size for a given treatment (figure 1). A treated area with a size just below a^* will be optimal for efficient control in the long term. Resistance will not develop, even if R alleles are already present.

(b) *Multiple insecticides: mixture, rotation and mosaics*

If different insecticides associated with different resistance genes are available, and in the absence of cross-resistance (i.e. resistance to each insecticide is independent of resistance to other insecticides), the strategy can be extended to a mosaic whereby each insecticide is used in a restricted area. It is possible to determine an optimal size for each of the areas treated with a given insecticide, so that genes that are resistant to one or other insecticide cannot increase in frequency in any area. This size depends mainly on the fitness-cost associated with the corresponding resistance gene, everything else—insecticide selection and gene flow—being roughly equal. This mosaic can be further optimized (i) by avoiding the selection of closely linked resistance genes in adjacent areas (details not shown); (ii) by avoiding treating adjacent areas with insecticides for which the corresponding resistance genes show residual cross-resistance; and (iii) by changing the distribution of insecticides through time. Let us note P, the period of rotation of insecticides (i.e. time separating the application of two different insecticides in a given area) and T, the generation time. The strategy where $P/T=1/2$ is equivalent to the case where the two insecticides are applied each generation (we will call this strategy a mixture, although it is slightly different from the case where every individual is exposed to both compounds or to neither). Strategies where $P/T \geqslant 1$ correspond to rotations, and mosaics correspond to the extreme case where $P/T \to \infty$. These three strategies are the three types of resistance management which occur when different insecticides are available. Their relative performance for delaying resistance have been extensively compared under various hypotheses and in general, mixtures or rotations were judged as preferable to mosaics (Comins 1986; Curtis 1985; Mani 1985, 1989; Roush 1989, 1993; Tabashnik 1989).

In a stable zone, what is the best strategy to use? Our results indicate that the efficiency of control increases with lower P/T values (figure 2), so that a mixture should be the favourable strategy. However before taking a final decision, the slight advantage of a mixture should be

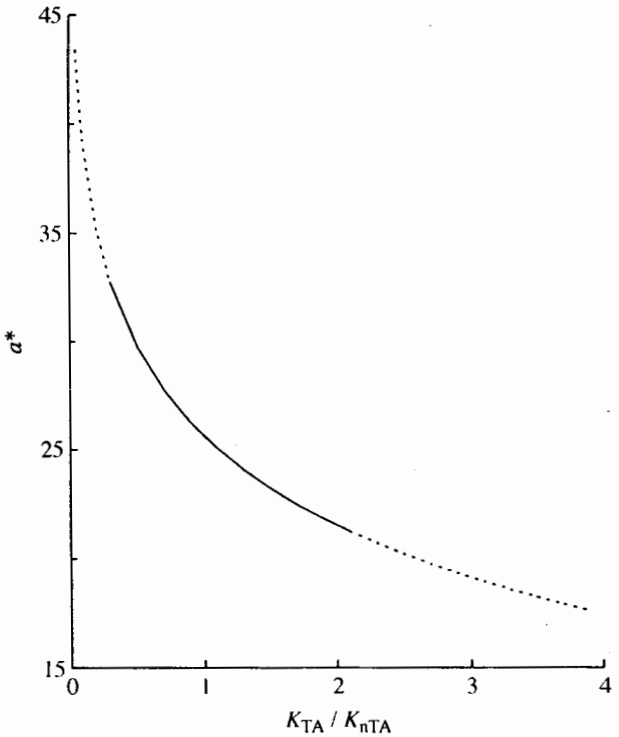

Figure 3. Critical size of the treated area (a^*) (km), as a function of the ratio of carrying capacities of treated (K_{TA}) and untreated area (K_{NTA}). Values of parameters are the same as in the case of the mixture in figure 2.

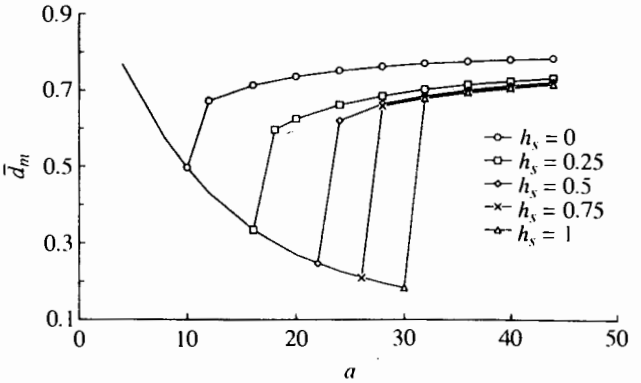

Figure 4. Average density ($\bar{d}_m$) after migration in a treated area of size a (km) at equilibrium, where two insecticides are used in a mixture (see figure 2). The example has the same values as those of the mixture in figure 2 except that h_s varies from 0 (R-gene dominant) to 1 (R-gene recessive). Variation of h_s corresponds to the variation of the effective dominance, i.e. to variations of insecticide dosage.

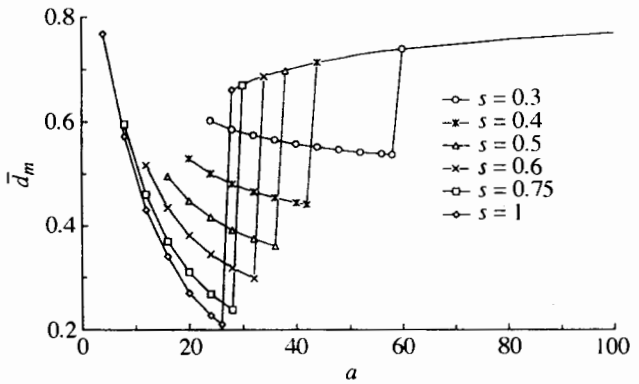

Figure 5. Average density ($\bar{d}_m$) after migration in a treated area of size a (km), at equilibrium, where two insecticides are used in a mixture (see figure 2). Again, the values are the same as those of the mixture in figure 2, except that s varies from 1 to 0.3. Variation of s corresponds to the variation of the intensity of treatment (the fraction of the population exposed to insecticide in the treated area).

weighed against the economic or ecological cost of insecticide usage (Tabashnik & Croft 1982; Tabashnik 1989), since a mixture doubles the quantity of insecticide used. Nevertheless, with the same overall insecticide usage, rotations are preferred to mosaics, although the difference between them is very slight.

(c) *Quality of the environment*

Insecticide selection in the treated area reduces the density of the pest. Any environmental manipulation that reduces the quality of the environment for the pest (i.e. the carrying capacity or its growth rate) should be viewed as the primary long-term strategy for population control, and therefore, should be recommended. However, it may be necessary to combine this with the use of insecticides. This combination has the advantage of minimizing insecticide use and reducing the population density in the treated area relative to the density in the untreated area. The principle is to make 'life' more difficult for resistance genes relative to susceptible ones: the density ratio between treated and untreated areas in fact resembles the effect of selection (Nagylaki 1978). Our results indicate that the critical size of the treated area (a^*) increases when this density ratio declines (figure 3).

(d) *Effective dominance*

In a stable zone, each resistance gene can only reach a low frequency and is therefore mainly present as a heterozygote. Therefore, an immediate improvement of the strategy would be to use a dose of a fast decaying insecticide that kills heterozygotes. This has already been suggested by many authors (Taylor 1986; Taylor & Georghiou 1979, 1982; Curtis *et al.* 1978; Wood & Mani 1981) and may be especially relevant in our situation: a^* greatly increases when h_s increases (figure 4).

(e) *Density tolerance*

The size of each area treated with a given insecticide depends on the pest density which can be tolerated. There is a long-term trade-off between full control of the pest population, and the size of the area where this pest can be controlled by an insecticide without the build-up of resistance. The size of the stable area can only be increased if the selective advantage of resistance genes is decreased through lower selection pressure (i.e a smaller fraction of the population is exposed to an insecticide in the treated area) and, consequently, poorer control of density (figure 5). If the area of insecticide application is restricted in order to prevent an increase in the frequency of resistance genes, this may prevent the control of density. If the treated area is too small relative to the scale of movement of individuals, the treated area will be invaded by a large number of individuals from surrounding untreated populations. Therefore, it is

advantageous to control the population in a larger area. By reducing s to 0.75, the strategy will permit the stable control of the population almost as efficiently as where $s=1$, and over a slightly larger area (figure 5).

4. DISCUSSION

(a) *Estimation of parameters*

A preplanned use of the stable zone strategy requires the estimation of critical sizes of treated areas. Many parameters have to be estimated to determine these critical sizes, and such estimates are notoriously difficult to make for wild populations. The genetic parameters are (i) the selective advantage of resistance genes in the presence of insecticides (and the dominance of this effect); and (ii) the selective disadvantage of resistance genes in the absence of insecticides (and dominance of this effect). The demographical and ecological parameters are (i) gene flow; and (ii) population densities in treated and untreated areas. However, the method could be operated on a trial and error basis in which the size of the differently treated areas would be increased or decreased—based on the monitoring of resistance gene frequencies. In addition, this trial and error approach may be analysed as field experiments, and may provide the data for estimating some of the parameters.

(b) *Multiple resistance gene for a same insecticide*

Although resistance is often a result of a major gene, other genes may also provide substantial resistance. In addition, positive linkage disequilibria will be generated between the resistance alleles at different loci, and each resistance allele will benefit from the selection at other loci (Slatkin 1975). In this context, epistasis as well as the different recombination rates must be considered in order to determine the critical size of treated areas. However, even if the presence of a major resistance gene can substantially increase the frequency of a minor one, the reverse is not true (Slatkin 1975).

(c) *Example:* Culex *mosquitoes in southern France*

All the above figures were computed using gene flow estimates ($\sigma=6.6\,\mathrm{km\,generation^{-1/2}}$) of *Culex pipiens* mosquitoes from southern France, and fitness costs associated with insensitive acetylcholinesterase ($c\approx 0.1$; Lenormand *et al.* (1998)). The densities and critical sizes of the treated areas are conservative, since fecundity was assumed to be high enough for the environment to be resaturated to the carrying capacity at each generation. This assumption, however, may not be true even though fecundity is very high in mosquitoes (and most other pests). In addition, this example illustrates that particular attention should be paid to the presence of barriers in gene flow. In southern France, only the coastal belt is subjected to insecticidal mosquito control. The presence of the sea along one side has an important impact since there is no influx of mosquitoes from that direction: it halves the critical size of the stable zone. In this context, a rotation involving an organophosphate insecticide and *Bacillus sphaericus* toxin could be used in two parallel 10 km belts (together approximating the actual width of the treated area). With $s_1=s_2=0.75$, $h_{s1}=h_{s2}=0.9$, $c_1=c_2=0.1$, $h_{r1}=h_{r2}=0.5$, $r=0.5$ and $\sigma=6.7\,\mathrm{km\,generation^{-1/2}}$, the population densities along the coast would be one-quarter of what they would reach if untreated, and the frequency of resistance genes would be zero at equilibrium.

There are cases where resistance has not developed despite long-term treatments (e.g. temephos resistance of *Culex pipiens* in Corsica (Raymond & Marquine 1994), or the resistance of *Ceratitis capitata* to various insecticides (Georghiou 1986)). It will be interesting to verify whether these situations correspond with the accidental implementation of a stable zone strategy. If the estimation of σ^2 of *C. pipiens* in the Montpellier area applies to Corsica, then the much smaller Corsican treated area could explain why effective temephos resistance genes (probably with high fitness costs) have still not invaded after 18 years of treatment.

From a practical point of view, the stable zone strategy can probably work in many situations, since most pest species have a high dispersal ability, allowing a relatively large optimal treated area. If the treated area required (e.g. for economic reasons) is larger than a^*, then a rotation could be implemented. Much effort has been devoted to the identification of resistance genes at the physiological and genetic levels. This is very useful in the context of the stable zone strategy, as monitoring resistance genes could be an efficient way of adjusting the size of the treated area to closer to its optimal size. However, little effort has been devoted to estimation of gene flow in pest species, a parameter which is crucial for an initial estimate of a^*.

On the assumptions so far, once a stable zone strategy is implemented, resistance genes would not be able to invade and an insecticide could be used for an indefinite period. Unfortunately, this is not the end of the story, as fitness modifiers can occur and invade (e.g. McKenzie & O'Farrell 1993). If these modifiers only slightly decrease the fitness cost, the value of a^* would be reduced, and the treated area would have to be reduced accordingly. If the fitness cost is completely suppressed by the modifier gene, then resistance genes would invade and new insecticides would have to be used. However, the selection of a modifier is slowed down by a low frequency of R alleles. The goal of well-known management strategies aims to delay the occurrence of R genes, but they are ineffective when these R genes become frequent. The goal of the stable zone strategy is to efficiently manage the frequency of R genes, so as to delay the occurrence of fitness modifiers. However, evolution toward lower fitness cost will not be prevented: the efficiency of this method will therefore decrease in the long term. We can only state that it will greatly delay the need for new compounds. Resistance management is a 'red queen' race where we need to be able to replace insecticides faster than resistance evolves. Slowing down the rate of adaptation of the pest's genome to below that of the discovery rate of new compounds, should be the fundamental goal of management and it must be planned on a long-term basis.

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