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ORIGINAL ARTICLE

Analysis of sustainable pest control using a pesticide and a screened refuge

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caged refuge, insecticidal crops, non-recessive resistance, pest control, population genetics, refuge, resistance evolution, screened refuge, sustainable, transgenic.

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Abstract

We describe and analyze a 'screened refuge' technique for indefinitely sustaining control of insect pests using transgenic pesticidal crops or an applied pesticide, even when resistance is not recessive. The screen is a physical barrier that restricts pest movement. In a deterministic discrete-time model of the use of this technique, we obtain asymptotic analytical formulas for the two important equilibria of the system in terms of the refuge size and the pest fitnesses, mutation rates, and mobility out of and into the refuge. One of the equilibria is stable and is the point at which the pest population is controlled. The other is a saddle whose stable manifold bounds the basin of attraction of the former: its location provides a measure of the tolerance of the control mechanism to perturbations in the resistant allele density.

Introduction

Blanket use of very strong pesticides is counterproductive to pest control because it can cause resistance to develop rapidly in the pest population (Georghiou 1986), rendering the pesticide useless. This applies in particular to the use of transgenic crop varieties that have been genetically engineered to produce proteins originating in the bacterium *Bacillus thuringiensis* (Bt) (Schnepf *et al.* 1998). These transgenic plants are highly toxic to insect pests, and are believed to be relatively harmless to humans and most nontarget species (Peairs 2007). The perceived benefits of their use include reduced reliance on sprayed pesticides (Ervin 2010) that run off into the environment and pose direct hazards to farm workers (Cattaneo 2006). A desire to preserve their usefulness for as long as possible has led the United States Environmental Protection Agency (USEPA) to mandate a 'high-dose/refuge' (HDR) technique for the use of Bt crop varieties (EPA 1998; FIFRA 1998; Gould 1998). 'High-dose' means the plant is engineered to produce a dose of the toxin orders of magnitude greater than what is required to kill all nonresistant pests. The 'refuges' are stands of either conventional nontoxic plants the farmer is required to grow interspersed among the toxic plantings, or noncrop hosts naturally occurring nearby. The role of the refuges is to lower the selection pressure favoring alleles that confer resistance to the pesticide (Andow 2008).

On the basis of mathematical analysis, simulations, and actual experiments (Andow 2008; Tabashnik 2008; Shelton *et al.* 2000), the HDR technique is believed to be potentially effective in at least delaying resistance evolution if the dose is high enough to render any gene for resistance functionally recessive (Liu and Tabashnik 1997; Gould 1998). The possibility of controlling the population indefinitely without resistance developing is a less settled issue (Lenormand and Raymond 1998; Carrière and Tabashnik 2001; Andow 2008). It is clear, in any case, that the movement of pests is a key factor and a delicate one: pest mobility can have multiple and competing effects, so that refuge efficacy can be a nonmonotonic function of the mobility or, equivalently, of the degree of fine-graining of the refuge arrangement, as seen in Fig. 3b of Sisterson *et al.* 2005. Coarse-graining on the one hand

enhances the ability of the refuges to protect susceptible pests in the cores of the refuges, but on the other hand makes parts of the toxic crop more remote from those sources of susceptible pests. While there appears to be some consensus among modelers that a degree of coarse-graining is beneficial in delaying control failure (Mallet and Porter 1992; Shelton *et al.* 2000; Caprio 2001; Sisterson *et al.* 2005), the idea of extreme fine-graining has not been abandoned (Griekspoor 2009; and see also the discussion of Fig. 1B of the current paper).

Pest movement also plays a key role in the technique that is the focus of this paper: we investigate what happens when pest movement between refuge and toxic crop is deliberately restricted by a physical barrier. We will refer to this idea as the 'screened refuge' technique, and we show that with sufficient fine-graining to justify the idealization of complete homogenization of the pest population within the refuge and toxic crop patches separately, the screened refuge technique can suppress the development of resistance indefinitely, and do it even when resistance is not highly recessive – the case where the usual 'open' refuge technique may fail. The nonrecessive case will be of interest if very high concentrations of insecticides in the crop were to be opposed by consumers or governments, or if a resistant pest strain were to arise with partial dominance for all technically achievable pesticide concentrations. In fact, Tabashnik *et al.* 2009 cite evidence of nonrecessive resistance to Bt crop varieties in two major lepidopteran pests.

It has long been recognized that resistance in a population can be prevented indefinitely by the continual migration from a source where selection pressure for resistance is absent (Haldane 1930; Comins 1977; Taylor and Georghiou 1979; Lenormand and Raymond 1998; Gomulkiewicz *et al.* 1999; Carrière and Tabashnik 2001; Vacher *et al.* 2003; Carrière *et al.* 2005). Actively providing a stream of such individuals has been proposed recently in the context of transgenic insecticidal crops by Alphey *et al.* 2007. A screened refuge, as proposed here, serves as a (passive) source of susceptible pests similar to that considered in the works cited above, and we provide what we believe are the first analytical results for the case where both the source (refuge) and the crop are finite, migration is bi-directional, and mutation and fitness costs of resistance in the refuge are included. We obtain asymptotic analytical formulas for the two important equilibria in a maximally simplified model of such a system. These formulas provide a quantitative relationship between the numerous parameters and the equilibrium pest densities that can be maintained, as well as a measure of the robustness of that equilibrium to perturbations in the prevalence of resistance.

How refuges work, or fail to

To establish context and make our level of idealization clear, we begin by illustrating quantitatively some basic principles of refuge functionality under the assumption that the adult pests are mobile enough prior to mating to homogenize the population, except where restricted by a physical barrier (the screen). In our models, we assume that in a diploid pest there is a single genetic locus for resistance to the toxin, with susceptible (S) and resistant (R) alleles. Generations do not overlap, and mating and egg deposition are random. In the very simple model we use in this section, the life cycle consists simply of reproduction and poisoning. The average number of offspring per individual surviving to adulthood in the absence of poisoning is denoted by F (for fecundity). Using N_R and N_S to denote the densities of R and S alleles respectively, reproduction is represented by the map

$$\mathrm{rep}\left(\begin{bmatrix} N_R \\ N_S \end{bmatrix}\right) = \begin{bmatrix} FN_R \\ FN_S \end{bmatrix}. \tag{1}$$

The genotype densities are obtained from the allele densities using the Hardy–Weinberg formulas for random mating (Hartl and Clark 2006):

$$\begin{bmatrix} N_{RR} \\ N_{RS} \\ N_{SS} \end{bmatrix} = \mathrm{typ}\left(\begin{bmatrix} N_R \\ N_S \end{bmatrix}\right) \equiv \begin{bmatrix} \frac{N_R^2}{N_R+N_S} \\ \frac{2N_RN_S}{N_R+N_S} \\ \frac{N_S^2}{N_R+N_S} \end{bmatrix}, \tag{2}$$

If ρ is the fraction of the total crop area allotted to open refuge, and if we assume there is full mixing of the adult pests between the open refuge and the toxic crop during mating, then the net rates of poisoning survival are (Carrière and Tabashnik 2001):

$$\begin{bmatrix} W_{RR} \\ W_{RS} \\ W_{SS} \end{bmatrix} = (1-\rho)\begin{bmatrix} W_{RR}^{tox} \\ W_{RS}^{tox} \\ W_{SS}^{tox} \end{bmatrix} + \rho\begin{bmatrix} W_{RR}^{ref} \\ W_{RS}^{ref} \\ 1 \end{bmatrix}, \tag{3}$$

where 'tox' superscripts on the survival rates (W's) denote values in the toxic crop, and 'ref' superscripts those in the refuge. In terms of these rates, the selection map is simply

$$\mathrm{sel}\left(\begin{bmatrix} N_{RR} \\ N_{RS} \\ N_{SS} \end{bmatrix}\right) \equiv \begin{bmatrix} W_{RR}N_{RR} \\ W_{RS}N_{RS} \\ W_{SS}N_{SS} \end{bmatrix}. \tag{4}$$

We then revert to allele densities:

$$\begin{bmatrix} N_R \\ N_S \end{bmatrix} = \text{all}\left(\begin{bmatrix} N_{RR} \\ N_{RS} \\ N_{SS} \end{bmatrix}\right) \equiv \begin{bmatrix} N_{RR} + \frac{1}{2}N_{RS} \\ N_{SS} + \frac{1}{2}N_{RS} \end{bmatrix}, \quad (5)$$

The overall dynamical map for this simple model is thus

$$\text{dyn}\left(\begin{bmatrix} N_R \\ N_S \end{bmatrix}\right) \equiv \text{all} \circ \text{sel} \circ \text{typ} \circ \text{rep}\left(\begin{bmatrix} N_R \\ N_S \end{bmatrix}\right) \quad (6)$$

This model is exactly equivalent to the one used in Carrière and Tabashnik 2001 and Tabashnik *et al.* 2008.

The recessive case: adding an open refuge is successful in controlling the pest

First of all, to be explicit about the hazard we are trying to avoid, we illustrate the readily understood failure to control the pest if a strong toxin is applied throughout the entire habitat, i.e., there is no refuge at all. For concreteness, if we start with a population of mating adults in which the R-allele fraction is 0.001 (Roush 1994, Gould 1998), then in their offspring there will be about one in 1 million with RR genotype. If poisoning of the offspring is performed throughout the entire habitat at an intensity great enough to kill all SS and RS individuals, the initial result is a dramatic reduction of the population to 1 millionth of its pre-poisoning size. But the survivors are all RR and resistant to the toxin. Chance extinction of the small population of highly fit survivors is possible at this point (though not in the deterministic model, where the population is treated as a continuum rather than as a collection of discrete individuals). But if extinction is avoided, the population rebounds, growing geometrically at a rate $FW_{RR} \approx F$ that can be quite high for well-fed insects (Fig. 1A).

In contrast, if a fraction of the habitat is maintained as an 'open' refuge of nontoxic plants, the results can be dramatically better from the poisoner's point of view – again, if resistance is recessive. Assuming for simplicity that the survival fractions in the refuge are high and the same for all three genotypes, then in the fully recessive case, the survival fractions for RS and SS pests, W_{RS} and W_{SS}, (see eqn 3) will be: (i) large enough that the RR fraction of the total population is negligible even right after initial poisoning (FW_{SS} much greater than the square of the initial R-allele fraction), (ii) small enough that the net growth rates FW_{RS} and FW_{SS} are still <1 as long as the refuge is not too large, and (iii) *equal to each other*. Indeed W_{RS} can even be a little smaller than W_{SS} if some small fitness cost of resistance in the refuge is included (Carrière and Tabashnik 2001), as we have done for Fig. 1. Then, the R-allele density decays geometrically at a slightly *faster* rate than the S-allele density, as in Fig. 1B, and (in the absence of immigration from elsewhere) the pest population is driven to *extinction*, because the R-allele fraction stays below its low initial value indefinitely as the population is driven down. In this way, the open-refuge technique can be highly effective when the dose is high and the resistant allele is completely recessive. Reports of extinction in the HDR modeling literature exist – see Carrière *et al.* 2001– but are scarce.

Table 1. Parameters of our model and their values in the examples of Figs 1A–F and 5. Without loss of generality, all SS pests in the refuge are assumed to survive the poisoning phase. (Any other desired value of 'W_{SS}^{ref}' could be absorbed into the fecundity F and a rescaling of the allele densities.)

Parameter description	parameter name	1a	1b	1c	1d	1e	1f	5
RR survival fraction on toxic plants	W_{RR}^{tox}	1	1	1	1	1	1	0.95
RS survival fraction on toxic plants	W_{RS}^{tox}	0	0	0.4	0.4	0.4	0.4	0.05
SS survival fraction on toxic plants	W_{SS}^{tox}	0	0	0.3	0.3	0.3	0.3	0
RR survival fraction on refuge plants	W_{RR}^{ref}	–	0.95	–	0.95	–	0.95	0.95
RS survival fraction on refuge plants	W_{RS}^{ref}	–	0.995	–	0.995	–	0.995	0.995
Pest fecundity	F	2	2	2	2	2	2	3
Crop area	A	1	1	1	1	1	1	1
Caged refuge area	B	0	0	0	0	0	0.01	0.05
Effective aperture size crop into cage	a	–	–	–	–	–	0.001	equal to b
Effective aperture size out of cage into crop	b	–	–	–	–	–	0.001	varied
Open-refuge fraction in crop	ρ	0	0.25	0	0.15	0	0	0
Mutation rate R to S	μ_{RS}	–	–	–	–	–	5×10^{-6}	5×10^{-7}
Mutation rate S to R	μ_{SR}	–	–	–	–	–	5×10^{-6}	5×10^{-7}
Density-dependent juvenile attrition survivors	$g(N)$	–	–	–	–	–	$1-e^{-N}$	$1-e^{-N}$
Delivery density	–	–	–	–	–	0.001	–	–
Delivery R fraction	–	–	–	–	–	0.001	–	–

This is due, we believe, to (i) the complicating effects of limited pest mobility as discussed in the Introduction (as in Sisterson *et al.* 2004), and (ii) the fact that absolute population densities are not always tracked in modeling studies (as in Mallet and Porter 1992; Roush 1994). The genius of the HDR technique, as idealized here, is that it provides net toxicities for SS and RS pests that are not too large, not too small, and approximately equal, for a wide range of pest resistance to smaller toxin concentrations, thereby obviating a calibration of toxin dose for each specific resistance gene. But the technique does hinge on the ability to render resistance nearly fully recessive.

Resistance not fully recessive: open refuge fails, screened refuge succeeds

If for any reason resistance cannot be made strongly recessive, even if it is not so dominant that $FW_{RS} > 1$, the results are not so satisfactory. This case is the focus of this paper. In the absence of any open refuge, and assuming again that the R-allele is rare at the onset of poisoning, the R- and S-allele densities initially decline geometrically at rates FW_{RS} respectively, now different from each other. With $FW_{SS} < FW_{RS}$, S catches up with R as they both decrease, as illustrated in Fig. 1C. At that point a significant fraction of the R allele in the popula-

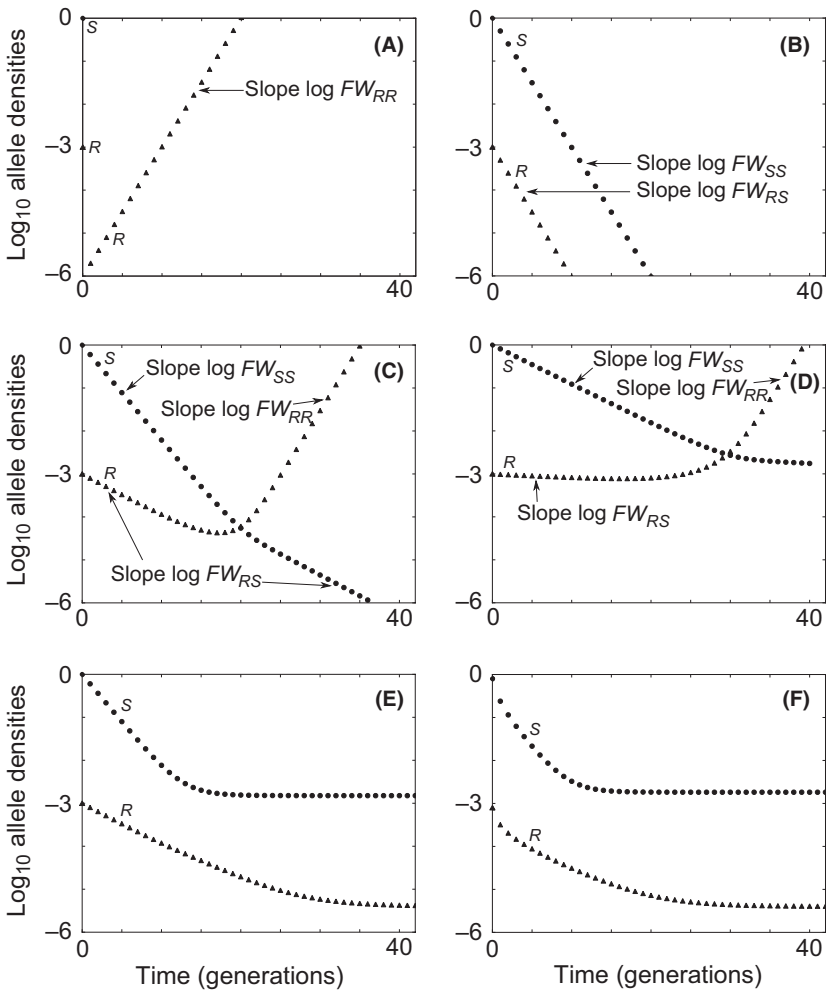

Figure 1 Illustration of basic ideas of refuge techniques. Parameter values are given in Table 1. (A) Applying very strong poison everywhere does not work. (B) Adding an open refuge is highly effective when resistance is recessive. (C) The population evolves resistance when resistance is not recessive and dose is not extremely high. (D) Adding open refuge is not very helpful in the case depicted in (C). (E) Active delivery at 0.1% of carrying capacity staves off resistance indefinitely for the parameters of (C). (F) A screened refuge occupying 1% of the land works successfully like active delivery in the non-recessive case: model of the next section, parameters the same as in (c), except that mutation is added to show the technique is not reliant on an absence of S to R mutation.

tion is carried by highly resistant RR individuals, and the R-fraction subsequently rises rapidly toward one and the population density rises rapidly to carrying capacity.

Moreover, in this nonrecessive case, the addition of an open refuge may not be very helpful, producing mere modest delays in resistance development as shown in Fig. 1D, or even actually accelerating resistance evolution (Mohammed-Awel *et al.* 2007). The reason is that although adding open refuge decreases the selective differential (Gould 1998) between RS and SS, hence delaying the time when S density becomes less than R density, it also increases the fitness of both RS and SS, so the population does not decline so low before it rises again.

A potentially effective alternative to the refuge technique that should work in the nonrecessive case, and that has been investigated previously, e.g. in Taylor and Georghiou 1979 and Alphey *et al.* 2007, is the continual *active release* of a supply of pests from a source where there is no selection pressure favoring the resistant allele. The effect of continual active release, shown in Fig. 1E, is that even if the R- and S-allele density trajectories are initially on a collision course (as in Fig. 1C) leading toward predominance of resistance, the collision is avoided because the delivery of (mostly susceptible) pests causes both curves to flatten out and remain well separated indefinitely, and at low levels if the delivery amount is small. This is the equilibrium described in Comins 1977 for the case of an infinite unpoisoned region.

A possible *passive* alternative to active release, that we investigate here, is to use a screened refuge. The idea is to emulate active release of mostly susceptible pests by shielding a refuge population from decimation and R-selection on the toxic crop, yet at the same time allowing sufficient numbers of susceptible pests to move to suppress resistance development in the toxic crop. Figure 1F shows an example of a screened refuge working in this way in the model that is analyzed in the remainder of this paper. The screened refuge can be viewed as implementing the conclusions of Caprio 2001; Ives and Andow 2002; Vacher *et al.* 2003; and Sisterson *et al.* 2005 on benefits of reducing pest mobility between refuge and toxic crop, while at the same time avoiding the isolation of some toxic regions found to be harmful when refuges are too far apart.

When the screening is extreme, meaning that the flow of pests between the crop and the refuge is heavily restricted, it may be more apt to describe the refuge as enclosed in a 'cage' (Fig. 2). In the following two sections, we obtain approximate analytical formulas for the equilibria in a maximally simplified model of the caged refuge mechanism, gaining quantitative information about how the many parameters of the problem determine the steady state population levels and about the robustness to perturbations of the steady state that corresponds to a sustainably controlled population. Unlike in previous studies of equilibria in related models we do not ignore pest flow back to the immigrant source (the cage)(Taylor and Georghiou 1979), nor fitness costs of resistance (Comins 1977).

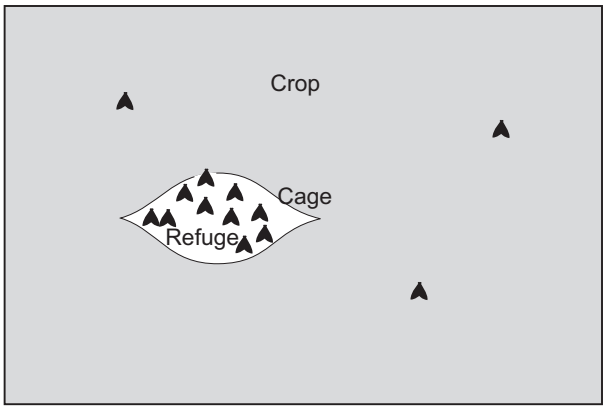

Figure 2 Pesticidal crop and a nontoxic refuge enclosed in a cage. Pests can leave and enter the refuge only through a gap in the cage.

Model of a toxic crop with a refuge in a cage

We now present the full model that we analyze in the sequel. As illustrated in Fig. 3, the same cycle of processes occurs in the crop and the cage (though the selection parameters are different). Interaction of crop and cage populations occurs only in an exchange phase marked Exc in the figure.

As in How refuges work, or fail to, allele densities are denoted by N_R and N_S respectively. Reproduction is represented by the map rep defined in the previous section (eqn 1). Population sizes are limited by a density-dependent process att, occurring at early juvenile stages (Vacher

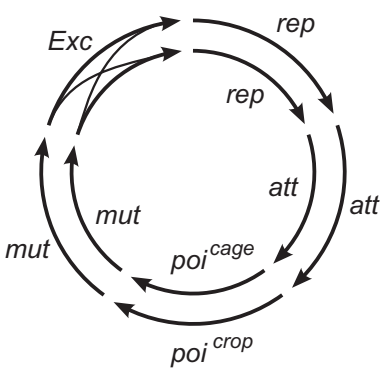

Figure 3 The model: a cycle of reproduction (rep), density-dependent genotype-neutral attrition (att), genotype-dependent poisoning (poi), mutation (mut), and exchange (Exc) between the crop (outer circle) and the refuge in a cage (inner circle).

et al. 2003), which is genotype-neutral, i.e., the densities of all genotypes change by the same proportion and the change depends only on the total number of individuals:

$$\mathrm{att}\left(\begin{bmatrix} N_\mathrm{R} \\ N_\mathrm{S} \end{bmatrix}\right) = \frac{g(N_\mathrm{R}+N_\mathrm{S})}{N_\mathrm{R}+N_\mathrm{S}} \begin{bmatrix} N_\mathrm{R} \\ N_\mathrm{S} \end{bmatrix}, \tag{7}$$

(and $\mathrm{att}([0,0]^\mathrm{T}) = [0,0]^\mathrm{T}$). The function g, which gives the density of survivors in terms of the density prior to attrition, can have rather general form (piecewise smooth is sufficient), save for the restrictions that $g(0) = 0$ and $g'(0) = 1$, so that $\mathrm{att}\left(\begin{bmatrix} N_R \\ N_S \end{bmatrix}\right) \approx \begin{bmatrix} N_R \\ N_S \end{bmatrix}$ in the low population limit. This encompasses all the following models: Ricker, $g(N) = N \exp(-cN)$ (Ricker 1954); Beverton-Holt, $g(N) = N/(1+cN)$ (Caswell 2001); Hassell, $g(N) = N/(1+cN)^b$ (Hassell 1975), and logistic, $g(N) = (1-cN)N$ (Hartl and Clark 2006). For the numerical illustrations in this paper, we choose $g(N) = 1 - \exp(-N)$ (Mohammed-Awel *et al.* 2007).

Genotype-specific density-independent attrition is represented by maps of the form all∘sel∘typ as defined in the previous section (eqns 2, 4 and 5) but the survival fractions are different in the crop and the cage:

$$\mathrm{sel}^\mathrm{crop}\left(\begin{bmatrix} N_\mathrm{RR} \\ N_\mathrm{RS} \\ N_\mathrm{SS} \end{bmatrix}\right) \equiv \begin{bmatrix} W_\mathrm{RR}^\mathrm{crop} N_\mathrm{RR} \\ W_\mathrm{RS}^\mathrm{crop} N_\mathrm{RS} \\ W_\mathrm{SS}^\mathrm{crop} N_\mathrm{SS} \end{bmatrix},$$

$$\mathrm{sel}^\mathrm{cage}\left(\begin{bmatrix} N_\mathrm{RR} \\ N_\mathrm{RS} \\ N_\mathrm{SS} \end{bmatrix}\right) \equiv \begin{bmatrix} W_\mathrm{RR}^\mathrm{cage} N_\mathrm{RR} \\ W_\mathrm{RS}^\mathrm{cage} N_\mathrm{RS} \\ N_\mathrm{SS} \end{bmatrix}. \tag{8}$$

In Fig. 3, we use the shorthand $\mathrm{poi}^\mathrm{crop}$ and $\mathrm{poi}^\mathrm{cage}$ for the respective *all*∘sel∘typ compositions:

$$\mathrm{poi}^{\mathrm{crop,cage}}\left(\begin{bmatrix} N_\mathrm{R} \\ N_\mathrm{S} \end{bmatrix}\right) \equiv \mathrm{all} \circ \mathrm{sel}^{\mathrm{crop,cage}} \circ \mathrm{typ}\left(\begin{bmatrix} N_\mathrm{R} \\ N_\mathrm{S} \end{bmatrix}\right) \tag{9}$$

We have chosen a life history in which density-dependent attrition occurs before poisoning, as in Vacher *et al.* 2003. It turns out that reversing the order of density-dependent attrition (att) and poisoning (poi), as in Alstad and Andow 1995; Ives *et al.* 1996, makes no difference to our asymptotic results. (The maps effectively commute because in each habitat section one of them has little effect: att in the crop, and poi in the cage.)

Next, we apply mutation using

$$\mathrm{mut}\left(\begin{bmatrix} N_\mathrm{R} \\ N_\mathrm{S} \end{bmatrix}\right) \equiv \begin{bmatrix} 1-\mu_\mathrm{RS} & \mu_\mathrm{SR} \\ \mu_\mathrm{RS} & 1-\mu_\mathrm{SR} \end{bmatrix} \begin{bmatrix} N_\mathrm{R} \\ N_\mathrm{S} \end{bmatrix}, \tag{10}$$

where μ_RS and μ_SR are the mutation rates from R to S and from S to R, respectively.

Finally, we account for exchange of mating adults between the crop and the cage. We assume that pest exchange occurs through some kind of apertures, and that the flux of alleles from one side to the other is proportional to the allele density on the source side. We call the constants of proportionality the effective outgoing aperture sizes (although they include a factor intrinsic to the pest, reflecting its mobility) and denote them by a (crop to cage) and b (cage to crop). We imagine that for an aperture like a simple hole in a net, the effective aperture size might be the same in both directions, i.e. $a = b$, but we allow them to be different because the greater generality does not make the calculations any more difficult. We denote the sizes (areas) of crop and the cage by A and B, respectively. An allele flux of aN^crop from the crop into the cage decreases the allele density in the crop by amount aN^crop/A and increases the density in the cage by amount aN^crop/B. Thus, the exchange map is

$$\mathrm{Exc}\left(\begin{bmatrix} N_\mathrm{R}^\mathrm{crop} \\ N_\mathrm{S}^\mathrm{crop} \\ N_\mathrm{R}^\mathrm{cage} \\ N_\mathrm{S}^\mathrm{cage} \end{bmatrix}\right) \equiv \begin{bmatrix} 1-\frac{a}{A} & 0 & \frac{b}{A} & 0 \\ 0 & 1-\frac{a}{A} & 0 & \frac{b}{A} \\ \frac{a}{B} & 0 & 1-\frac{b}{B} & 0 \\ 0 & \frac{a}{B} & 0 & 1-\frac{b}{B} \end{bmatrix} \begin{bmatrix} N_\mathrm{R}^\mathrm{crop} \\ N_\mathrm{S}^\mathrm{crop} \\ N_\mathrm{R}^\mathrm{cage} \\ N_\mathrm{S}^\mathrm{cage} \end{bmatrix} \tag{11}$$

The allele densities after one full generation are then given by the following composition of the maps described above:

$$\mathrm{Dyn}\left(\begin{bmatrix} N_\mathrm{R}^\mathrm{crop} \\ N_\mathrm{S}^\mathrm{crop} \\ N_\mathrm{R}^\mathrm{cage} \\ N_\mathrm{S}^\mathrm{cage} \end{bmatrix}\right) \equiv \mathrm{Exc}\left(\begin{bmatrix} \mathrm{mut} \circ \mathrm{all} \circ \mathrm{sel}^\mathrm{crop} \circ \mathrm{typ} \circ \mathrm{att} \circ \mathrm{rep}\left(\begin{bmatrix} N_\mathrm{R}^\mathrm{crop} \\ N_\mathrm{S}^\mathrm{crop} \end{bmatrix}\right) \\ \mathrm{mut} \circ \mathrm{all} \circ \mathrm{sel}^\mathrm{cage} \circ \mathrm{typ} \circ \mathrm{att} \circ \mathrm{rep}\left(\begin{bmatrix} N_\mathrm{R}^\mathrm{cage} \\ N_\mathrm{S}^\mathrm{cage} \end{bmatrix}\right) \end{bmatrix}\right) \tag{12}$$

with the census taken just prior to mating ('12 o'clock' in Fig. 3).

Results: formulas for the equilibria of the dynamical map

Numerically, we have observed that for a wide range of parameter values, the dynamical system Dyn has four equilibria in the relevant region of the state space where the allele densities are nonnegative. A 2D caricature of the situation, with the cage variables 'projected out', is given in Fig. 4. The true picture is in the 4-dimensional state space $\{[N_\mathrm{R}^\mathrm{crop}, N_\mathrm{S}^\mathrm{crop}, N_\mathrm{R}^\mathrm{cage}, N_\mathrm{S}^\mathrm{cage}]\}$, but it is

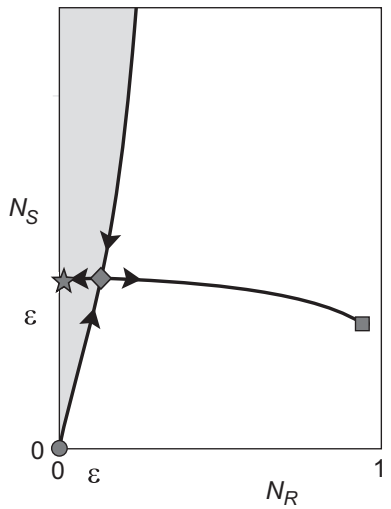

Figure 4 Schematic depiction of the equilibria of the model (12) and the basins of attraction of the 'control' equilibrium (★) and the 'failure' equilibrium (■). In this paper, analytical formulas are provided for the control equilibrium and the 'separator' equilibrium (♦). ϵ refers to the small parameter in our asymptotic analysis.

analogous in terms of the dynamical roles played by the four equilibria. One of the equilibria is the extinction state (no pests of any type anywhere), and this is unstable for parameter values of interest. In the figure, the extinction state is marked by the ●. Another equilibrium is a stable one with a large, predominantly resistant, population in both crop and refuge, a state which corresponds to agricultural disaster. We call this the 'failure' state (■ in Fig. 4).

The remaining two equilibria are the focus of this paper. One is a stable, low-population, predominantly susceptible state that farmers would be happy for the system to occupy, which we call the 'control' state (★ in Fig. 4). The other is also a low-population state whose stable manifold bounds the basin of attraction (shaded gray) of the control state and separates it from the basin (unshaded) of the failure state. We call this last one the 'separator' state (♦ in Fig. 4). The R-allele fraction in the crop at the separator equilibrium provides a measure of the robustness to perturbations of the control state, i.e. how small of an injection of R allele could knock the system out of the basin of attraction of the control state.

It is not possible to obtain *exact* formulas for the control state and the separator state in terms of the parameters. However, we have found that asymptotic approximations can be obtained by scaling the parameters as follows:

$$\mu_{RS} \sim \hat{\mu}_{RS}\epsilon^2, \quad \mu_{SR} \sim \hat{\mu}_{SR}\epsilon^2, \quad a \sim \hat{a}_{crop}\epsilon, \quad b \sim \hat{a}_{cage}\epsilon, \qquad (13)$$

$$W_{RS}^{cage} \sim 1 - \hat{W}_{RS}^{cage}\epsilon, \qquad W_{RR}^{cage} \sim 1 - \hat{W}_{RR}^{cage}\epsilon, \qquad (14)$$

and by assuming that the equilibrium values of $(N_R^{crop}, N_S^{crop}, N_R^{cage}, N_S^{cage})$ have asymptotic expansions as $\epsilon \to 0$ of the form

$$N_R^{crop} \sim \hat{N}_{R,1}^{crop}\epsilon + \hat{N}_{R,2}^{crop}\epsilon^2, N_S^{crop} \sim \hat{N}_S^{crop}\epsilon, N_R^{cage} \sim \hat{N}_R^{cage}\epsilon,$$
$$N_S^{cage} \sim N_0 - \hat{N}_S^{cage}\epsilon, \qquad (15)$$

where N_0 is a carrying capacity parameter that is explained below. Our small parameters are the mutation rates, the exchange rates between the cage and the toxic crop, and the fitness costs of resistance in the refuge. The smallness of the mutation rates is consistent with the values of $\sim 10^{-6}$ that are commonly cited, e.g. in Sisterson *et al.* 2004. Fitness costs in the refuge were detected in more than half of the experiments surveyed by Gassmann *et al.* 2009. Our assumption that these costs are not large is made to render the mathematical analysis tractable, but fairly small values such as $1 - W_{RS}^{ref} = 0.05$, $1 - W_{RR}^{ref} = 0.10$ have been considered appropriate in other modeling studies (Tabashnik *et al.* 2008, Supplementary Information). For larger fitness costs, the control and separator equilibria still exist, and the caged refuge technique works even more robustly, but we are not able to obtain compact and accurate mathematical formulas for the equilibria using the methods of this paper. The case of complete absence of fitness costs is discussed in Conclusions. We stress that neither W_{RS}^{tox} nor W_{SS}^{tox}, the fitnesses of the heterozygotes and the susceptible homozygotes in the crop, are assumed very small: we require only that the growth rates FW_{RS}^{crop} and FW_{SS}^{crop} be <1.

The parameter N_0 is the carrying capacity (density) for SS pests in a pure-refuge habitat and is determined by the attrition function g used in the density-dependent attrition map, att, and the fecundity parameter, F, which is the number of offspring per individual that survive to adulthood in the absence of poisoning and density-dependent attrition. Specifically, N_0 is the positive solution of the equation $N_0 = g(FN_0)$, meaning that it is the (positive) equilibrium population density that exists for a pure-S population in the absence of poisoning. The function g enters our calculations at leading order only through N_0 and the derivative of g at FN_0, which we call $\sigma(F)$. (We require $|F\sigma(F)| < 1$.) For example, if $g(N) = 1-exp(-N)$, and $F = 3$, then numerically we can find that $N_0 = 0.94047979...$, and $\sigma(F) = 0.059520209....$

We substitute the expressions (13–15) into the equation that defines equilibrium,

Table 2. Asymptotic approximations of the ''control'' equilibrium and the ''separator'' equilibrium. N_R and N_S are the equilibrium allele densities just prior to mating.

Allele density	Control equilibrium (★)	Separator equilibrium (♦)
N_R^{crop}	$\frac{b}{A}\frac{1}{1-FW_{RS}^{crop}}N_{R\bigstar}^{cage}$	$\frac{1-FW_{RS}^{crop}}{FW_{RR}^{crop}-1}N_{S\blacklozenge}^{crop}$
N_S^{crop}	$\frac{b}{A}\frac{1}{1-FW_{SS}^{crop}}N_0$	$\frac{b}{A}\frac{F(W_{RR}^{crop}-W_{RS}^{crop})}{(1-FW_{RS}^{crop})^2+(1-FW_{SS}^{crop})(FW_{RR}^{crop}-1)}N_0$
N_R^{cage}	$\frac{\mu_{SR}}{1-W_{RS}^{cage}}N_0$	$\frac{\mu_{SR}}{1-W_{RS}^{cage}}N_0+\frac{a}{B}\frac{1}{1-W_{RS}^{cage}}N_{R\blacklozenge}^{crop}$
N_S^{cage}	$(1-\frac{b}{B}\frac{1}{1-F\sigma(F)})N_0-N_{R\bigstar}^{cage}$	$(1-\frac{b}{B}\frac{1}{1-F\sigma(F)})N_0-N_{R\blacklozenge}^{cage}$

$$\mathrm{Dyn}\left(\begin{bmatrix}N_R^{crop}\\N_S^{crop}\\N_R^{cage}\\N_S^{cage}\end{bmatrix}\right)=\begin{bmatrix}N_R^{crop}\\N_S^{crop}\\N_R^{cage}\\N_S^{cage}\end{bmatrix}, \qquad (16)$$

and solve for the allele densities, retaining only terms of leading order in ϵ. We obtain the asymptotic approximations given in Table 2 for the control equilibrium and the separator equilibrium in terms of the parameters. We have made the formulas look as simple as possible by expressing some of the equilibrium allele densities in terms of others. The details of the calculations are omitted because they are routine and rather lengthy. As a check against calculational mistakes, the asymptotic accuracy of the approximations given in the tables was tested numerically at some 'generic' locations in the parameter space: the errors relative to the last retained term were all observed to be $O(\epsilon)$ as $\epsilon \to 0$. The asymptotic order of each term in Table 2 can be seen by substituting the scalings (13) and (14).

We can also obtain asymptotic expressions for the eigenvalues and eigenvectors of the linearized dynamical map at the control and separator equilibria. Table 3 gives the asymptotic approximations for the control equilibrium. We see that it is asymptotically stable, because all eigenvalues are <1 in absolute value as long as $FW_{SS}^{crop} < 1$, $FW_{RS}^{crop} < 1$, $W_{RS}^{cage} < 1$, and $|F\sigma(F)| < 1$. For the separator state, we can also obtain asymptotic formulas for the eigendata, and two eigenvalue-eigenvector pairs are shared with the control state, namely λ_3, v_3 and λ_4, v_4. Although we can also write down the formulas for the other two, the expressions are too complicated to be easily interpreted.

Table 3. Asymptotic formulas for the eigenvalues and eigenvectors at the control equilibrium.

Asymptotic eigenvalue	Asymptotic eigenvector
$\lambda_1 \sim FW_{RS}^{crop}$	$\vec{v_1} \sim \begin{bmatrix}1\\1\\0\\0\end{bmatrix}$
$\lambda_2 \sim FW_{SS}^{crop}$	$\vec{v_2} \sim \begin{bmatrix}0\\1\\0\\0\end{bmatrix}$
$\lambda_3 \sim W_{RS}^{cage}$	$\vec{v_3} \sim \begin{bmatrix}0\\0\\1\\-1\end{bmatrix}$
$\lambda_4 \sim F\sigma(F)$	$\vec{v_4} \sim \begin{bmatrix}0\\0\\0\\1\end{bmatrix}$

Discussion

The *existence* of a stable control equilibrium is certainly to be expected, for (i) a refuge in a cage that is completely sealed will sustain a predominantly susceptible population in the cage at carrying capacity; (ii) a small leakage out of the cage into the toxic crop can suppress resistance development there by the mechanism illustrated in Fig. 1E; and (iii) a small enough back-flow from the crop into the cage plausibly will not destabilize the equilibrium in the cage. Equally, the *existence* of the separator equilibrium may be forced on topological grounds given the presence of two locally attracting fixed points (control and failure equilibria). Nevertheless, the asymptotic formulas in Table 2 provide a way to understand what is the dominant balance of processes that give rise to each of the equilibria (as we spell out below), and provide quantitative approximations of the allele densities at which those balances occur - all in terms of parameters that may be estimated for applications of interest. Our formulas also provide some information about where the caged refuge technique will break down.

The processes that balance to create the equilibria

We stress that the following discussion is *interpretation* of the results in Table 2, not our *derivation* of them: it is the results in Table 2 that justify the assertions in this section.

In the cage

The density of S allele in the cage, N_S^{cage}, at both equilibria is essentially the carrying capacity N_0, which is the leading O(1) term in the expressions in row 4 of Table 2. This is a balance between reproduction and the density-dependent attrition that limits the population. The density of R-allele in the cage, N_R^{cage}, is essentially determined, at the control equilibrium, by a balance between mutation and selection at the well-known equilibrium R-fraction $\mu_{SR}/(1 - W_{RS}^{cage})$ (see e.g. Hartl 1988; Gillespie 2004; or Rice 2004). Self-consistency of our formulas therefore requires that there be enough fitness cost for heterozygotes in the refuge that $1 - W_{RS}^{cage} >> \mu_{SR}$. With μ_{SR} typically $\sim 10^{-6}$, this is not a great imposition: $W_{RS}^{cage} = 0.999$, or even as high as 0.9999, would be satisfactory. At the separator equilibrium, we see that balance involves a term (the one proportional to a) that represents back-flow of R allele from the crop. For this term to have a magnitude consistent with our assumptions, it is necessary that $1 - W_{RS}^{cage} >> \frac{a}{A}\frac{b}{B}$ which can be accomplished for any nonzero fitness cost by making the effective aperture sizes a, b sufficiently small. (See Effect of the aperture size for the consequences of reducing the aperture sizes.) The issue of fitness costs is discussed further in Conclusions.

In the crop

At the *control* equilibrium, the S-allele density in the crop is a balance between immigration from the cage and reproduction/poisoning in the crop itself: balance is achieved when amount of S allele in the offspring that survive poisoning plus the amount in immigrants from the cage equals the amount in the parent generation:

$$N_{S\bigstar}^{crop} \cdot FW_{SS}^{crop} + \frac{b}{A} N_0 = N_{S\bigstar}^{crop}. \quad (17)$$

Likewise, the R-allele density is a balance in which the net decrease in density resulting from reproduction and poisoning is restored by immigration from the cage:

$$N_{R\bigstar}^{crop} \cdot FW_{RS}^{crop} + \frac{b}{A} N_{R\bigstar}^{cage} = N_{R\bigstar}^{crop}. \quad (18)$$

(Solving eqns (17) and (18) for $N_{S\bigstar}^{crop}$ and $N_{R\bigstar}^{crop}$ respectively gives the expressions in column 2, rows 2 and 1, of Table 2.)

At the *separator* equilibrium, the expression for the S-allele density is more complicated for general values of the fitnesses of RS and SS individuals in the crop, but it is readily verified that when $W_{RR}^{crop} \approx 1$, and $FW_{RS}^{crop} << 1$, the value is close to the value at the control equilibrium (as exemplified in Fig. 5) and is, at least in these circumstances, a balance between S-allele immigration and reproduction/poisoning.

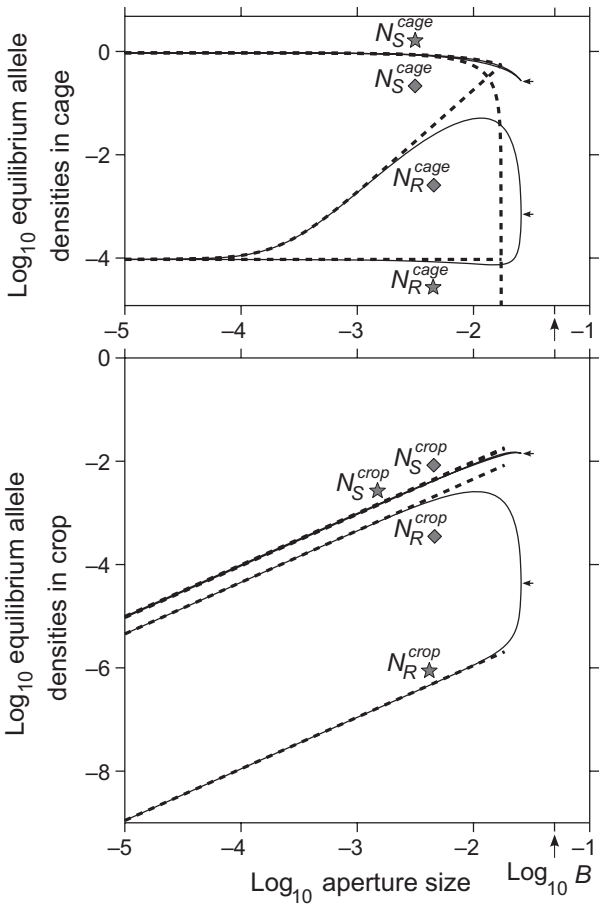

Figure 5 Dependence of equilibrium allele densities on the size of the hole in the cage, $a = b$: analytical approximations (thick dashed curves), and numerical results for comparison (thin unbroken curves). Parameter values are given in Table 1. The accuracy of the analytical approximations is very good when the fraction of pests leaving the cage, b/B, is not more than 5% or so. The analytical asymptotic formulas are plotted only up to the aperture size at which the expression for $N_{S\blacklozenge}^{cage}$ first goes negative. The small arrows locate the saddle-node bifurcation observed numerically.

The balance for N_R^{crop} at the separator equilibrium is quite different from that at the control equilibrium. At the separator, RR individuals play a role, and immigration from the cage does not. The balance is R allele in surviving RR offspring + R allele in surviving RS offspring = R allele in parents, or

$$Nr^2 \cdot FW_{RR}^{crop} + Nr(1-r) \cdot FW_{RS}^{crop} = Nr^2 + Nr(1-r), \quad (19)$$

where N is the total population density in the crop and r is the R-allele fraction, $N_{R\blacklozenge}^{crop}/N$. Equation (19) can be rewritten (dividing through by r and using $N(1-r) = N_{S\blacklozenge}^{crop}$) as

$$N_{R\blacklozenge}^{crop} \cdot (FW_{RR}^{crop} - 1) = N_{S\blacklozenge}^{crop} \cdot (1 - FW_{RS}^{crop}) \qquad (20)$$

which gives the expression in column 3 row 1 of Table 2 when solved for $N_{R\blacklozenge}^{crop}$.

Effect of the aperture size

The accuracy of the asymptotic formulas for the equilibria is illustrated in Fig. 5, where the approximations are compared with numerically obtained equilibrium values. As would be expected, agreement is good in the left half of the figure where the aperture is small. The aperture(s) permitting pest flow between the cage and the crop should be small because, as understood from 'How refuges work, or fail to', it is the restriction of pest flow that makes the refuge in a cage work. We see from the formulas in Table 2 that as the cage-to-crop aperture size b tends to zero, the control equilibrium population densities in the crop go to zero. It might seem desirable to make the aperture extremely small, for we would want the control equilibrium pest population – the population we hope to maintain in the crop – to be as small as possible. However, we also see from the formulas that the separator equilibrium goes to zero too as $b \to 0$, which means that the width of the basin of attraction of the control equilibrium shrinks to zero, i.e. the robustness of the control equilibrium with respect to additions of resistant allele is lost: for very small b, very small additions of R allele can knock the system into the basin of the failure state. Thus, there is a trade-off between how low the equilibrium pest population is pushed and the robustness of the protection against resistance evolution. Determining an actual optimal value of b will involve factors not treated here, because the perturbations in N_R^{crop} that one wants to protect against are most likely to arise from demographic or environmental stochasticity or other influences that are not included in our model.

At the other extreme of aperture size, numerically we find that as the aperture size is increased, the control and separator equilibria eventually collide and annihilate each other in a saddle-node bifurcation (indicated by small arrows in Fig. 5). For apertures this large and larger, control failure is inevitable, as the 'failure' equilibrium is the only stable one and is likely the limit of all trajectories except the extinction state. In the formulas for N_S^{cage} in Table 2, we have included not just the leading O(1) term, but also the $O(\epsilon)$ term, which captures the effect of outflow from the refuge. From this, it can be seen that a crude estimate for a maximum aperture size above which the asymptotics for the equilibria cannot be relied upon, because the leading order term does not dominate the next one, and therefore where absence of the control and separator equilibria is possible, is

$$\left(\frac{b}{B}\right)_{max} \approx 1 - F\sigma(F). \qquad (21)$$

A numerical sensitivity analysis around the case shown in Fig. 4 and some other numerical spot-checks suggest that this estimate correctly predicts that the maximum aperture size relative to cage size for which the refuge works as indented depends on only the pest fecundity F and the derivative $\sigma(F)$ of the attrition map, among all the parameters, and the estimate predicts rather well the relative change of the bifurcation value caused by a given small relative change in F, athough not the actual number very accurately.

Making the caged refuge small

A trade-off also exists with respect to the size, B, of the caged refuge. On the one hand, B should be small, if possible, because a cage may have high cost per unit size to maintain, and any land allocated to the cage, which is always heavily infested with the pest, will produce low-quality crops or none at all (Pech *et al.* 2009). On the other hand, to avoid the mutual annihilation of the control and separator equilibria as described in the previous subsection, the aperture size, b, must be reduced in proportion as $B \to 0$. Therefore, a compromise must be struck between low population levels at the control equilibrium and high robustness of that equilibrium, just as in the previous subsection. Nevertheless, simulations to be described elsewhere lead us to believe that caged refuges *very* much smaller than the 20% of crop mandated by the USEPA for open refuges can be effective.

Conclusions

Our asymptotic analysis reveals which processes balance to give rise to two important equilibria of the pest population in the presence of a heavily screened refuge (a caged refuge). It also provides quantitative estimates of where these equilibria are in terms of parameters that can be estimated for applications of interest, as well as of the size of perturbations that can be withstood when employing this technique of pest suppression.

We have described in the previous section how some fitness cost for heterozygotes in the refuge is necessary for the existence of the equilibria we have analyzed. Such a cost has been observed for some pests, but may not exist for all (Foster *et al.* 2000; Gassmann *et al.* 2009). If there is absolutely no fitness cost for heterozygotes in the refuge, then a true control equilibrium may not exist, but the control state can still be expected to be metastable, i.e., transient but long-lived. For example, turning fitness

costs off completely in Fig. 1F, even for both heterozygotes and resistant homozygotes (i.e., $W_{RS}^{cage} = W_{RR}^{cage} = 1$), does not change the picture discernibly over the 40 generations shown; the resistant allele fraction rises extremely slowly and explodes only after 2500 generations. On the other hand, *higher* costs than considered here will only make the control of the population by the caged refuge technique *more* robust, athough our formulas for the equilibria are not expected to be accurate in this case. Fitness costs can be significantly beneficial in the open-refuge context, at least in the case of fully recessive resistance, as shown by Gassmann *et al.* 2009b. But our results indicate that the caged refuge technique can control the population in the case of nonrecessive resistance where fitness costs of resistance cannot enable an open refuge to perform its intended function, or are too small do so.

The model we used is intended to be one of the simplest possible that incorporates the essential processes, and for that reason ignores, or represents only crudely, many features that belong in a realistic model of an agricultural pest. Demographic and environmental stochasticity, explicit spatial structure besides the crop/refuge dichotomy, overlapping generations, host plant development, pest development, and all other processes that occur on a subgenerational timescale are omitted. We have therefore also explored the use of the screened refuge technique in an implementation of a detailed stochastic model of pink bollworm (*Pectinophora gossypiella*, Noble 1969) on Bt cotton formulated in Sisterson *et al.* 2004, which is in the same class of models as those of Peck *et al.* 1999; Caprio 2001; and Storer *et al.* 2003. We have observed that the screened refuge mechanism does suppress resistance in this context also, and a detailed comparison with the current model results will be presented elsewhere.

We are not equipped to assess possible practical obstacles and burdens (Hargrove 1999) of implementing a caged refuge for moth species like the major pests of corn or cotton. But we have seen fruit crops grown in Taiwan inside large netted enclosures for the purpose of keeping insect pests off. Such technology could perhaps be used for a refuge cage in some contexts. The potential for reducing refuge size relative to that required for open refuges might offset the burden of maintaining cages, and of course open refuges may simply not be an option if resistance is not strongly recessive.

We remark parenthetically that *remoteness* of a refuge cannot necessarily substitute for a cage as a means of refuge isolation. Spatial effects create an additional layer of complexity whose consequences are not simple to predict. But we can say that any spatial gradient provides a spectrum of conditions, and if any point on that spectrum promotes resistance development, a resulting local development of resistance could potentially serve as the nucleus for its spread throughout the habitat.

Finally, we note that although we have described the screened refuge technique in the context of insects on genetically modified insecticidal crops, the formulation is sufficiently abstract that it could be applied to a variety of other situations where the development of resistance to toxins in a sexually reproducing species is to be averted: even to weed control with a herbicide if both pollen and seeds are dispersed widely relative to refuge size and spacing.

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