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REPORT

Evolution of resistance to *Bt* crops: directional selection in structured environments

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Abstract

Widespread use of transgenic crops that express an insecticidal endotoxin from *Bacillus thuringiensis* increases the risk of evolution of resistance by the European corn borer and other insect pests. To delay resistance evolution, the high-dose/refuge strategy is being implemented for *Bt* maize and *Bt* cotton. We develop a general modelling framework to understand the invasion and spread of alleles conferring resistance. We show that at least three processes are involved in explaining the effectiveness of the high-dose/refuge strategy: the intensity of selection, assortative (non-random) mating due to spatial subdivision, and variation in male mating success also due to spatial subdivision. Understanding these processes leads to a greater range of possible resistance management tactics. For example, efforts to encourage adults to leave their natal fields may have the unwanted effect of speeding rather than slowing resistance evolution. Furthermore, when *Bt* maize causes high mortality to susceptible target pests, spraying insecticides in refuges to reduce pest populations may not greatly disrupt resistance management.

Keywords

Resistance evolution, directional selection, *Bt* corn, European corn borer, *Bacillus thuringiensis*, *Ostrinia nubilalis*, high-dose/refuge strategy, resistance management.

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INTRODUCTION

The purpose of this article is to provide a general mathematical framework to understand the processes underlying directional selection in a spatially structured environment. Specifically, given a single-locus, diallelic trait, we ask how spatial structure affects the rate of evolution of the trait in a sexual population. We illustrate this framework using a simple simulation model for the evolution of resistance to *Bt* toxins by the European corn borer, *Ostrinia nubilalis* (Hübner), a major insect pest of maize in the USA and elsewhere (Mason *et al.* 1996). Although we illustrate the framework using a specific simulation model for *O. nubilalis*, the framework can be applied broadly to understand both other models of *O. nubilalis* resistance evolution and, more generally, models of directional selection in other systems.

The high-dose/refuge strategy is recommended for *Bt* maize to slow the evolution of resistance by *O. nubilalis* (Anonymous 1998; FIFRA 1998; Gould 1998). This strategy consists of planting fields as pure stands of either *Bt* or non-*Bt* varieties, so that pests are exposed to either high doses of *Bt* toxins or remain unexposed in refuges. A high dose of a toxin is one that renders resistance functionally recessive or nearly recessive (Tabashnik & Croft 1982). Thus, the high-dose/refuge strategy creates strong spatial structure in selection for resistance, with selection very intense in *Bt* fields but non-existent in refuge fields. This is thought to delay resistance because the non-*Bt* refuge provides a source of susceptible insects to mate with resistant ones so their offspring will be *RS* (resistant/susceptible) heterozygotes that can be killed by *Bt* maize (Comins 1977; Taylor & Tabashnik 1979; Roush 1989; Alstad & Andow 1995; Anonymous 1998; FIFRA 1998; Gould 1998; Rausher 2001). Despite extensive modelling efforts, however, the mechanisms underlying resistance evolution are incompletely understood, impeding the search for effective management strategies.

AN APPROXIMATION FOR RESISTANCE EVOLUTION

To illustrate the processes affecting resistance evolution, we first describe a numerical simulation model developed by Alstad & Andow (1995) and Andow & Ives (2002), based

on the original model of Comins (1977; May & Dobson 1986). The simulation model has the same basic features as other, more complex models of resistance evolution to transgenic crops (Onstad & Gould 1998; Peck *et al.* 1999; Caprio 2001). Although specific assumptions in the model (e.g. the order of dispersal and mating, the form and timing of density dependence, and the manner of distributing dispersing individuals among field types) may differ from other models for *O. nubilalis* or models for other systems, many details can be changed without compromising the use of the model to illustrate our general mathematical framework. The simulation model is depicted in Fig. 1 and derived mathematically in Appendix S1.

For the simulation model, the environment contains two field types planted with either *Bt* (field type $i = 1$) or non-*Bt* maize ($i = 2$) making up fractions $1 - Q$ and Q of the total maize fields, respectively. A proportion r_{im} of males disperse from natal fields before mating, while females mate within their natal fields before a proportion r_{if} disperse. Mating within fields is random, and following dispersal females lay F_1 and F_2 eggs in *Bt* and refuge fields. As in the other models of resistance evolution to transgenic crops, resistance is governed by a single locus with *R* and *S* alleles, and expression in the heterozygous *RS* individuals is governed by a parameter h ($h = 0$, fully recessive; $h = 1$, fully dominant). After mortality due to *Bt* toxins, density dependence occurs among the remaining larvae.

To characterize the invasion dynamics of a rare resistance allele, we derived an approximation of the simulation model (Appendix S2). An approximation of the same form would apply to a broad class of models for this problem, and thus the approximation provides a general framework to understand the processes underlying resistance evolution. The approximation is a second-order Taylor expansion of the simulation model around the case in which the frequency of the *R* allele among fields is low and expression of resistance is recessive. Provided the area of refuge is large enough to support a purely susceptible population following the introduction of *Bt* crops,

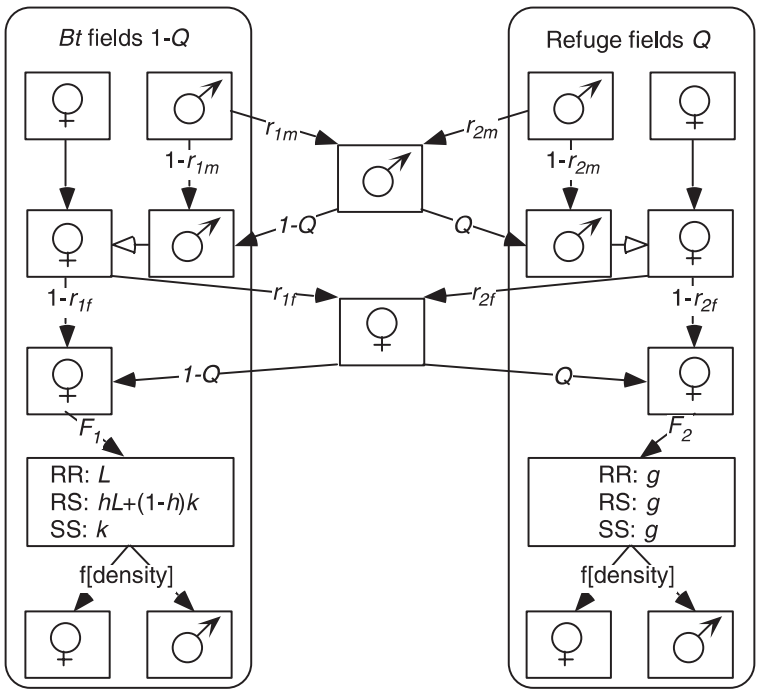

Figure 1 Graphic representation of the model for *O. nubilalis* control and resistance evolution. A proportion Q of fields are refuges. A fraction r_{1m} and r_{2m} of males disperse from their natal *Bt* fields and refuge fields, and dispersing males are redistributed between field types in proportion to their area. Random mating then occurs within fields, and fractions r_{1f} and r_{2f} of the mated females disperse from their natal *Bt* and refuge fields, and are redistributed between field types in proportion to their area. Females produce F_1 and F_2 eggs in *Bt* and refuge fields. In *Bt* fields, homozygous resistant larvae (*RR*) have survival *L*, homozygous susceptible larvae (*SS*) have survival *k*, and heterozygotes have survival $hL + (1 - h)k$, where h governs the dominance of expression of resistance. Larvae of all genotypes have survival g in refuge fields. The larvae within each field type experience density-dependent survival governed by the function $f[x] = x(1 + ax)^{-b}$, where x is the density of larvae, and a and b are parameters governing density dependence (Hassell 1975). Larvae then pupate and produce equal numbers of males and females for the next generation. We assume no developmental delays (Liu *et al.* 1999) or costs of resistance (McKenzie 1996). A developmental delay could accelerate resistance, and a cost of resistance could delay it.

$$P(t+1) \cong (\boldsymbol{\Theta}_{\mathbf{RR}} - \mathbf{I})\mathbf{M}_{\mathbf{f}}(P(t) \times (\mathbf{M}_{\mathbf{m}}P(t))) + \frac{1}{2}\boldsymbol{\Theta}_{\mathbf{RS}}\mathbf{M}_{\mathbf{f}}[\mathbf{I} + \mathbf{M}_{\mathbf{m}}]P(t) \quad (1)$$

where $P(t)$ is a vector containing the frequencies of the R allele in Bt and refuge fields in generation t. The matrices $\boldsymbol{\Theta}_{\mathbf{RR}}$ and $\boldsymbol{\Theta}_{\mathbf{RS}}$ contain elements θ_{ii} ($i = 1$ for Bt and 2 for refuge fields) along the diagonals which are the relative fitnesses of RR homozygotes and RS heterozygotes in each field type; for example, for the simulation model (Fig. 1) the diagonal elements of $\boldsymbol{\Theta}_{\mathbf{RR}}$ are $\theta_{11} = L/k$ and $\theta_{22} = 1$. Movement of males and females among field types is given by the matrices $\mathbf{M}_{\mathbf{m}}$ and $\mathbf{M}_{\mathbf{f}}$ whose elements m_{ij} give the proportion of the male or female population in fields of type i that moved from fields of type j. Finally, $\mathbf{I}$ is the 2×2 identity matrix, and '$\times$' represents the element-by-element (or Schur) product of vectors. Although the approximation is derived formally for the case when the R allele is rare ($P \rightarrow [0, 0]$) and resistance is recessive ($h \rightarrow 0$), numerical explorations with the simulation model demonstrate that equation 1 performs well provided the average R allele frequency $\bar{p} < 0.2$ and $h < 0.1$. These two conditions are appropriate for a model of the high-dose/refuge strategy, since they include the generally acknowledged conditions for the high-dose/refuge strategy to be effective (Roush 1989; Caprio & Tabashnik 1992; Anonymous 1998; Gould 1998).

The value of equation 1 is that, although derived formally for the simulation model, it is the approximation to a broad class of patch models of resistance evolution. Equation 1 shows that the rate of resistance evolution depends only on the intensity of selection for RR and RS individuals in each of the field types, θ_{ii}, and the redistribution of individuals among field types, m_{ij}. Equation 1 can be explained by recognizing that the first and second terms correspond to selection on resistant homozygotes and heterozygotes, respectively. For a given distribution of frequencies among field types in generation t, $P(t)$, the distribution of the R allele among males following dispersal is $\mathbf{M}_{\mathbf{m}}P(t)$. Females mate in their natal fields with these males, so the proportion of RR offspring that would be produced by these females is $P(t) \times (\mathbf{M}_{\mathbf{m}}P(t))$ in the first term of equation 1. Females disperse before depositing eggs, so the distribution of RR offspring among field types is $\mathbf{M}_{\mathbf{f}}(P(t) \times (\mathbf{M}_{\mathbf{m}}P(t)))$. These are then subject to selection which is given by $(\boldsymbol{\Theta}_{\mathbf{RR}} - \mathbf{I})$; the relative fitnesses of resistant to susceptible homozygotes, $\boldsymbol{\Theta}_{\mathbf{RR}}$, is reduced by $\mathbf{I}$, because in the absence of selection on the resistant homozygotes (i.e. $\boldsymbol{\Theta}_{\mathbf{RR}} = \mathbf{I}$), resistant homozygotes make up a vanishingly small component of the total population in the limit as $P \rightarrow [0, 0]$, and consequently they contribute nothing to the frequency of resistance in the following generation. For the second term of equation 1, the frequency of RS heterozygotes in a field type is approximately equal to the frequency of the R allele. The frequency of the R allele in the different field types is the average of the maternal frequency following dispersal into fields and the paternal frequency of alleles which the females bring with them, $(\mathbf{M}_{\mathbf{f}}P(t) + \mathbf{M}_{\mathbf{f}}\mathbf{M}_{\mathbf{m}}P(t))/2$. The heterozygotes are then subject to selection which depends on $\boldsymbol{\Theta}_{\mathbf{RS}}$.

Equation 1 is a multidimensional form of the second-order approximation to equations governing directional selection in a spatially unstructured environment (Crow & Kimura 1970) or in a spatially structured environment (Levene 1953). Due to its general structure, it can accommodate any number of field types, where field types can be defined either by the strength of selection within the fields given by θ_{ii} or the movement of individuals from other field types, m_{ij}. Therefore, it is not limited to the case of only Bt and refuge fields, and can account for simple patterns of spatial location of field types. Furthermore, by imposing a selective advantage to SS individuals in refuge fields, equation 1 can address the case of a cost to resistance (Roush & McKenzie 1987; Groeters 1995). Finally, even for complex, computer-intensive simulations (Onstad & Gould 1998; Peck *et al.* 1999; Caprio 2001), equation 1 can be used as an approximation in which the terms θ_{ii} and m_{ij} are hyperparameters calculated during the simulations. Because equation 1 defines how selection and movement combine to drive resistance evolution, it can be used as a probe to understand the output of complex models.

COMPONENTS OF RESISTANCE EVOLUTION

Equation 1 can be decomposed to reveal the underlying mechanisms of resistance evolution. Specifically, the proportional change in the average frequency of the R allele among field types, $\bar{p}(t)$, is (Appendix)

$$\frac{\bar{p}(t+1) - \bar{p}(t)}{\bar{p}(t)} \cong s_0 + \Delta s + \eta + \mu. \quad (2)$$

Here, s_0 denotes the effect of selection on resistance evolution for the case of complete mixing ($r_{im} = r_{if} = 1$), in which each generation adults are distributed among field types according to the relative area of field types in the environment. The term Δs denotes the change in the effect of selection caused when there is incomplete mixing. If, for example, movement of female adults from refuge fields is reduced, then fewer will fly into Bt fields, thereby decreasing the mortality of susceptibles caused by Bt toxins and reducing the rate of resistance evolution. The term η involves assortative mating caused by the spatial structure of the environment. Because limited dispersal will create differences in the frequencies of the R allele between field types, the proportion of RR homozygous offspring averaged between field types will be different from that predicted for the case of complete mixing. If spatial structure increases

the proportion of *RR* homozygous offspring in *Bt* fields, this will increase the rate of resistance evolution for the case when resistance is partially or completely recessive (the most likely scenario) by increasing the proportion of the population that is phenotypically resistant. Finally, μ is the result of variation in male mating success caused by spatial structure. Differences in male and female population sizes among field types may change the number of mates available to males with the *R* allele. If this increases the mate competition experienced by males carrying the *R* allele, it will lower their average reproductive success relative to males carrying only the *S* allele, thereby delaying resistance evolution. Thus, for example, if large numbers of susceptible males disperse from refuge to *Bt* fields, this will reduce the reproductive success of males carrying an *R* allele in *Bt* fields by intensifying competition for the relatively rare females.

EFFECTS OF DISPERSAL

To illustrate the combined effects of the components of resistance evolution given by equation 2, Fig. 2 uses the simulation model to examine the consequences of reducing dispersal from natal fields. In the case illustrated (and for all cases we have found with the simulation model) reducing dispersal increases the time to control failure (Fig. 2a). Consistent with other simulation studies (Caprio & Tabashnik 1992; Caprio 2001), this result shows that high dispersal is not necessary for effective resistance management under the high-dose/refuge strategy.

To understand this result, we have plotted descriptors of the genetic structure of the population (Fig. 2b) and values for the components of equation 2 (Fig. 2c) measured at the point at which the *R* allele frequency is $\bar{p}(t) = 0.001$ (i.e. the starting frequency from which time to control failure is measured, where control failure is defined as $\bar{p}(t) = 0.5$). It is true that reducing dispersal from natal fields increases the average frequency of *RR* homozygotes among both field types relative to the case of complete mixing ($\bar{p}_{RR}$ in Fig. 2b), and this by itself would increase the rate of resistance evolution (η in Fig. 2c). Nonetheless, the effect is small. Reducing dispersal decreases the mortality of susceptible pests relative to the case of complete mixing (*mort* in Fig. 2b), because fewer susceptible insects disperse from refuge fields to be killed in *Bt* fields. This reduces the fitness advantage of resistance and slows resistance evolution ($s_0 + \Delta s$ in Fig. 2c). Reducing dispersal also decreases the mating success of males carrying the *R* allele, because these males are more likely to occur in *Bt* fields where there is a relatively low abundance of females and a large number of *SS* males dispersing in from the refuge. This lowers the frequency of the *R* allele that males pass to females during mating relative to the frequency of *R* allele in the male population averaged among field types ($\bar{p}_m$ in Fig. 2b), thereby slowing resistance evolution (μ in Fig. 2c).

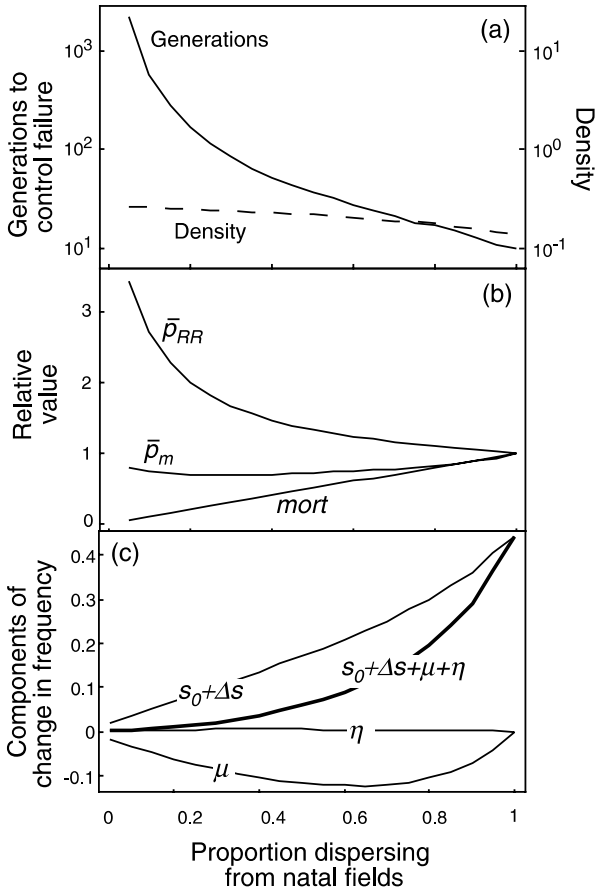

Figure 2 Factors affecting the rate of resistance evolution when the proportions of males and females leaving natal fields are the same for both field types ($r_{im} = r_{if}$ for all i). (a) Generations to control failure (when *R* allele frequency reaches 0.5) from a frequency of 0.001 (solid line) and average population density across *Bt* and refuge fields (dashed line). (b) The frequency of *RR* homozygotes when dispersal is limited relative to that when all *O. nubilalis* disperse from natal fields, labelled $\bar{p}_{RR}$; the change in the *R* allele frequency due to reduced mating success of males with the *R* allele, labelled $\bar{p}_m$; and the change in the proportion of the susceptible population killed in *Bt* fields when limited dispersal lowers the chance of exposure to *Bt* crops, labelled *mort*. All of these values are calculated when the frequency of the *R* allele is 0.001. The simulations were performed by starting the frequency of the *R* allele at 0.0005 and counting the generations to control failure after the frequency reached 0.001. (c) The components of resistance evolution, $s_0 + \Delta s$, μ, and η, and the total rate of increase in the frequency of the resistance allele, $s_0 + \Delta s + \mu + \eta$, measured when the frequency of the *R* allele is 0.001. Parameter values are: $F_1 = F_2 = 50$, $L = 1$, $k = 0.001$, $a = 4$, $b = 0.7$.

The effect of dispersal on the rate of resistance evolution is only weakly coupled with its effect on the total pest population size. While reducing dispersal increases the number of generations to control failure by 2 orders of magnitude, it increases the pest population density by less than a factor of 2 (Fig. 2a).

TRANSIENT POPULATION PERSISTENCE AND QUASI-STATIONARITY

Equation 1 was derived under the assumptions that (i) a purely susceptible population can persist in the environment of *Bt* and refuge fields, and (ii) the densities of susceptible individuals among field types is at quasi-stationarity (i.e. the densities of a purely susceptible population after they have equilibrated following *Bt* crop introduction). We examined the consequences of these assumptions with the simulation model (Fig. 1) for the case in which female fecundity is low ($F_1 = F_2 = 10$). For this case, when the proportion of the environment consisting of refuge fields drops below 10%, a purely susceptible population cannot persist (Fig. 3a), violating assumption (i). We explored the other assumption by changing the initial frequency of the *R* allele when *Bt* maize is introduced. Specifically, we let the initial frequency of the *R* allele in *Bt* and refuge fields be 0.0001, 0.0002, 0.0005 and 0.001. In all of the simulations, we count the time to control failure as the number of generations for the frequency of the *R* allele to go from 0.001 to 0.5. Thus, when the initial frequency of the *R* allele is 0.001, we start counting when *Bt* maize is introduced. With lower initial *R* allele frequencies, after introducing *Bt* maize we wait for the *R* allele frequency to increase to 0.001 before counting. In this case, the density of the susceptible population has time to approach its quasi-stationary density (if stationarity exists) to satisfy assumption (ii).

When the purely susceptible population is not persistent, violating assumption (i), the time to control failure drops rapidly, and equation 1 over-estimates the time to control failure (Fig. 3b). The smaller the refuge, the faster the total population tends to extinction, and the more rapid the rate of resistance evolution. This occurs because the non-*Bt* fields no longer have a sufficient susceptible population to serve as a refuge. When the purely susceptible population can persist, however, the results for the cases in which the initial *R* allele frequency is less than 0.001 are almost identical to each other, and almost identical to the predictions of equation 1 (Fig. 3b).

When the population is not at quasi-stationarity, violating assumption (ii), the rate of evolution differs from that predicted by equation 1. This occurs most dramatically for the case in which the initial frequency of the *R* allele is 0.001, and for the simulations we conducted (Fig. 3b), the rate of resistance evolution is faster than the quasi-stationary cases in which the initial *R* allele frequency is < 0.001.

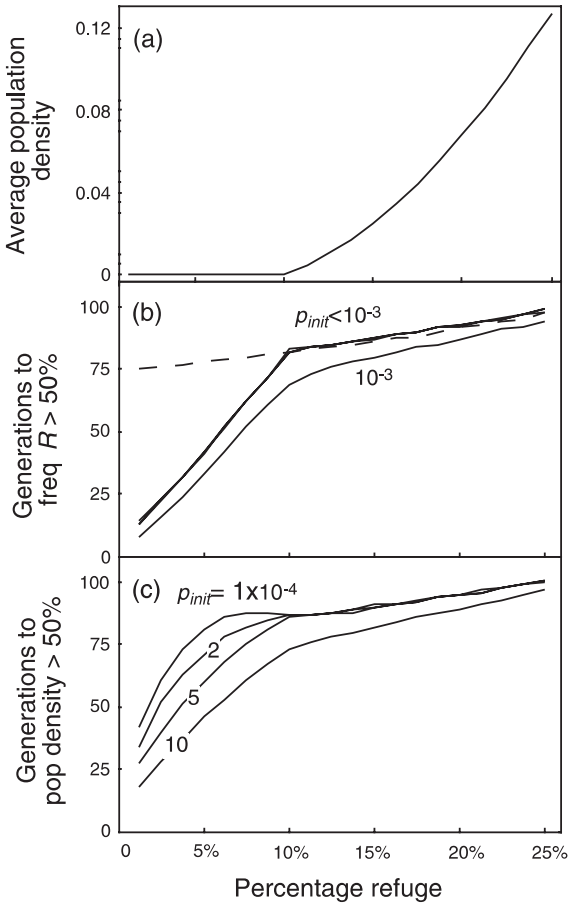

Figure 3 Transient population dynamics affect the generations to control failure. (a) Stationary density of a purely susceptible population when the percentage refuge varies from 0% to 25%. Below 10%, the susceptible population becomes extinct. (b) Generations to control failure counted as the number of generations required for the frequency of the *R* allele to go from 0.001 to 0.5. Simulations were started when the *Bt* crop was introduced, and the initial frequency of the *R* allele was 0.0001, 0.0002 and 0.0005 (collectively labelled $p_{init} < 10^{-3}$), and 0.001 (labelled $p_{init} = 10^{-3}$). When the initial frequencies were less than 0.001, the generations until the frequency reached 0.001 were not counted in the generations to control failure. The dashed line gives the prediction using equation 1, with values of m_{ij} calculated at the point at which the frequency of the *R* allele was 0.001. (c) Same as (b), but the time to control failure is measured as the number of generations from a *R* allele frequency of 0.001 to when the population density in *Bt* fields reached 50% of the density in the simulation before *Bt* maize was introduced. Parameter values are: $r_{1m} = r_{2m} = r_{1f} = r_{2f} = 1$, $F_1 = F_2 = 10$, $k = 10^{-4}$, $b = 0.001$, $a = 4$, $b = 0.7$.

As pointed out by Peck *et al.* (1999), measuring the time to control failure using the frequency of the *R* allele can be misleading, whereas using instead the population density of pests in *Bt* fields is a more pertinent measure of control failure. Figure 3(c) is generated like Fig. 3(b), except the time to control failure is measured as the time from which the frequency of the *R* allele is 0.001 to the time at which the population density in *Bt* fields reaches half that of the stationary population density before *Bt* maize is introduced. Using this measure of control failure, the time to control failure increases slightly when the susceptible population is not persistent (refuge < 10%) for small initial frequencies of the *R* allele ($\bar{p} = 0.0001$). This is because the susceptible population is driven to very low densities before resistance spreads significantly in the population. Although the *R* allele frequency may reach 0.5 more quickly than in the case in which the susceptible population persists, by the time this occurs the population density is so low it requires a long time to recover and regain its pre-*Bt* densities.

COMPLETE MIXING AND INSECTICIDES

Complete mixing occurs when all adults disperse from their natal fields ($r_{im} = r_{if} = 1$ for all i) and are distributed at random among *Bt* and refuge fields. This special case is instructive, because equation 1 is greatly simplified. Complete mixing leads to $\Delta s = \eta = \mu = 0$ in equation 2, so the rate of resistance evolution depends only on s_0 (Appendix). This case isolates the effect of the difference in fitness between individuals carrying the *R* and *S* alleles. If, in addition, *Bt* maize has high control efficacy so that survival of susceptibles, k, is very low ($k \to 0$), then for the simulation model (Fig. 1)

$$\frac{\bar{p}(t+1) - \bar{p}(t)}{\bar{p}(t)} \cong (1 - Q)LF_1(\bar{p}(t) + h) \qquad (3)$$

where Q is the proportion of fields in refuge, and F_1 is the fecundity of surviving females in *Bt* fields. In equation 3, increasing the size of the refuge, Q, slows resistance evolution by decreasing the exposure of the susceptible population to selection.

Importantly, equation 3 demonstrates that the rate of resistance evolution does not depend on the survival, g, and reproduction, F_2, of the pest in refuges. This lack of dependence on survival and reproduction in refuge fields means that insecticide application in refuges would have little effect on resistance evolution when *Bt* crops have high dose (so resistance is mostly recessive) and high efficacy (so survival of susceptibles is very low). This result has a simple explanation. For the case of complete mixing, the rate of evolution depends only on the intensity of selection for resistance. The intensity of selection depends on the relative fitnesses of *RR*, *RS*, and *SS* individuals, which in turn depend on the proportion of each genotype exposed to selection in *Bt* fields. When the *Bt* fields are highly toxic to phenotypically susceptible individuals, the entire susceptible population (before dispersal) resides within the refuges. Therefore, the selective advantage of resistance depends only on the proportion of the susceptible population that moves from refuge to *Bt* fields, which is $(1 - Q)$. Consequently, spraying insecticides in the refuge will not affect the intensity of selection for resistance.

The key to understanding this result is that the intensity of selection depends on the relative fitness of the different genotypes, not on the population size. Although spraying insecticides in the refuge will decrease the size of the population, it will not affect the proportion of the population exposed to *Bt* toxins (provided dispersal itself is not density dependent) and hence will not affect relative fitnesses. This argument breaks down when the *R* allele frequency becomes high enough for a sizeable population to occur in *Bt* fields (roughly $\bar{p}(t) > 0.2$), but this case is of little consequence for the high-dose/refuge strategy, because control failure will occur soon thereafter. The argument also breaks down when the susceptible population is not persistent, which we will address next.

LIMITED DISPERSAL AND INSECTICIDES

Under limited dispersal, the rate of resistance evolution remains insensitive to insecticide application in refuges. Using the simulation model, we ran 4000 simulations with values of $F_1 = F_2$, $r_{1m} = r_{2m}$, $r_{1f} = r_{2f}$, Q, and h randomly selected from a wide range of possible values. We measured the time to control failure either as the time for the frequency of the *R* allele to go from 0.001 to 0.5 or from the time the *R* allele frequency was 0.001 to the time at which the density in *Bt* fields was half the stationary density before *Bt* maize was introduced. Furthermore, the 4000 simulations were divided according to whether a purely susceptible population was persistent vs. non-persistent for the case with insecticide application. In these simulations, we selected an initial frequency of the *R* allele of 0.0001, so considerable time elapsed before the frequency reached 0.001 and we commenced counting the time to control failure. We did this to increase the contrast between the two measures of time to control failure we used (Fig. 4c vs. Figure 4d). The consequences of spraying insecticide are depicted in Fig. 4 as the proportional change in the number of generations to control failure.

When control failure is assessed by the time required for the *R* allele frequency to reach 0.5, spraying insecticide in refuges causes at most a modest reduction in the time to control failure, provided the susceptible population is persistent (Fig. 4a). These modest reductions generally occurred in simulations in which the time to failure

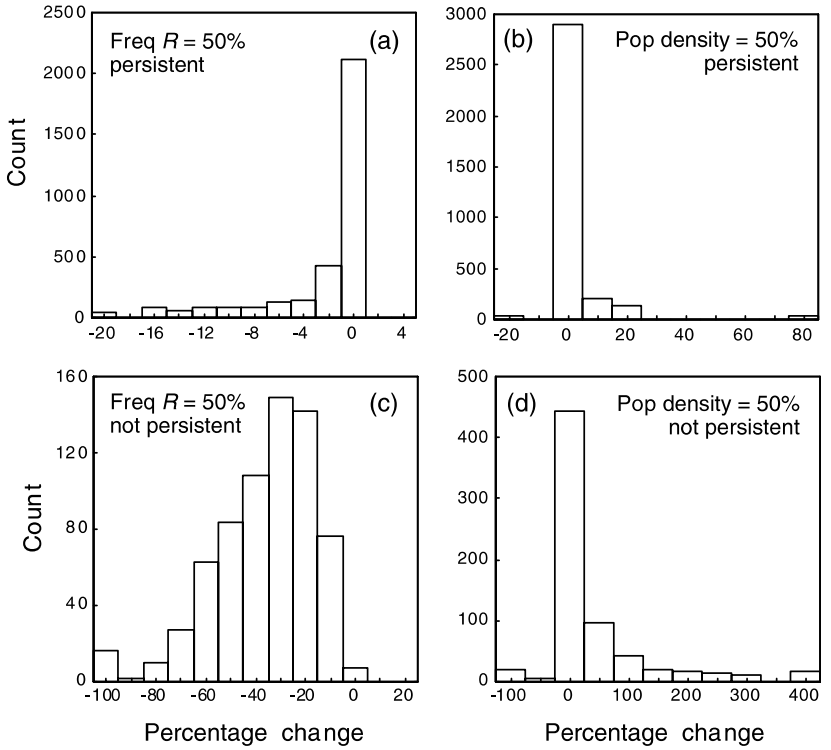

Figure 4 Percentage change in the number of generations before control failure [(generations without insecticide minus generations with insecticide)/generations without insecticide] in the simulation model when insecticide application in refuges kills 90% of larvae ($g = 0.1$). Control failure is assumed to occur when either the frequency of the *R* allele reaches 0.5 ((a) and (b)), or the density in *Bt* fields reaches 50% of the stationary density before the introduction of *Bt* crops ((b) and (d)). Four-thousand simulations were run with $g = 1$ and $g = 0.1$ selecting values of $r_{1m} = r_{2m}$, $r_{1f} = r_{2f}$, $F_1 = F_2$, and Q from uniform random distributions with ranges [0.1, 1], [0.1, 1], [10, 50], and [10%, 50%], respectively, and $h = 10^x$ where x was selected from a uniform distribution with range [− 4, – 0.5]. For parameter combinations in (a) and (b), the purely susceptible population in the presence of insecticides ($g = 0.1$) is persistent, whereas for (b) and (d), a purely susceptible population would go extinct. No purely susceptible populations went extinct in the absence of insecticides ($g = 1$). Survival of susceptible *SS* individuals is assumed to be zero in *Bt* fields ($k = 0$). Other parameter values are: $L = 1$, $a = 4$, $b = 0.7$.

was ≤ 20 generations. In these cases, insecticides decreased the time to failure by only 1–2 generations. When the susceptible population is not persistent, however, spraying insecticide in refuges can substantially decrease the time to control failure when measured by the *R* allele frequency (Fig. 4c).

In contrast to these results, when control failure is measured by population density, insecticides on average increase the time to control failure, with this increase being greater for non-persistent than for persistent cases (Figs 4b,d). For the case of non-persistent susceptible populations, very low densities were reached before the frequency of the *R* allele was high enough to allow the population to increase. Therefore, it often took a much longer time for the population to rebound to densities near those before *Bt* crop introduction.

The requirement that *Bt* fields are highly toxic to susceptible *O. nubilalis* genotypes (survival, $k < 10^{-3}$) is satisfied for common commercial transgenic *Bt* maize varieties (based on the Mon 810 and Bt-11 transformation events) (Andow 2001). Looking beyond *O. nubilalis*, *Bt* cotton causes high mortality to *Heliothis virescens*; resistance is probably rare and largely recessive (Gould 1991; Gahan *et al.* 2001); and movement of this pest between fields can be extensive (Haile *et al.* 1975; Schneider 1999). Therefore, these conclusions for the high-dose/refuge strategy for *Bt* maize likely also apply in some cases to *Bt* cotton. Of course, there are numerous other factors that could influence the effect of insecticide application in refuges on the rate of resistance evolution, such as whether insecticides are applied only when pest densities exceed their economic threshold. Nonetheless, the lack of a strong effect of insecticide use in refuges when the conditions of the high-dose/refuge strategy are met is a general result of the analytical and simulation models we have studied.

IMPLICATIONS

The high-dose/refuge strategy for resistance management is thought to delay resistance because the refuge provides a source of susceptible insects to mate with resistant ones so their offspring will be *RS* heterozygotes that are killed by *Bt* maize (Anonymous 1998; Gould 1998). While elements of this explanation are correct, it is incomplete, and it can lead to incorrect conclusions about factors affecting resistance evolution. Specifically, this explanation leads to two major recommendations for the design of resistance management strategies. First, movement from refuges should be encouraged to ensure random mating between susceptible adults from the refuge and resistant adults in *Bt* fields (Roush 1997; Gould 1998). Second, if refuges are sprayed with insecticides, they would provide a smaller number of susceptible insects to mate with resistant individuals in *Bt* fields, thereby reducing the benefit of the refuge (Roush 1989; Ostlie *et al.* 1997; Roush 1997; Gould & Tabashnik 1998; Shelton *et al.* 2000). Our results demonstrate that these recommendations are not general and are based on an incomplete understanding of the processes affecting resistance evolution under the high-dose/refuge strategy.

We have developed a general second-order approximation to a wide class of patch models for insect resistance evolution, in which selection occurs on larvae, followed by density dependent mortality and dispersal of adults among patch types. Using this approximation, we have shown that three processes are involved in resistance evolution in the high-dose/refuge strategy. By reducing the exposure of the susceptible population to *Bt* crops, refuges slow resistance evolution by decreasing selection favouring resistant phenotypes. Therefore, reducing dispersal between refuge and *Bt* fields will decrease the selection intensity for resistance (Δs), delaying resistance evolution. Reduced dispersal will also generate assortative mating (η) and change the mating success of males carrying the *R* allele (μ), processes that occur because of the non-uniform *R* allele frequencies among different patch types. Under typical high-dose conditions, assortative mating will accelerate resistance evolution whereas changing the mating success of males with the *R* allele will delay it; the magnitude of these two effects may be difficult to generalize. Therefore, although we can conclude that increasing movement among *Bt* and refuge fields does not necessarily delay resistance evolution, we cannot conclude that it will always increase the rate of resistance evolution. Nonetheless, equation 2 may help to derive a general understanding of factors governing the consequences of movement among field types, suggest novel approaches for resistance management, and support results from simulation models such as those by Caprio (2001).

Our analyses also show that, for the important, high efficacy ($k < 10^{-3}$) subclass of the high-dose/refuge strategy, the null expectation should be that spraying insecticides in the refuges has no effect on resistance evolution. This expectation comes from the simple argument that spraying insecticide in the refuges will not change the proportion of the susceptible population exposed to *Bt* toxins, and therefore will not change the intensity of selection for resistance. Although we view this as the null expectation, our analyses show that this expectation can be modified by several factors. If the susceptible population is not persistent, then the refuge will not contain a permanent population much greater than that found in the *Bt* fields, and the time to control failure of *Bt* crops depends on the relative rates at which the total population declines and the *R* allele spreads among the survivors. There are also cases under limited dispersal where insecticide spraying slightly accelerates resistance evolution, although this does not occur across a wide range of parameter values in the simulation model, and when it does occur, the reduction in time to control failure is generally only 1–2 generations. Finally, we caution that our results are restricted to the case of high efficacy, high-dose *Bt* crops. Simulations show that when the survival of susceptibles in *Bt* fields exceeds 10^{-3}, spraying refuges begins to increase the rate of resistance evolution.

The key to successful resistance management is decoupling the benefits of the management strategy (durability of *Bt* crops) from the potential costs (increased pest abundance). Understanding the three processes affecting resistance evolution may increase the diversity of resistance management practices to supplement the high-dose/refuge strategy. For example, because male dispersal does not affect the strength of selection for resistance (s_0 and Δs in equation 2), manipulating male dispersal or male mating propensities may delay resistance evolution (Andow & Ives 2002) if the deceleration in resistance evolution caused by reducing *R* male mating success (μ) outweighs the acceleration caused by increasing the proportion of *RR* homozygotes in the population (η). As another example, equation 2 focuses attention on the role of selection in resistance evolution, s_0 and Δs, where selection depends on the proportion of the female population killed by *Bt* toxins. As demonstrated for the case of spraying insecticides in refuges, the proportion of the population killed by *Bt* toxins does not necessarily depend strongly on the size of the population, implying that there may be other management strategies in which *Bt* crops reduce population size without greatly increasing the rate of resistance evolution. Finally, we note that, even though our discussion has centred around only two types of fields (*Bt* fields and refuges), equation 1 can be applied to any number of field types. Therefore, the same three processes – difference in fitness, assortative mating, and variation in male mating success – can be used

to understand more complex spatial structures, and correspondingly more complex management strategies.

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SUPPLEMENTARY MATERIAL

The following material is available from http://www.blackwell-science.com/products/journals/suppmat/ELE/ELE392/ELE392sm.htm:

Appendix S1 Simulation model of resistance evolution

Appendix S2 Approximation to the model of resistance evolution

APPENDIX

The terms of equation 2 are

$$
\begin{aligned}
s_0 &= \frac{1}{\bar{p}(t)} X^* \left\{ (\boldsymbol{\Theta}_{\mathbf{RR}} - \mathbf{I}) \mathbf{M}_0 (P(t) \times (\mathbf{M}_0 P(t))) \right. \\
&\qquad \left. + \frac{1}{2} \boldsymbol{\Theta}_{\mathbf{RS}} \mathbf{M}_0 [\mathbf{I} + \mathbf{M}_0] P(t) \right\} - 1 \\
\Delta s &= \frac{1}{\bar{p}(t)} X^* \left\{ (\boldsymbol{\Theta}_{\mathbf{RR}} - \mathbf{I})(\mathbf{M}_\mathbf{f} - \mathbf{M}_0)(P(t) \times (\mathbf{M}_0 P(t))) \right. \\
&\qquad \left. + \frac{1}{2} \boldsymbol{\Theta}_{\mathbf{RS}} (\mathbf{M}_\mathbf{f} - \mathbf{M}_0)[\mathbf{I} + \mathbf{M}_0] P(t) \right\}, \\
\mu &= \frac{1}{\bar{p}(t)} X^* \left\{ (\boldsymbol{\Theta}_{\mathbf{RR}} - \mathbf{I}) \mathbf{M}_\mathbf{f} (P(t) \times (\mathbf{M}_0 (\mathbf{M}_\mathbf{m} - \mathbf{M}_0) P(t))) \right. \\
&\qquad \left. + \frac{1}{2} \boldsymbol{\Theta}_{\mathbf{RS}} \mathbf{M}_\mathbf{f} [\mathbf{M}_\mathbf{m} - \mathbf{M}_0] P(t) \right\}, \\
\eta &= \frac{1}{\bar{p}(t)} X^* \left\{ (\boldsymbol{\Theta}_{\mathbf{RR}} - \mathbf{I}) \mathbf{M}_\mathbf{f} (P(t) \right. \\
&\qquad \left. \times ((\mathbf{I} - \mathbf{M}_0)(\mathbf{M}_\mathbf{m} - \mathbf{M}_0) P(t))) \right\}, \qquad \text{(A1)}
\end{aligned}
$$

where X^* is the row vector containing the relative densities in each field type that would occur at stationarity for a purely susceptible population; specifically, if x_i^* is the density in field type i ($i = 1, \ldots, n$), then $X^* = (q_1^*, q_2^*, \ldots, q_n^*)$ where $q_i^* = x_i^*/(x_1^* + x_2^* + \cdots + x_n^*)$. The matrix $\mathbf{M}_0$ gives the redistribution of males and females when there is complete mixing; each element in column i of $\mathbf{M}_0$ equals q_i^*.

The term s_0, which gives the case of complete mixing, can be shown to simplify to

$$s_0 = \sum_{i=1}^{n} ((\theta_{RR,ii} - 1)\bar{p}(t) + \theta_{RS,ii}) q_i^* - 1, \qquad \text{(A2)}$$

where $\theta_{RR,ii}$ and $\theta_{RS,ii}$ are the diagonal elements of $\boldsymbol{\Theta}_{\mathbf{RR}}$ and $\boldsymbol{\Theta}_{\mathbf{RS}}$, respectively. For the specific case of the simulation model (Fig. 1, Appendix S1), this becomes

$$s_0 = \left(\frac{L}{k} - 1 \right) \left(\frac{x_1^*}{x_1^* + x_2^*} \right) (\bar{p}(t) + h) \qquad \text{(A3)}$$

Equation 3 is derived from this expression in the limit as $k \to 0$.

The components Δs, μ, and η can be explained by considering the consequences of female vs. male movement. Female movement governs the distribution of offspring among field types and thereby determines the proportion of susceptibles in *Bt* fields. Hence, changes in natural selection favouring resistance (Δs) depend on the deviation of female movement from random $\mathbf{M}_\mathbf{f} - \mathbf{M}_0$. Because in our model only males disperse before mating, they alone determine the effects of assortative mating (η) and male mating success (μ) which depend on the deviation of male movement from random $\mathbf{M}_\mathbf{m} - \mathbf{M}_0$. To clarify the partitioning between η and μ, we note that the mean change in allele frequency in males due to limited dispersal is $\mathbf{M}_0(\mathbf{M}_\mathbf{m} - \mathbf{M}_0)P(t)$, a vector with identical top and bottom elements. Therefore, Hardy–Weinberg equilibrium is satisfied for any $P(t)$ giving the allele frequencies in females before female dispersal, and $P(t) \times (\mathbf{M}_0(\mathbf{M}_\mathbf{m} - \mathbf{M}_0)P(t))$ is the Hardy–Weinberg frequencies of homozygous resistant offspring accounting for differential male mating success. By subtracting this effect from the overall effect associated with limited male dispersal, we obtain $P(t) \times ((\mathbf{I} - \mathbf{M}_0)(\mathbf{M}_\mathbf{m} - \mathbf{M}_0)P(t))$, from which we calculate the change in R allele frequency caused by assortative mating.

Although equations 1 and 2 are derived for the case in which females disperse after they mate, the equations can be modified in a straight-forward manner for the case in which females also disperse before mating (Appendix S2).