Preventing insect adaptation to insect-resistant crops: are seed mixtures or refugia the best strategy?

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SUMMARY

Transgenic crops expressing insecticidal toxins could soon provide safe, clean and effective means of pest control, but their usefulness will be short-lived if insects adapt to the toxins. Two planting strategies are among those that have been recommended to delay crop failure: susceptible insects could be conserved by planting either 'refugia', i.e. separate fields of toxic and toxin-free crop, or 'seed mixtures' of toxic and toxin-free plants in the same fields. However, we show that if insects can move from plant to plant, seed mixtures may actually hasten insect resistance compared with pure stands of toxic plants. Insect movement causes an increase in effective genetic dominance which can counteract reduced selection due to the mixture. This failure of seed mixtures is likely under just those conditions, low genetic dominance of resistance, which predict a good chance for resistance to the toxin to evolve slowly. Seed mixtures, unlike refugia, are therefore failure prone. This result also suggests potential problems with a third strategy, tissue-specific expression of toxins, which essentially provides a mixture of toxin-free and toxin-containing tissues on the same plant. However, better information and modelling are urgently required to evaluate alternative means of slowing insect adaptation to resistant crop plants. Legislation for toxin-free refugia may provide one of the best available means for conserving insect susceptibility.

1. INTRODUCTION

In the next few years, genetically engineered insect-tolerant crops containing endotoxins from *Bacillus thuringiensis* will be available commercially. These plants will have enormous environmental advantages over current pest control techniques, which often rely on multiple applications of broadcast insecticides. Field tests with endotoxin-containing cotton on private farms are already being done by Monsanto in Mississippi and other areas in the U.S.A. But a major potential problem with the use of these insect-resistant crops is the possibility of insect adaptation, which could render the plants useless in a small number of insect generations (Gibbons 1991; Harris 1991). Transgenic insect control may be especially vulnerable because insects are treated with a high dose of toxin even when they are not causing economic damage. Researchers have recommended a number of strategies to delay the build-up of resistance to these crops. Two potentially valuable planting strategies to reduce selection are: (i) to grow some stands of the traditional toxin-free crop alongside toxin-containing crop to maintain a 'refugium' of susceptible insect populations (Comins 1977; Georghiou & Taylor 1977; Wood & Mani 1981; Tabashnik & Croft 1982; Mani 1985, 1989; Gould 1986; Curtis 1987; Roush & McKenzie 1987; Mallet 1989; Altman *et al.* 1992); and (ii) to use 'seed mixtures' of toxin-free and toxin-containing plants within fields (Gould 1988, 1989; Altman *et al.* 1992). A third strategy, 'tissue-specific expression', advocated especially by Gould (1988, 1989, 1991), is similar to seed mixtures in that a mixture of toxin-free and toxic tissues is used within plants; the theory for seed mixtures developed here is applicable to tissue-specific expression as well. Seed mixtures have already been used in field trials of transgenic cotton in Mississippi. The usefulness of seed mixtures (or tissue-specific expression) and refugia is investigated in the following model.

2. THE MODEL

Suppose that an insect has a two-stage life cycle. Assuming toxin-free seed is a fraction V of the total in the crop area, and oviposition is at random, a fraction $(1-V)$ of eggs laid in the transgenic mixture will be placed on toxic plants. It is assumed that with probability M an insect will move to another plant after the first stage. In both stages, the insect consumes parts of the plant on which it finds itself, and suffers mortality according to its stage-specific susceptibility to any toxin present. At the end of the second stage, the insect matures, mates at random with other members of the population (including those raised in the toxin-free refugia, fraction C), and subsequently lays eggs at random on the toxic and toxin-free crop and in the refugia. This life cycle is simplified from that of larval pests such as *Heliothis* on cotton, but may also apply approximately to adults of insects such as Colorado potato beetles (*Leptinotarsa decemlineata*). 'Stages' are not intended to correspond to larval instars; in a

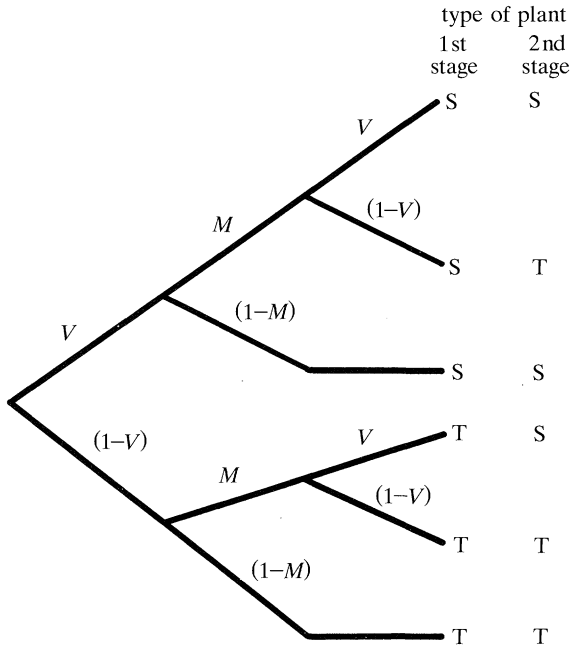

Figure 1. Complete probability tree for insect development on susceptible (S) and toxic (T) plants in first and second stage when exposed to a seed mixture. For example, the proportion of larvae developing on toxic plants in both stages is given by the two bottom branches of the tree. The first stage is laid on a toxic plant with probability $1-V$. Subsequently, it may move with probability M, in which case it ends up on a toxic plant again in the second stage with probability $1-V$; the probability of the fifth branch is thus $(1-V)\,M(1-V)$. Alternatively, the insect may not change plants, with probability $1-M$; the probability of this bottom branch is then $(1-V)\,(1-M)$. Together, the proportions of individuals given by these two branches sum to $(1-V)\,M(1-V)+(1-V)\,(1-M)$. If we are considering, say, heterozygotes for the resistance gene, the fitness of these individuals exposed to toxic plants in both first and second stage will be $[1-(1-h_1)\,s_1]\,[1-(1-h_2)\,s_2]$. Finally, the sum of all probabilities, each one multiplied by its respective fitness, gives the overall survival of the heterozygous genotype in the cropping area, as shown in the text.

lepidopteran, stage 1 might be roughly equivalent to instars 1–early 5, and stage 2 might correspond to late instar 5.

Consider an insect allele for resistance, A_R, and the susceptible allele at the same gene, A_S. Insecticide resistance in the field is rarely perfectly monogenic but usually seems to consist of one or a few genes with major effects on fitness, and a larger number of minor modifiers, so that single-gene models are usually appropriate (Roush & McKenzie 1987; Mallet 1989). Firko (1991*a*, *b*) has claimed that single-gene inheritance of resistance is a 'myth'; however, the abundance of cases of resistance at genes for the protein targets of insecticide action suggests that single-locus models are most relevant. Insect resistance so far confirmed against bacterial endotoxins includes altered targets (Van Rie *et al.* 1990; Van Rie 1991). Three insect genotypes at such a locus are then possible: $A_S A_S$, $A_R A_S$, and $A_R A_R$. In the first stage these will have survival (relative fitnesses) $1-s_1$, $1-(1-h_1)\,s_1$, and 1 with respect to the toxins on the toxic plants, and all will have fitness 1 on toxin-free plants. In the second stage, the genotypes will similarly have fitnesses $1-s_2$, $1-(1-h_2)\,s_2$, and 1 on toxic plants. The parameters h_1, h_2 are the stage-specific genetic dominances of resistance, and s_1, s_2 are the stage-specific selection coefficients. These stage-specific fitnesses are assumed multiplicative across stages, as are the probabilities of movement and oviposition, because all probabilities will be independent to a first approximation (the assumption of independence can of course be relaxed, but this would require further parameters). Other types of mortality are assumed constant across genotypes and plants, so that the relative fitnesses here represent only selection by the insecticidal plant, and include selection against resistance in the absence of the toxin. It is assumed here that $0 \leqslant h_1, h_2, s_1, s_2 \leqslant 1$, as selection is likely to be directional. Then, combining across the life cycle, expressions for the overall ('effective') dominance H of A_R, and overall selection pressure S, which determine the rate of evolution, can be found in terms of V, M, h_1, h_2, s_1, and s_2 as follows.

Consider the probability that an insect spends both first and second stage on toxic crop individuals, ignoring refugia for the moment. The probability that it is laid as an egg on a toxic plant, and therefore spends its first stage there, is $1-V$. With probability $1-M$ it will complete its life cycle on this same plant. If it moves, with probability M, it may establish on another toxic plant, again with probability $1-V$. Thus, for example, the fraction on toxic plants for both stages is $(1-V)\,(1-M)+(1-V)\,M(1-V)$. The entire range of possibilities is shown in figure 1. Survival for $A_S A_S$ individuals spending both stages on toxic plants will be $(1-s_1)\,(1-s_2)$; for $A_R A_S$ this is $[1-(1-h_1)\,s_1]\,[1-(1-h_2)\,s_2]$, and for $A_R A_R$ it is 1. In contrast, all individuals spending both stages on toxin-free plants will have fitness 1. After adding all the other possibilities, the overall relative fitness of $A_S A_S$ insects within the crop (ignoring refugia) is:

$$W_{SS} = V^2M + V(1-M) + V(1-V)\,M(1-s_1) + V(1-V)\,M(1-s_2) + (1-V)^2\,M(1-s_1)\,(1-s_2) + (1-V)\,(1-M)\,(1-s_1)\,(1-s_2),$$

which simplifies to:

$$W_{SS} = 1-(1-V)\,(s_1+s_2)+\{1-V[1+M(1-V)]\}\,s_1\,s_2.$$

Similarly,

$$W_{RS} = 1-(1-V)\,[(1-h_1)\,s_1+(1-h_2)\,s_2] + \{1-V[1+M(1-V)]\}\,(1-h_1)\,s_1(1-h_2)\,s_2,$$

and $W_{RR} = 1$, by definition. The overall selection against $A_S A_S$ insects in the crop is $S = 1-W_{SS}$, and the overall effective dominance of the resistance allele is $H = 1-(1-W_{RS})/S$, leading to a standard single-locus model with $W_{SS} = 1-S$, $W_{RS} = 1-(1-H)\,S$, and $W_{RR} = 1$.

In contrast to this rather complex effect of larval movement in a seed mixture, a refugium has the simple effect of reducing the overall selection, S, without altering overall dominance, H. A refugium, C, provides an unselected part of the population so that $W_{SS} = 1-(1-C)\,S$ and $W_{RS} = 1-(1-C)\,(1-H)\,S$.

Thus, with primes representing the overall effect including the effect of refugia, $S' = (1-C)S$; and $H' = H$. It is readily seen that if there is no larval movement, the seed mixture V acts as a refugium. For example, inserting $M = 0$ into the formulae for fitness above gives overall selection $S = (1-V)(s_1+s_2-s_1s_2)$, whereas effective dominance is $H = 1-\{[(1-h_1)s_1+(1-h_2)s_2-(1-h_1)s_1(1-h_2)s_2]/(s_1+s_2-s_1s_2)\}$, which does not depend on V. In the absence of insect movement between plants, refugia or seed mixtures thus reduce overall selection S by a fraction C or V, respectively, without altering dominance. This equivalence is of course expected, because with movement $M = 0$ both refugia and mixtures simply reduce the proportion of the population exposed to the toxin.

3. RESULTS

It is somewhat difficult to analyse this standard population genetic model when overall effective dominance H is nearly but not exactly zero (May & Dobson 1986). However, it is easy to show numerically that adding toxin-free plants to a seed mix can hasten insect resistance. The generation in which resistance is said to have evolved is here defined operationally to be the generation in which the frequency, q, of a resistance allele reaches or exceeds 50%. The results of figures 2 and 3 were obtained by iterating the genetic model in double precision on a personal computer. Resistance can evolve faster in seed mixtures than in toxic pure stands when h_1, h_2 are low and $s_1 s_2$ are high, and where $h_1 \approx h_2$ and $s_1 \approx s_2$ (figure 2). Similarly, when h_1 is low and s_1 is high, there are a range of possible values of h_2 and s_2 which cause more rapid failure of the transgenic crop when there is a seed mixture, $V > 0$ (figure 3). It is known that the refugium strategy might be more useful than the same proportion of toxin-free crop grown in a seed-mixture strategy because, in the latter case, individuals might move between toxic and toxin-free plants (Gould 1988, 1989; Gould *et al.* 1992; R. G. Luttrell, personal communication), and thereby increase the survival of heterozygotes for rare resistance alleles. However, it has not been realized that using toxin-free seed in a transgenic crop (or tissue-specific expression of a toxin) to reduce selection may paradoxically increase the rate of evolution of resistance in the pest compared with planting pure stands. These conditions occur with high s_1, s_2 and low H (with H near but not equal to 0), and seem likely or at least possible on the basis of the arguments about the strength of selection and dominance given below.

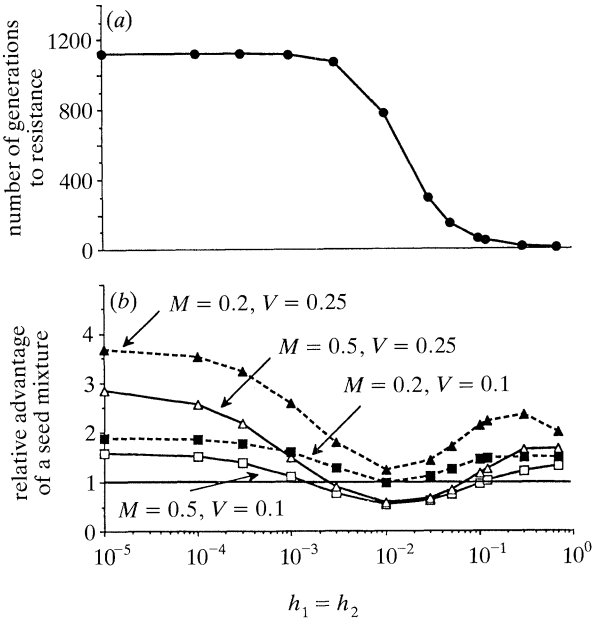

Figure 2. (*a*) No seed mixture. Time until evolution of resistance ($q = 0.5$) when $C = 0.1$, $V = 0$, $s_1 = s_2 = 1.0$, initial gene frequency, $q_0 = 0.0001$, for various values of $h_1 = h_2$. The figure shows the existence of a threshold level of dominance ($h_1 = h_2 \approx 0.01$) below which resistance evolution is effectively recessive. (*b*) Advantage of two rates of seed mixtures V, given two rates of movement between plants M, relative to the time to resistance when $V = 0$ under the same conditions as in (*a*), for various values of $h_1 = h_2$. For example, with $h_1 = h_2 = 0.01$, if $V = 0$ then the number of generations to resistance is 779. If $V = 0.1$ and $M = 0.5$ then the number of generations to resistance is 419, so the relative advantage of a seed mixture with 10% susceptible is $419/779 = 0.54$; in this case the 'advantage' is less than 1 (solid horizontal line), i.e. there is a disadvantage to a seed mixture. The figure shows the region near $h_1 = h_2 = 0.01$ where there is a clear disadvantage to a seed mixture when $M = 0.5$.

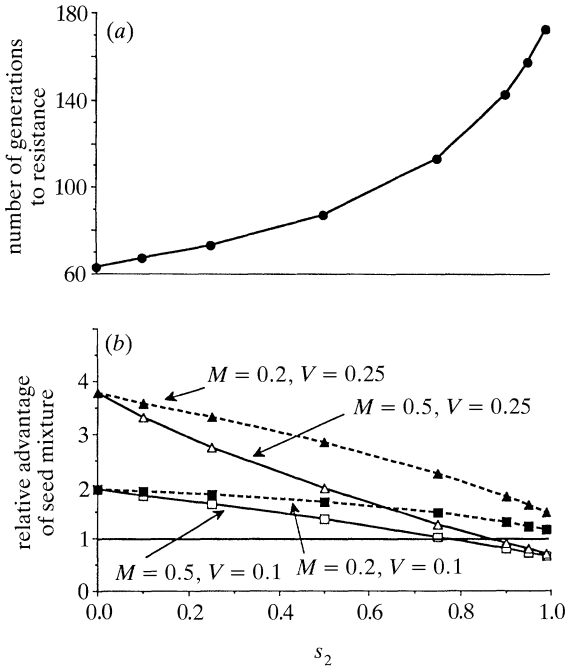

Figure 3. (*a*) No seed mixture. Time until evolution of resistance with $C = 0.1$, $V = 0$, $h_1 = 0.01$, $s_1 = 0.99$, $h_2 = 0.1$, and $q_0 = 0.0001$, for various levels of selection in the second stage, s_2. This figure shows the masking effect of higher levels of s_2, which paradoxically increases the overall time to resistance, because of the reduction in effective dominance H as s_2 increases. (*b*) Advantage of two rates of seed mixture V, given two rates of larval movement M, relative to the situation where $V = 0$ for various values of s_2 (see figure 1 for further explanation). The figure shows a region where high late-stage selection ($s_2 > 0.8$–0.9) can mask appreciable late-stage dominance ($h_2 = 0.1$) in the absence of seed mixtures, giving an overall advantage to a pure stand of the transgenic plant when $M = 0.5$.

Figures 2 and 3 also show that movement between plants (or tissues) must be high ($M \geqslant \approx 0.2$) for pure stands to perform better than seed mixtures, so that seed mixtures could be beneficial under many circumstances other than those which cause more rapid failure.

The paradoxical failure of seed mixtures to delay resistance if larvae move can be understood intuitively as follows. A mixture containing some plants without toxin is obviously expected to reduce selection, S. However, in insecticide resistance, effective dominance H is a negative function of dose (or in this case time of exposure to a constant dose) and so H can be increased in heterozygous larvae move between toxic and non-toxic plants. Dominance strongly affects resistance evolution: when a resistance allele is rare, its homozygote will be far rarer, so that increased survival of the merely rare heterozygotes will greatly speed the important early stages of evolution, provided that there is an appreciable refugium ($C \approx 5\,\%$) of untreated larvae (Curtis 1987; Roush & McKenzie 1987; Mallet 1989). The reduction in selection caused by the seed mixture can then potentially be outweighed by a steep increase in dominance. There is a threshold dominance of about $h_1 \approx h_2 \approx 0.01$ below which resistance is effectively recessive and the evolution of resistance takes an extremely long time (see figure 2*a*). If movements between plants cause effective dominance to increase near this threshold, the reduction in selection due to a seed mixture can be counteracted by an increase in effective dominance, causing more rapid evolution of resistance. In general, high selection pressures and low levels of dominance in two (or more) stage-specific fitnesses will mask each other, making selection for resistance almost completely recessive overall ($H \approx 0$). When insects spend only part of their larval life on a toxic plant because they can move, increases in overall effective dominance can have a catastrophic effect on resistance evolution.

4. DISCUSSION

Figures 2 and 3 demonstrate that the seed mixture strategy under some conditions can give worse results than no strategy at all. These conditions are high levels of selection ($s_1, s_2 \approx 1$; although overall selection, S, S', may be lower), low levels of dominance ($H \approx 0$), and intermediate to high levels of movement ($M \geqslant \approx 0.2$).

But how likely are these conditions? Experience with insecticide resistance suggests that insect resistance alleles will be at low frequency initially, say $\leqslant 0.001$, and that selection on each allele will be high in the presence of insecticide (Curtis *et al.* 1978; Curtis 1987; Roush & McKenzie 1987; Gould 1991). It is not so clear what the stage-specific and overall dominances, h_1, h_2, and H of resistance alleles will be. If the insect resistance were completely recessive ($h_1, h_2, H = 0$), it could take thousands of generations for resistance to evolve, but this time would drop precipitously if resistance was partly dominant (Georghiou & Taylor 1977; Wood & Mani 1981; Tabashnik & Croft 1982; Mani 1985; Curtis 1987; see figure 2*a*). Therefore the earliest resistance alleles to evolve will almost always be partly dominant. However, if the toxin is a useful insecticide, any highly effective insect alleles for tolerance must be relatively rare. Over all genes, it seems likely that alleles causing low levels of tolerance are commoner than alleles causing high levels of tolerance. It is known that alleles causing low levels of tolerance will also be more effectively recessive than those causing high tolerance, for a given dose of toxin, because genotypes with but a single low tolerance allele will still have high mortalities (Curtis *et al.* 1978; Curtis 1987). Combining these arguments, the first insect resistance alleles to evolve will probably be those whose genotypic tolerances make them nearly, but not completely, effectively recessive. With recessive resistance, conditions are also best for a delay in resistance evolution using the refugium strategy (Curtis 1987).

Movement rates of crop pests are poorly known; in pests like *Heliothis* on cotton, larval movement will depend on planting density and other factors. However, it seems at least possible that movement is high enough in some pest–crop systems to have the effect found here, especially if the movement is between parts of the same plant as in the tissue-specific expression strategy. The model assumes only two stages, whereas in a real pest movement might occur continuously over a period of time. *Heliothis* larvae may avoid artificial diets containing bacterial endotoxins; this means that insect movement between plants might not be independent of whether plants contain toxin, as assumed here. If this is the case in the field, pest evolution could be slowed when a choice of food is provided via a seed mixture or tissue-specific expression (Gould & Anderson 1991; Gould *et al.* 1991). However, because the choice may also increase effective dominance, results will be unpredictable and situation dependent. More information on particular insect life histories, movement between plants, avoidance of toxins, stage-specific mortalities, possible gene dominance, the effect of multiple loci, and the actual strength of selection are needed. More complex models will also be required to understand more realistic situations.

The model of transgenic crop failure proposed here is deliberately simplified. However, because some possible, perhaps even likely, conditions can cause seed mixtures or tissue-specific expression to perform worse than pure-line transgenic crops, these strategies are inherently risky. Sadly, the strategies are most risky when there is a good chance that insects will take a long time to adapt, i.e. when insect resistance alleles are nearly completely recessive. But seed mixtures or tissue-specific expression are also among the few strategies that companies selling bioengineered plants can implement; seed companies can hardly expect farmers to plant refugia for resistance management if short-term economic considerations dictate planting a pure-line transgenic crop. Even in the absence of detailed knowledge, a good case can therefore be made for legislation to enact refugia. Refugia always perform as well as or better than seed mixtures (or tissue-specific expression) for a given level of selection reduction, because they do not increase effective dominance. Refugia would be in the public interest, but it is hard to imagine farmers or industry voluntarily

creating refugia if they are competing against other farmers or companies not promoting such strategies. Legislation could take the form of an expanded 'set-aside' program, under which land is currently left fallow to maintain crop prices. In the modified program, pure toxin-free seed could be grown on a fraction (possibly 5–10%) of cropland, which would provide a sufficient refugium of insect susceptibility to reduce selection. The program would be enhanced if broadcast pesticide use were prohibited on this land. The ban should, for obvious reasons, include *Bacillus thuringiensis* and derivatives, and pest control should be only by means of 'bio-rational' or 'ecologically sustainable' means such as cultural practices, biological control, and control using pheromones or bait trapping. Crops should be grown on this new set-aside land to ensure survival of susceptible pests. This program would provide refugia for susceptibility genes to all present and future toxin-based means of pest control, including pesticides as well as transgenic crops, so the measures would not unfairly favour new technology. A useful by-product of the modified program would be a great encouragement of research into sustainable pest control to be applied in refugia. In general, it seems clear that wildlife and fisheries conservation philosophy could and should be applied to endangered susceptibility genes within pests, as well as to endangered biodiversity in natural habitats. Both types of threatened resources will be lost if we fail to act for their preservation.

We are very grateful to F. Gould, D. G. Heckel, S. D. Knapp, A. Liebowitz, R. G. Luttrell, A. Pomiankowski, R. Roush and J. C. Schneider for comments and discussions. The work benefited from USDA-CSRS grant 90-37250-5358 to J. M., and Hatch funds from Mississippi State University.

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Received 17 August 1992; accepted 17 September 1992