III digestion (Promega Erase-a-Base System) of bases 4210 through 4405 (clone pDKΔ4210), followed by digestion and fill-in of the Bbs I site at nucleotide 1198 and the Apa I site in the polylinker outside of ORFIII. This fragment was cloned into the blunted Bam HI site of pPL2.7. The plasmid pDK3 is an Apa I–Pfl MI fragment from pDKΔ4210 inserted into the deletion clone pDKΔ2013 at the Apa I and Aat II sites of the polylinker. The outer four bases of the 3′ end of the Pfl MI end and the outer five bases of the 3′ end of the Aat II end were removed with T4 DNA polymerase (Pharmacia Biotech) before ligation. This resulted in an in-frame deletion of 525 amino acids and addition of a codon for an arginine residue. The insert was excised from this construct with Apa I and Bbs I and cloned into pPL2.7 (as for pDK2). For pDK4, pDKΔ4210 was digested with Bbs I and Bsa WI, and the ends of the excised fragment were end-filled and cloned into pPL2.7 (as for pDK2). Junctions of all constructs and pDK1 were sequenced with an ABI PRISM 310 genetic analyzer.

27. Cell lines were transformed as described in (*25*). After transformation, samples were divided in two, and each portion was plated onto solid BG-11 medium [M. M. Allen, *J. Bacteriol.* **96**, 836 (1968)] containing kanamycin (25 μg/ml). Plates were incubated at 25°C in 35 $\mu E\ m^{-2}\ s^{-1}$ of either constant red light (Westinghouse 20-watt red fluorescent tube, F20T12/R) or constant green light (Westinghouse 20-watt green fluorescent tube, F20T12/G) for 2 weeks.
28. Database searches were conducted with the BLAST Network Service at the National Center for Biotechnology Information [S. F. Altschul, W. Gish, W. Miller, E. W. Myers, D. J. Lipman, *J. Mol. Biol.* **215**, 403 (1990)]. Alignments were performed with the Bestfit program from the GCG Wisconsin Sequence Analysis Package, Madison, WI.
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31. We acknowledge the Carnegie Institution of Washington for providing an enlightened research environment and, in particular, W. Briggs, who has always shared his enthusiasm and encouragement with us and has been instrumental in bringing this work to fruition. We also thank E. Casey, D. Bhaya, and B. Kehoe for thoughtful discussions and K. Bump for help in preparing the manuscript. Supported by a NSF 1993 Postdoctoral Fellowship in Plant Biology to D.M.K. and NSF award MCB 9513576 to A.R.G. The GenBank accession number for *rcaE* is U59741. This is Carnegie Institution of Washington publication number 1303.

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TECHNICAL COMMENTS

Evolution of Insect Resistance to *Bacillus thuringiensis*–Transformed Plants

More than 30 crop species have been genetically engineered to express *Bacillus thuringiensis* endotoxins which are highly toxic to specific insect pests (*1*). However, several insect species have evolved resistance to *B. thuringiensis* toxins, and resistance evolution could seriously compromise the success of *B. thuringiensis*–transformed crops in controlling pests (*2*).

Recently, D. N. Alstad and D. A. Andow (*3*) proposed a strategy to slow the rate of resistance evolution in the European corn borer to *B. thuringiensis*–transformed maize. Below, I demonstrate that their conclusions are based on an inappropriate comparison of models. Then I use a general model to demonstrate why their strategy does not substantially reduce resistance evolution. I conclude that changing the distribution of toxic plants among fields is not a silver bullet to combat resistance evolution.

A critical feature of corn borer natural history is its preferential migration into the most mature stands (the "preferred crop") during the first of its two annual generations. Alstad and Andow state that resistance evolution can be slowed by using *B. thuringiensis*–toxic plants in the preferred crop, thereby creating a "trap crop." Insect densities predicted by Alstad and Andow's model (Fig. 1A) are compared in their report to those obtained in a model without preference-biased migration (*4*). Because preference-biased migration concentrates insect densities, it increases density dependent mortality and reduces insect abundance. The improvement Alstad and Andow attribute to the "trap crop" strategy is actually caused by preference-biased migration itself (*5*).

The correct comparison of densities would be among cases having different distributions of toxic plants among fields, but retaining preference-biased migration. For example, consider the case when 72% of the fields contain toxic plants, regardless of whether they are preferred or nonpreferred (Fig. 1B), or the case when mixtures of toxic and nontoxic plants are used to reduce insect survival to the same rate in both preferred and nonpreferred crops (Fig. 1C) (*6*). In all three cases, the reduction in the total insect density is the same, so strategies can be evaluated in terms of the rate of the evolution of resistance (*7*). Alstad and Andow's strategy is only slightly better than the second (Fig. 1B), and worse than the third (Fig. 1C) example.

I developed a general model, appropriate for a large number of insect pests, to ask how the distribution of toxic plants among fields affects resistance evolution (*8*). The rate of resistance evolution increases with the average per capita reproductive potential of resistant insects, R, at the reduced insect density, n_{min}, created by mortality of susceptible insects (*9*). R is calculated as

$$R = rf\mathrm{F}[fK_1n_{\min}] + r(1-f)\mathrm{F}[(1-f)K_2n_{\min}] \quad (1)$$

where F is a function giving density-dependent survival, r denotes the insect's intrinsic rate of increase, f is the fraction of insects in the preferred crop, and K_1 and K_2 are the fractions of susceptible insects surviving the toxic plants in preferred and nonpreferred crops. This equation demonstrates the trade-off between reducing insect density and slowing resistance evolution. Because F is a decreasing function, lower $n_{\min}$ produces higher R and more rapid resistance evolution. It is possible to mitigate this trade-off by changing values of K_2 and K_1. In the nonpreferred crop, density dependence is weak (because low immigration produces smaller populations), and therefore the per capita reproductive potential of resistant insects is greater than in the preferred crop. Thus, R is lowest when the proportion of insects killed by toxic plants is higher in the nonpreferred crop ($K_2 \leq K_1$) (*10*).

An example for the rate of resistance

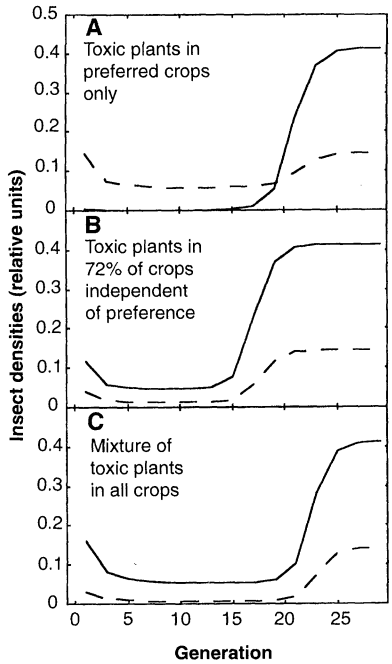

Fig. 1. Insect densities during the first annual generation in preferred (solid) and nonpreferred (dashed) crops. Densities increase around generation 20 because of the increase in resistance allele frequency. (**A**) From the model presented by Alstad and Andow (*1*) [with the typo in equation 9 in the report corrected: $X'''' = X'''(1 + aX''')^{-b}$] in which toxic plants in preferred crops reduce survival to 0.1%. (**B**) A modified model in which 72% of both preferred and nonpreferred fields contain toxic plants that reduce survival to 0.1%. (**C**) The case with density-independent survivals of $K_1 = K_2 =$ 15.25% in both preferred and nonpreferred crops.

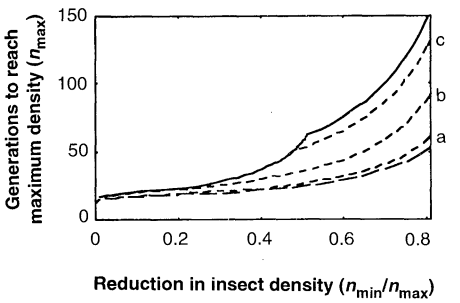

Fig. 2. From the general model, the number of generations required for the return of the insect population to 0.99 n_{max}, where n_{max} is the insect density before introduction of toxic plants. Solid line indicates the strategy of confining toxic plants to the nonpreferred field until $K_2 = 0$, and then adding toxic plants to the preferred fields. Dashed line, indicates the strategy of confining toxic plants to the preferred fields until $K_1 = 0$, and then adding toxic plants to the nonpreferred fields. Strategies a, b, and c (dotted lines) have the ratio $(1-K_2)/(1 - K_1) = 0.25$, 0.5, and 0.75, respectively. Other parameters are $r = 10$, $f = 0.8$, $h = 0$, and $F[x] = (1 + a\,x)^{-1}$ with $a = 9$.

evolution in the general model is given (Fig. 2). The strategy giving slowest resistance evolution always has $K_2 \leq K_1$. However, as found for the corn borer model (Fig. 1), there is little difference among strategies when substantial reductions (>80%) in insect density are desired. This result suggests that changing the distribution of toxic plants among fields has little potential for controlling resistance evolution. Therefore, other types of strategies to mitigate resistance evolution should be investigated (*11*).

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2. W. H. McGaughey and M. E. Whalon, *Science* **258**, 1451 (1992).
3. D. N. Alstad and D. A. Andow, *ibid.* **268**, 1894 (1995).
4. In the model without preference-biased migration, the rate of resistance evolution is roughly the same as in the model with preference-biased migration, but there is a much smaller reduction in total insect density.
5. Alstad and Andow imply that their results stem from greater frequency of heterozygotes than predicted at Hardy-Weinberg equilibrium. Although this can slow resistance evolution [H. N. Comins, *J. Theor. Biol.* **64**, 177 (1977)], Alstad and Andow's model assumes 95% of the population disperses each generation. Analyzing their model in more detail shows that this leads to a frequency of heterozygotes very close to Hardy-Weinberg equilibrium.
6. It is assumed that mixtures do not change the rate of resistance evolution within fields. Although mixtures potentially increase resistance evolution [J. Mallet and P. Porter, *Proc. R. Soc. London B* **250**, 165 (1992)], Alstad and Andow's argument is about the distribution of toxic plants among rather than within fields.
7. It is also possible to compare strategies by standardizing the rate of resistance evolution and calculating the maximum reduction in insect densities. However, the reductions in insect densities are so similar in the three cases that differences are not easily visible.
8. A general model for the evolution of resistance is

$$n_1 = f\{p_t^2 + 2p_tq_t[K_1 + h(1 - K_1)] + K_1q_t^2\}n_t$$

$$n_2 = (1 - f)\{p_t^2 + 2p_tq_t[K_2 + h(1 - K_2)] + K_2q_t^2\}n_t$$

$$n_{t+1} = r\,n_1F[n_1] + r\,n_2F[n_2]$$

$$p_1 = f\{p_t^2 + p_tq_t[K_1 + h(1 - K_1)]\}n_t$$

$$p_2 = (1 - f)\{p_t^2 + p_tq_t[K_2 + h(1 - K_2)]\}n_t$$

$$p_{t+1} = (rp_1F[n_1] + rp_2F[n_2])/n_{t+1}$$

where n_t and p_t are the total insect density and frequency of the resistance allele in generation t, and h is the level of dominance (dominant when $h = 1$, recessive when $h = 0$).
9. The rate at which a resistance allele is fixed from an initial frequency of p_0 is approximately proportional to $\log[Rp_0 + (1 - p_0)]$ when resistance is completely recessive, and $\log[1 + h(R - 1)]$ when not.
10. It is possible to prove for all functions F[ ] having the properties

$$\frac{\partial F[x]}{\partial x} < 0,\ \frac{\partial (xF[x])}{\partial x} > 0,\ \text{and}\ \frac{\partial}{\partial x}\left(x\frac{\partial F[x]/\partial x}{F[x]}\right) < 0$$

that the lowest value of R given n_{min} occurs when $K_2 \leq K_1$. A proof will be provided on request.
11. R. M. May, *Nature* **361**, 593 (1993); F. Gould, *Am. Sci.* **79**, 496 (1991); R. M. May and A. P. Dobson, in *Pesticide Resistance: Strategies and Tactics for Management* (National Academy Press, Washington, D.C., 1986) pp. 170–193.

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Response: Ives makes a formally correct argument that presents problems in practical application. He shows that evolution of resistance to genetically engineered crop varieties expressing insecticidal crystal (*cry*) proteins of *B. thuringiensis*, can be slowed by minimizing *cry*-induced pest mortality and maximizing pest mortality attributable to other causes. In an array of preferred and unpreferred fields, this can be accomplished by restricting *cry*-induced mortality in unattractive units (minimizing *cry*-toxin exposure and selection), and allowing insects to pile up in attractive, untreated refuges, maximizing the potentially beneficial effects of density-dependent mortality. We do not disagree with his argument. The problem with his analysis is that significant density-dependent mortality will be accompanied by significant crop losses. Growers are not in business to raise insect pests and may be unmotivated to plant attractive refuges.

We proposed the more practical opposite: restricting the *B. thuringiensis* crop to preferred fields (*1*). This reduces density-dependent mortality, increases *cry*-toxin selection, and as we showed in our paper, speeds the evolution of resistance relative to the case where insects exhibit no preference and movement is unbiased. Ives' alternative will delay the evolution of resistance more than either our scheme or the unbiased case; however, as Ives correctly states, differences in the evolutionary rates among these alternatives are very subtle. In contrast, with Ives' and our models, insect densities and damage in the refuge plots are very different. When the *cry* crop is used as an attractive trap crop, insects are drawn out of the refuge, reducing pest density and damage in the refuge. This extends pest protection afforded by a *B. thuringiensis* field into adjacent refuges, leveraging a grower's investment in transgenic seed and providing an economic incentive to plant a refuge. We suggested a strategy that does not maximize the delay of resistance because we believe that implementation is the principal challenge of resistance management. Growers will not adopt a recommendation that increases the risk of crop loss in their refuges, and regulatory imposition of such practices would be costly.

In addition to this basic difference of perspective, there are technical problems resulting from the assumptions underlying Ives' general analytical model. For example, the compression of our four-stage model into a single set of recursion equations imposes life history events in an inappropriate sequence. Ives' life history sequence is adult migration → selection → density-dependent survival → reproduction. The sequence of corn borer life history (and our model) is migration → reproduction → selection → density-dependent survival; Ives' assumption would cause density-dependent mortality to operate incorrectly on post-migratory adult population sizes. Analytical simplification also requires Ives to assume panmictic mating, while we model assortative mating by spatial proximity after migration. The difference will affect the relative distribution of genotypic frequencies in proportion to migration rates and the difference in allelic frequency between refuge and *cry*-toxin units. The two models are equivalent when rates of migration are high, but with moderate migration and the high-dose assumption that heterozygotes do not survive *B. thuringiensis* exposure, this difference in assumptions can significantly affect the evolutionary trajectory.

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