F2 Screen for Rare Resistance Alleles

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F_2 Screen for Rare Resistance Alleles

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ABSTRACT The refuge plus high-dose resistance management strategy for transgenic crops producing *Bacillus thuringiensis* Berliner crystal protein toxins (*Bt* crops) assumes that resistance is rare and functionally recessive. We propose an F_2 screening procedure to estimate the frequency of rare resistance alleles in natural insect populations and acquire them for further testing. The procedure preserves genetic variation in isofemale lines and concentrates the resistance alleles in homozygous genotypes where they can be detected, whether they are recessive or dominant. Bayesian statistics for estimating allele frequency and credibility intervals, and the probability that the screen will not detect a resistance allele are derived. Compared with other methods for detecting and estimating resistance alleles, the F_2 screen will be efficient and accurate, extend the sensitivity of allele-frequency estimation for recessive traits by more than an order of magnitude compared with a discriminating-dose assay, and detect all resistance traits segregating in the initial sample of field-collected insects rather than a subset that responds most rapidly to laboratory selection.

KEY WORDS *Bacillus thuringiensis* endotoxin, resistance management, transgenic crops, monitoring, screening, allele frequency

SUCCESSFUL INSECT RESISTANCE management has used 2 strategies. In preventative or preemptive resistance management, rotations, refuges, multiple toxins, and other actions are implemented without respect to the frequency of resistant insects. These actions are intended to delay or arrest the evolution of resistance and are based on theoretical projections (Comins 1977a, b; Gould 1986; Roush 1994, 1996; Tabashnik 1990; Alstad and Andow 1995). In the absence of knowledge about initial resistance allele frequency, gene expression (especially dominance), and relative fitness, practitioners of preventative management guess at these values and the validity of the theoretical assumptions. The refuge plus high-dose resistance management strategy proposed for *Bacillus thuringiensis* Berliner (*Bt*) crystal protein genes in transgenic crops (*Bt* crops) exemplifies this approach. The actions make theoretical sense, but without estimates of the frequency, dominance, and fitness of resistance alleles in feral populations, we will not know whether the theory is appropriate (Alstad and Andow 1996).

The alternative, adaptive resistance management, is applicable when resistance alleles have been identified and their frequencies of occurrence can be measured and used to make management decisions. Under these conditions, theory can provide a concrete basis for action. For example, in the absence of selection, *mfo* pyrethroid resistance (mixed-function oxidases) is slightly deleterious in *Helicoverpa armigera* (Hübner) (Daly 1993). This resistance has been managed with some success in Australian cotton by restricting pyrethroid use to a short period each season, and further restricting this use interval as resistance has become more common (Forester and Fitt 1992). Coupled with migration of susceptible types from refuges, the cost of resistance then reduces resistant allele frequency in the absence of pyrethroid selection (Daly 1993, Forrester et al. 1993).

The use of adaptive resistance management is limited, in part, by our ability to estimate resistance allele frequencies in the wild. Rare or recessive resistance alleles are particularly problematic (Roush and Miller 1986). To illustrate, consider a monitoring method that screens field-collected individuals at a discriminating dose. We expect a recessive, resistant allele at frequency p to be expressed in only 1 among $1/p^2$ individuals. Ignoring issues of statistical confidence (worked out by Roush and Miller 1986), monitoring allele frequencies near $p = 10^{-3}$ would require screening at least 10^6 random individuals, whereas frequencies near $p = 10^{-1}$ would require $\approx 10^2$ individuals. For a dominant resistance allele near $p = 10^{-3}$, we still expect only 1 resistant phenotype among 500 individuals sampled ($=1/\{2p(1-p)\}$). These limitations imply that only large changes in resistance frequency can be detected, and adaptive resistance management is possible only when resistance is common. When resistance is common, however, evolution can proceed very rapidly, and a correspondingly strong selection pressure is required to arrest and reverse the process. Consequently, adaptive resistance management has rarely been successful.

To enable planning and implementation of resistance management on sound scientific bases, it is essential to improve the sensitivity of our estimates of

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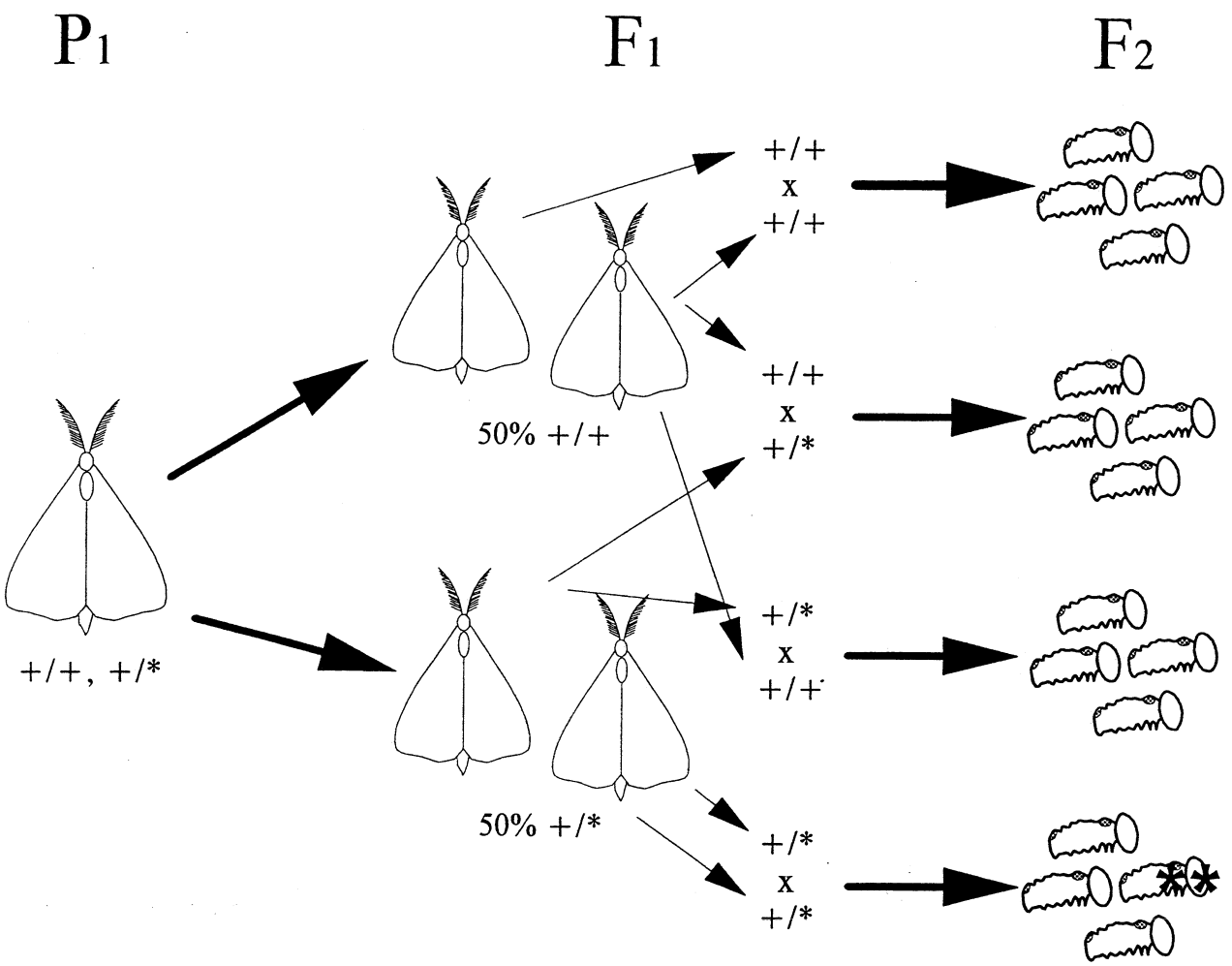

Fig. 1. F_2 screen for a rare resistance allele (*) in European corn borer. P_1s are the parental females caught in the field, here indicated with 1 copy of the * allele and 3 copies of the wild-type allele (+). Her F_1 generation is sib-mated to produce the F_2 generation. In the F_2 generation, only 1/16 of the larvae are expected to be homozygous for the rare resistance allele (**).

resistance allele frequencies and our ability to identify and recover resistance alleles from natural populations. The need is particularly acute for rare recessive traits, which are expressed at the square of their frequencies. Because the objective of the refuge plus high-dose strategy is to make resistance traits functionally recessive, such improvements are likely to be widely applicable in transgenic crops. In this article we describe an F_2 screening method for estimating resistance allele frequency and identifying and recovering resistance alleles from natural populations. The key to our method is to preserve genetic variation among isofemale lines and concentrate all alleles into homozygous, detectable, F_2 genotypes. Compared with field-screening methods for estimating the frequency of recessive alleles, the F_2 screen reduces the number of individuals that need to be tested by $1/p$, where p is the resistance allele frequency. If $p = 10^{-3}$, the F_2 screen is expected to reveal 1 resistance allele among 250 isofemale lines, allowing frequency estimates at least an order of magnitude below the logistical limits of a discriminating-dose assay on feral insects. Although the F_2 screen is particularly efficient for recessive alleles, it will detect both recessive and dominant alleles with similar precision.

F_2 Screen

To provide a concrete example, we describe the F_2 screen for European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), but our procedure should be applicable to many insects that can be reared in the laboratory. It also can be adapted for field rearing. The F_2 screen (Fig. 1) is conducted by sampling mated females from natural populations; rearing the progeny of each female as an isofemale line and sib-mating her F_1 offspring; rearing eggs from the F_1 parents and testing the F_2 larvae using an appropriate screening procedure; followed by statistical analysis. After sibling mating the F_1 generation, 1/16 of the F_2 larvae are expected to be homozygous for each grandparental allele. Because each female carries at least 4 gametic haplotypes (2 of her own and 2 of her mate's), each isofemale line allows us to characterize 4 genomes. The method also limits the influence of vertically transmitted diseases, such as *Nosema pyrausta*

(Paillot), because infected lines can be discarded early in the process.

Sampling. Mated females should be sampled from field populations so that at least 4 random haplotypes are evaluated for each female sampled. For European corn borer, females can be caught at light traps dispersed over space and time. Dispersing the traps will maximize the likelihood that females are a random sample of the local population. These sampled females are the P_1 parents of the isofemale lines.

Sibling Mating. Offspring from each P_1 female should be reared as separate family lines on an appropriate diet. The number of F_1 males and females should be estimated, and these individuals then sibmated within female lines.

F_2 Screening. An appropriate screen on the F_2 generation is critical for successful isolation of the resistance alleles. The screen should provide unambiguous, repeatable results. In our companion article (Andow et al. 1998) we describe 2 screens on neonate European corn borer larvae for resistance to Cry1Ab toxin of *B. thuringiensis*.

Statistical Analysis. There are 2 statistical problems associated with any resistance screening or monitoring procedure, including the F_2 screen. The 1st is estimation of resistance frequency in the sampled population. Here we use Bayesian inference (Brunk 1975) to estimate the expected allelic frequency and the 95% credible intervals. Bayesian inference enables us to make statistical inferences about the population that we sampled. In contrast, conventional statistical approaches (e.g., Gould et al. 1997) enable us to make statistical inferences that are applicable to a large number of populations that are identical to the one that we sampled. Because we are more interested in the actual allele frequency of our sampled population than in the average allele frequency of a large number of similar populations, Bayesian inference is our preferred approach.

To calculate allele frequency, we note that each isofemale line represents 1 Bernoulli trial, where a success is defined as a true positive in the F_2 offspring. The probability of success is $4q$ for monandrous P_1 females, where q is the frequency of the resistant allele, and $1\text{-}q$ is the frequency of the susceptible allele. A success occurs when the line foundress carries a resistance allele, either in her own genotype, or among the patrilineal haplotypes of her fertilized eggs. If n is the number of females tested, S is the number of successes, and we assume a uniform prior distribution, then

$$\mathrm{E}[q] = \frac{(S + 1)}{4(n + 2)} \qquad [1]$$

$$\mathrm{Var}\,[q] = \mathrm{E}[q]\frac{(1 - \mathrm{E}[q])}{(n + 3),} \qquad [2]$$

where $\mathrm{E}[q]$ is the expected frequency of resistance alleles, and $\mathrm{Var}[q]$ is the variance associated with the expected frequency. A uniform prior distribution implies that we expect all allele frequencies to be equally likely. When n is large (i.e., $\approx$100), the influence of the prior distribution is negligible. Moreover, q will be distributed normally with mean equal to $\mathrm{E}[q]$ and variance equal to $\mathrm{Var}[q]$ and standard statistical methods can be used to estimate Bayesian credibility intervals. Table 1 indicates the potential power of this method. If no true positives are detected and there are no false negatives (see below), screening of 1,200 isofemale lines will establish with 95% probability that resistance allele frequency is $\leq 10^{-3}$. If there are multiple loci or alleles, or dominant or recessive resistance alleles, this effort will enable us to conclude that no resistance allele is more common than 10^{-3}. If females are polyandrous, these formulas will overestimate q and its variance.

Table 1. Estimated frequencies of dominant or recessive resistant alleles in European corn borer, given the hypothetical result that no true positives were found in a sample and there are no false negatives

No. isofemale lines screened	Expected frequency of resistant allele $\mathrm{E}[q]$	Variance of frequency of resistant allele $\mathrm{Var}[q]$	95% credible interval of frequency (95% CI)
100	2.5×10^{-3}	2.37×10^{-5}	$0\text{–}1.20 \times 10^{-2}$
500	5.0×10^{-4}	9.9×10^{-7}	$0\text{–}2.45 \times 10^{-3}$
1,000	2.50×10^{-4}	2.49×10^{-7}	$0\text{–}1.23 \times 10^{-3}$
1,200	2.08×10^{-4}	1.73×10^{-7}	$0\text{–}1.02 \times 10^{-3}$
1,500	1.66×10^{-4}	1.11×10^{-7}	$0\text{–}8.19 \times 10^{-4}$

A 2nd statistical problem is to minimize false inferences. The chance that we will identify an isofemale line as carrying a resistance allele (a positive) when it is actually susceptible can be minimized by having a reliable screening procedure, which is not a statistical issue. The screening procedure can be made more reliable by retesting all potential positives to verify that they are resistant. The other false inference is that we identify a line as susceptible when it actually had resistance alleles. This is a sampling problem that has been largely ignored in the literature on resistance monitoring. It can occur, for example, when an isofemale line starts with a resistance allele, but the allele is lost or reduced to a low frequency in the F_1 or F_2 generation and becomes difficult to detect.

The probability that we will not detect a resistance allele (a false negative) is related to a series of sampling processes in the screening procedure. This problem is not unique to our proposed F_2 screen, but occurs in any method that screens a sample of individuals. For the F_2 screen, it is possible that either mother or father was heterozygous for resistance, yet none of the F_2 offspring survives the screening procedure. This probability (designated as P_{No}) is the probability of not detecting a resistance allele. There are 3 sequential sampling problems that must be considered to calculate P_{No}. Assuming that 1 of the P_1 parents has 1 resistance allele, the allele must be passed to the F_1 offspring, matings between the appropriate F_1 genotypes must occur, and the F_2 offspring must inherit the trait and live long enough to be screened. Here we develop an explicit probability for recessive alleles. The situation for dominant alleles is

much less problematic because the 2nd sampling problem is irrelevant.

The 1st sampling problem involves inheritance of the resistance allele by the F_1 offspring. Because Mendelian segregation is usually random, it is possible that none or few of the offspring might actually inherit the resistance allele. The expected number of female and male *RS* heterozygotes (where *S* is a susceptible allele and *R* is a resistant allele) in the F_1 generation is 50% of the total number of adults. The actual number of female and male *RS* heterozygotes will be distributed as binomial distributions, $B(F,0.5)$ and $B(M,0.5)$, where F is total number of females and M is the total number of males in the F_1 generation.

The 2nd sampling problem involves mating by the F_1 adults. To concentrate the resistance alleles in homozygous genotypes, it is essential that *RS* females mate with *RS* males. If mating is random, the expected frequency of F_1 heterozygote by heterozygote matings ($SR \times SR$) is 0.25 (or 0.5×0.5, the product of the expected frequencies of heterozygote females and males). The actual number of $SR \times SR$ matings will depend on the number of heterozygote F_1 females and males in a family line, which can be expressed as a conditional probability. Because we cannot know how many of the F_1s actually are heterozygous, we must consider all possibilities. We sum over all possible combinations of numbers of heterozygous females and males weighted by their probabilities of occurrence (determined by the binomial probabilities in the previous paragraph).

The final sampling problem involves the possibility that no offspring from a $SR \times SR$ F_1 mating survive the screening procedure. This can occur because no homozygote offspring are produced, or because the homozygotes die for other reasons. The number of resistant homozygotes is expected to be 25% of the total number of offspring of $SR \times SR$ matings. The actual number of resistant homozygotes from a $SR \times SR$ mating will have a binomial distribution, $B(J,0.25)$, where J is the number of offspring from a $SR \times SR$ mating.

These sampling problems can be quantified in a series of conditional probabilities. Let P_{No} = Prob(no F_2 survivors $|JF$ F_2 offspring screened and F and M F_1 females and males), $P_{(No|I=1)}$ = Prob(no F_2 survivors $|$ J F_2 offspring screened and the F_1 mating is $SR \times SR$), P_I = Prob(I F_1 matings are $SR \times SR$ $|$ F and M F_1 females and males). The probability that we miss a recessive resistance allele, P_{No} (a false negative), is equal to:

$$P_{No} = \sum_{I=0}^{F} P_I \, P^I_{(No|I=1)} \qquad [3]$$

$P_{(No\,|I=1)}$ is equal to the probability that no resistant homozygotes are produced from a single $SR \times SR$ mating, including the probability that homozygotes die for other reasons. If μ is the mortality rate of larvae for other reasons, then $P_{(No|I=1)}$ is

$$P_{(No|I=1)} = \sum_{k=0}^{J} \mu^k \binom{J}{k} \left(\frac{3}{4}\right)^{(J-k)} \left(\frac{1}{4}\right)^k \qquad [4]$$

P_I is complicated because it combines the probability of inheritance of the resistance allele to the F_1 generation and the probability of $SR \times SR$ matings. Let $P_{(I|FmM)}$ = Prob(I $SR \times SR$ matings $|f$ SR females and F total females, and m SR males and M total males), $P_{(f|F)}$ = Prob(f SR females$|F$ total F_1 females), $P_{(m|M)}$ = Prob(m SR males$|M$ total F_1 males). Consequently, for a polygynous mating system P_I is equal to

$$P_I = \begin{cases} \text{Prob}(f = 0 \text{ or } m = 0|FM) & \text{for } I = 0 \\ \sum_{m=1}^{M}\left[\sum_{f=1}^{F}\left(P_{(I|\,fFmM)}P_{(f\,|F)}\right)P_{(m|M)}\right] & \text{for } I > 0. \end{cases} \qquad [5]$$

In addition,

$$P_{(I|\,fFmM)} = \binom{f}{I}\left(\frac{m}{M}\right)^I\left(1 - \frac{m}{M}\right)^{f-I}. \qquad [6]$$

With binomial distributions of $P_{(f|F)}$ and $P_{(m|M)}$ and some rearranging, P_I is equal to

$$P_I = \begin{cases} \left(\frac{1}{2}\right)^F + \left(\frac{1}{2}\right)^M - \left(\frac{1}{2}\right)^{(F+M)} & \text{for } I = 0 \\ \left(\frac{1}{2}\right)^{(F+M)}\binom{F}{I}\left[\sum_{m=1}^{M}\binom{M}{m}\left(\frac{m}{M}\right)^I\left\{\sum_{k=1}^{F-I}\binom{F-I}{k}\left(1-\frac{m}{M}\right)^k\right\}\right] & \text{for } I > 0. \end{cases} \qquad [7]$$

We have written a computer program to calculate P_{No} given M males and F females produced in the F_1 generation and FJ larvae screened among the F_2. Calculation of the probability of not detecting a resistance allele (P_{No}) for different M, F, and J for an isofemale line (Fig. 2) indicates that a P_{No} <1% requires each isofemale family to have >10 F_1 males, >10 F_1 females, and >10 larvae per F_1 female screened. When the number of offspring in the F_1 generation is low, the probability of a false negative is high, implying that these family lines are uninformative. If the field-collected females are polyandrous, our calculation underestimates the probability of a false negative, and if the resistance allele is dominant, it overestimates this probability.

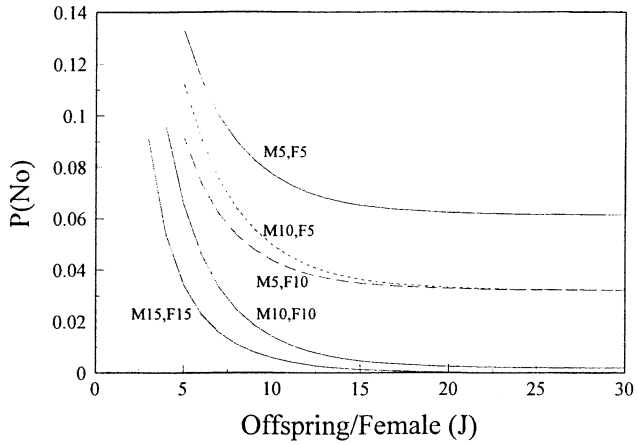

Fig. 2. The probability (P[No]) that we will not detect a recessive resistance allele in an isofamily line (P_{No} in text, the probability of a false negative) that produce F_1 families with 5, 10, or 15 males (M) and 5, 10, or 15 females (F), and F_2 families with J offspring per F_1 female.

The experimentwise probability of not detecting a resistance allele can be calculated as the average P_{No}. When resistance alleles are rare, the experimentwise probability of not detecting a resistance allele is the same as the probability of not detecting a resistance allele in any one of the isofemale lines, which is the same as the average P_{No}. If resistance alleles are common, the calculation is more complicated, but if this occurs, resistance should be readily detected in the field, and use of the F_2 screen may be less critical.

Discussion

To provide a sound scientific basis for planning and implementing resistance management and to evaluate the appropriateness of the refuge plus high-dose strategy for resistance management in transgenic *Bt* crops, we need to estimate the frequency of resistance alleles in natural populations, and recover those alleles to characterize their fitness. There are at least 5 different methods by which allele frequencies could be estimated or resistant alleles recovered, each with strengths and weaknesses.

Enumeration of Insects that Survive Toxin Exposure in Farmer's Fields. In principle, every insect that survives toxin exposure in the field could be a resistant genotype. This approach does not yield a direct estimate of allelic frequency because one cannot know how many insects were originally present, but using population densities on an appropriate nontoxic control field could provide an indirect estimate of allele frequency. A significant limitation of this method is the logistical burden of looking for survivors in large areas and confirming that the survivors are resistant. If the resistance allele is recessive and at frequency 10^{-3}, then 10^6 random (not known to be sibs) individuals must be exposed in the field before we expect to find 1 resistant individual. For *O. nubilalis,* if we assume that every plant receives 1 egg mass and all egg masses are unrelated, then >33 acres of corn must be examined to find 1 survivor. If oviposition can be concentrated on fewer plants, as in sweet corn, this logistical burden would be reduced. An additional problem is that many survivors will be false positives—susceptibles that avoided exposure to the toxin. Pesticide applications are uneven and concentrations change with time, allowing susceptible individuals to avoid the toxin and survive. Although toxin concentrations in transgenic *Bt* crops are probably more uniform than insecticide applications, the seed is not perfectly uniform and some plants will not express the toxin. Moreover, crops may show a decline in the concentration of Cry toxin as the plant ages (cf. Fitt et al. 1994). Consequently, it will be necessary to verify any potential positive to confirm that it is truly resistant, by using laboratory methods similar to those described here for the F_2 screen.

Laboratory Selection. Mass selection on a laboratory colony can identify resistance alleles (Tabashnik 1994) that can be used subsequently to estimate allele frequencies in natural populations (Gould et al. 1997). However, it is an inefficient method for estimating allele frequencies directly because only a limited number of founders can be incorporated into a laboratory colony. An additional limitation is that laboratory selection will recover only a subset of the possibly many major and minor resistance alleles segregating in natural populations. Specifically, mass selection in the laboratory will recover the resistance genotype that responds most rapidly to laboratory selection by virtue of its initial variance in the colony, dominance, and fitness advantage under mass rearing. Genotypes that might respond more rapidly under field selection may be missed. In addition, field-collected colony founders may harbor parasites, such as *N. pyrausta,* a microsporidian parasite of *O. nubilalis* that can debilitate or destroy a colony. These considerations suggest that laboratory selection will allow us to acquire 1 or a few resistance traits segregating at high frequency (Tabashnik et al. 1997), but may not suffice for predicting evolution of resistance in natural populations.

Screening Natural Populations with a Discriminating Dose. This method, called the discriminating-dose assay (Roush and Miller 1986), has been used with apparent success in managing mite resistance to dicofol (Dennehy et al. 1987, 1990; Martinson et al. 1991a, b). Because it is a quantitative screen of field-collected insects, potential genotypic complications stemming from multiple genetic mechanisms, major and minor genes, and quantitative versus single-gene resistance are avoided. The principal limitation of this method is its low sensitivity (Roush and Miller 1986). If resistance is recessive (as is hoped for Cry toxin resistance alleles) then it will be expressed at the square of its allele frequency. If the frequency is low (e.g., 10^{-2}), routine screening (requiring 10^4 field-collected individuals) with a discriminating-dose assay would be a logistical nightmare. Consequently, the discriminating-dose assay is most useful for resistance alleles that are partially to completely dominant or at relatively high frequency in natural populations.

Screening Against Test-Stocks. Gould et al. (1997) used an elegant series of genetic crosses with test-

stocks of resistant insects to estimate resistance frequency in a natural population. Using a colony of *Heliothis virescens* (F.) that can survive on transgenic *Bt* cotton, they crossed field-collected males with virgin colony females so that all F_1 progeny would be *RS* heterozygotes. By using an assay that discriminates between *RS* and *RR* genotypes, they could establish which wild males carried an *R* allele. Although they do not explicitly consider the probability that they would obtain a false negative, it is similar to our $P_{(No|I=1)}$ above, and in their case is exactly

$$\sum_{k=0}^{I} \mu^k \binom{J}{k} \left(\frac{1}{2}\right)^{(J-k)} \left(\frac{1}{2}\right)^{k}, \qquad [8]$$

where J is the number of offspring screened in a family, and μ is the mortality of offspring from other causes. When $J > 10$ and $\mu < 0.25$, this probability is small.

The primary limitation of this method is that one must have a resistance allele identified before one can estimate its frequency. If there is more than 1 genetic mechanism of resistance, as there are for some insecticide resistance traits (e.g., Gunning et al. 1991; Gould et al. 1992, 1995), the method will tabulate only the previously identified resistance alleles. This information enables calculation of the longest possible time to the evolution of resistance, a sort of best-case analysis. To develop a realistic case it may be necessary to identify and estimate the frequency of all resistance alleles in a population.

F_2 Screen. Screening of inbred F_2 insects preserves virtues of the discriminating-dose assay, but extends its sensitivity for rare, recessive alleles. F_2 screening allows us to acquire all dominant and recessive resistance alleles present by using a method that is slightly more intensive than the test-stock screen (Gould et al. 1997). The F_2 screen requires significantly more effort for each insect collected from the field than the discriminating-dose assay, but inclusion of an inbreeding step extends sensitivity substantially. In a companion paper we estimate the effort needed to conduct the F_2 screen (Andow et al. 1998). It can be used to confirm 1 of the assumptions underlying the refuge plus high-dose strategy. In addition, if it is cost-effective, it could be incorporated into an adaptive resistance management strategy that could manage the evolutionary process at low frequencies of resistance.

One concern with the F_2 screen is the possibility of complications from inbreeding depression. Inbreeding depression may limit the number of F_2 progeny that survive in each family or may cause some families to die out before the F_2 generation, reducing precision of the allele-frequency estimate. This will not affect the estimate of the expected resistance allele frequency unless the resistance alleles contribute to inbreeding depression. An additional potential complication is that the identified alleles may be associated with families suffering inbreeding depression. This can be compensated by back-crossing the resistant families to a parental stock so that fitness estimates for the resistant genotypes are unaffected.

The F_2 screen should be useful for estimating the frequency of rare, recessive alleles, and recovering those alleles for additional testing. Because nearly all known *Bt* resistance alleles are partially recessive (Tabashnik 1994, Gould et al. 1997), the F_2 screen should be especially pertinent in resistance management for transgenic *Bt* crops. The F_2 screen can be used to verify assumptions underlying refuge plus high-dose resistance management for *Bt* corn. If we find no resistance alleles among 1,200 isofemale lines, we can conclude with 95% confidence that the initial frequency of resistance in the sampled population is $p < 10^{-3}$. If resistance alleles are found, they can be characterized to estimate the fitness of the genotypes, determine if there is a cost of resistance, and enable realistic predictions of the evolution of resistance. Until these data are acquired, growers and seed companies that had ≈3 million ha of *Bt* corn planted during the 1997 season will continue to base resistance management on faith and guesses.

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References Cited

Alstad, D. N., and D. A. Andow. 1995. Managing the evolution of insect resistance to transgenic plants. Science (Wash. D.C.) 268: 1394–1396.

1996. Implementing management of insect resistance to transgenic crops. AgBiotec News Info. 8: 177–181.

Andow, D. A., D. N. Alstad, Y.-H. Pang, P. C. Bolin, and W. D. Hutchison. 1998. Using an F_2 screen to search for resistance alleles to *Bacillus thuringiensis* toxin in European corn borer (Lepidoptera: Crambidae). J. Econ Entomol. 91: 579–584.

Brunk, H. D. 1975. An introduction to mathematical statistics. Xerox College Publishing, Lexington, KY.

Comins, H. N. 1977a. The development of insecticide resistance in the presence of migration. J. Theor. Biol. 64: 177–197.

1977b. The management of pesticide resistance. J. Theor. Biol. 65: 399–420.

Daly, J. C. 1993. Ecology and genetics of insecticide resistance in *Helicoverpa armigera:* Interactions between selection and gene flow. Genetica 90: 217–226.

Dennehy, T. J., E. E. Grafton-Cardwell, J. Granett, and K. Barbour. 1987. Practitioner-assessable bioassay for detection of dicofol resistance in spider mites (Acari: Tetranychidae). J. Econ. Entomol. 80: 998–1003.

Dennehy, T. J., J. P. Nyrop, and T. E. Martinson. 1990. Characterization and exploitation of instability of spider mite resistance to acaricides, pp. 77–91. *In* M. B. Green, H. M. LeBaron, and W. K. Moberg [eds.], Managing resistance to agrochemicals: From fundamental research to practical strategies. American Chemical Society, Washington, DC.

Fitt, G. P., C. L. Mares, and D. J. Llewellyn. 1994. Field evaluation and potential ecological impact of transgenic

cottons (*Gossypium hirsutum*) in Australia. Biocontrol Sci. Technol. 4: 535–548.

Forrester, N. W., and G. P. Fitt. 1992. Management of insecticide resistance in *Heliothis armigera* in Australia - Ecological and chemical countermeasures, pp. 88–99. *In* I. Denholm, A. L. Devonshire, and D. W. Holloman [eds.], Resistance 91: achievements and developments in combating pesticide resistance. Elsevier, London.

Forrester, N. W., Cahill, M., Bird, L. J., and J. K. Layland. 1993. Management of pyrethroid and endosulfan resistance in *Helicoverpa armigera* (Hübner) in Australia. Bull. Entomol. Res. Spec. 1: 1–144 (suppl.).

Gould, F. 1986. Simulation models for predicting durability of insect-resistant germ plasm: Hessian fly (Diptera: Cecidomyiidae)-resistant winter wheat. Environ. Entomol. 15: 11–23.

Gould, F., A. Martinez-Ramirez, A. Anderson, J. Ferre, F. J. Silva, and W. J. Moar. 1992. Broad-spectrum resistance to *Bacillus thuringiensis* toxins in *Heliothis virescens*. Proc. Natl. Acad. Sci. U.S.A. 89: 7986–7990.

Gould, F., A. Anderson, A. Reynolds, L Bumgarner, and W. Moar. 1995. Selection and genetic analysis of a *Heliothis virescens* (Lepidoptera: Noctuidae) strain with high levels of resistance to *Bacillus thuringiensis* toxins. J. Econ. Entomol. 88: 1545–1559.

Gould, F., A. Anderson, A. Jones, D. Sumerford, G. G. Heckel, J. Lopez, S. Micinski, R. Leanard, and M. Laster. 1997. Initial frequency of alleles for resistance to *Bacillus thuringiensis* toxins in field populations of *Heliothis virescens*. Proc. Natl. Acad. Sci. (U.S.A.) 94: 3519–3523.

Gunning, R. V., C. S. Easton, M. E. Balfe, and I. G. Ferris. 1991. Pyrethroid resistance mechanisms in Australian *Helicoverpa armigera*. Pesticide Sci. 33: 473–490.

Martinson, T. E., J. P. Nyrop, T. J. Dennehy, and W. H. Reissig. 1991a. Field measurements of selection for European red mite (Acari: Tetranychidae) resistance to dicofol in apple orchards. J. Econ. Entomol. 84: 1–6.

Martinson, T. E., T. J. Dennehy, J. P. Nyrop, and W. H. Reissig. 1991b. Field measurements of selection for twospotted spider mite (Acari: Tetranychidae) resistance to dicofol in apple orchards. J. Econ. Entomol. 84: 7–16.

Roush, R. T. 1994. Managing pests and their resistance to *Bacillus thuringiensis*: can transgenic crops be better than sprays? Biocontrol Sci. Technol. 4: 501–516.

1996. Can we slow adaptation by pests to insect-resistant transgenic crops?, pp. 242–263. *In* G. Persley, [ed.], Biotechnology and integrated pest management. CAB, Wallingford, UK.

Roush, R. T., and G. L. Miller. 1986. Considerations for design of insecticide resistance monitoring programs. J. Econ. Entomol. 79: 293–298.

Tabashnik, B. E. 1990. Modeling and evaluation of resistance management tactics, pp. 153–182. *In* Roush, R. T. and B. E. Tabashnik [eds.], Pesticide resistance in arthropods. Chapman & Hall, New York.

1994. Evolution of resistance to *Bacillus thuringiensis*. Annu. Rev. Entomol. 39: 47–79.

Tabashnik, B. E., Y. Liu, N. Finson, L. Masson, and D. G. Heckel. 1997. One gene in diamondback moth confers resistance to four *Bacillus thuringiensis* toxins. Proc. Natl. Acad. Sci. U.S.A. 94: 1640–1644.

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