Pro-active Monitoring

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The aim of monitoring is to provide timely information to trigger alternative management options, that is, monitoring is linked to adaptive management. One goal of monitoring is to document the failure of resistance management and the need to stop sales and move to an alternative control tactic. A second, pro-active goal of monitoring is to provide information on the progress of resistance evolution so that resistance management tactics can be altered to delay resistance more aggressively. In this short discussion, I will sketch an approach for realizing this pro-active monitoring goal.

Need for Pro-Active Monitoring

Resistance management for *Bt* corn is based on the high-dose plus refuge strategy. A central feature of this approach is the 20% refuge for susceptible corn borers. Two years ago at the time of the publication of NCR-602 (Ostlie et al. 1997), the recommended 20% refuge was considered possibly higher than necessary as a safety margin to hedge against uncertainties in the assumptions of the high-dose plus refuge strategy (Andow and Hutchison 1998). By the time of the publication of the Supplement to NCR-602 (NC205 1998) sufficient data had accumulated about the genetic structure of European corn borer populations to eliminate this safety margin. Indeed, when the genetic structure of corn borers is included in evolutionary models, a 20% unsprayed refuge barely preserves corn borer susceptibility for 15 years under the remaining assumptions of the high-dose plus refuge strategy (ILSI 1998, NC205 1998, Andow and Alstad unpublished data). These assumptions include that resistance will be nearly completely recessive, that the structured refuge will allow for random mating between resistant and susceptible corn borer genotypes, and that compliance will be complete. Although recessivity had been assumed on theoretical grounds (Andow and Hutchison 1998), some researchers had criticized this assumption (Tabashnik, personal communication) and recent evidence indicates that resistance possibly could be dominant (Huang et al. 1999). Little data exist on mating behavior of corn borers, and compliance mechanisms are undetermined.

Given the absence of a safety margin in the present high-dose plus refuge strategy for *Bt* corn, it would be quite risky indeed to rely only on this static strategy. A robust resistance management strategy will include pro-active monitoring that will allow management to adapt to the evolution of resistance as it occurs but before there are control failures. Such a monitoring system will provide the safety margin necessary to hedge against violations in the scientific assumptions of the high dose plus refuge strategy. Indeed, although there are no data to support these speculations, with effective pro-active monitoring refuges managed by IPM practices may become an effective resistance management tactic.

Necessary Precision to Allow Timely Response

For any monitoring system it is essential to understand the time frame in which an effective response can be made. For resistance management, the primary goal is to avoid control failures attendant with resistance evolution. These control failures will become common when the allele frequency gets high enough so that most insects are resistant. Based on theoretical models (Alstad and Andow 1995), pest density will reach potentially damaging levels about 1 year after the allele frequency reaches 0.5. Using this model and the model developed by Caprio (1998), I estimate that the maximum time from detection of a resistance allele at given frequency to the time of control failures will vary from 2 to 4 years for a fully dominant allele (Figure 1a), and 2 to 7 or 12 years for a fully recessive allele (Figure 1b). For a recessive allele, the earlier that that resistance can be detected, the more time there is to react to the information by implementing alternative management tactics. For a dominant allele, there is very little time to react even when the allele is detected at frequencies of 0.0001. Because a dominant allele would invalidate the high-dose plus refuge strategy, a very sensitive method for monitoring for dominant alleles needs to be implemented.

Potential Monitoring Methods and Their Probable Cost

The monitoring methods that are available are screening field-collected egg masses, screening field-collected larvae, in-field *Bt* sweet corn screen (Andow and Hutchison 1998, Venette et al., submitted), and F2 screen (Andow and Alstad 1998). The first 2 rely on laboratory discriminating dose assays on either neonate or older larvae. The other 2 rely on *Bt* plants to supply the discriminating dose. Two additional methods are an in-field Bt field corn screen (Pierce et al 1998) and screening against test stocks (Gould et al. 1997). The field corn screen may be logistically inefficient (Andow, unpublished data), and will not be discussed further. Use of test stocks is not yet possible because resistance in corn borer has not yet been recovered.

A screen of field collected egg masses could involve collecting the egg masses from the field, during either the first or second corn borer generation, delivering them to a lab for testing, hatching the egg masses in the lab, and screening the neonates using a laboratory discriminating dose assay. The costs for conducting this assay are not known, but

assuming typical egg mass density in field corn, and an assay that screens each larva individually, I estimate about $2.00 per egg mass to collect it and $2.21 to assay the larvae. In addition, travel time to and from the field site will add an additional $60 to collect the egg masses. The sample unit is the egg mass, not the larva, because all larvae in an egg mass are related (i.e., they are not independent samples from the population). Although statistical analysis of the data should be adjusted for egg mass size, for simplicity I assume that an egg mass will be unerringly determined as resistant or susceptible. Because the method determines phenotype, when resistance is rare, its statistical precision is $1/2N$ for dominant alleles and $1/N^{1/2}$ for recessive alleles. The method is statistically inefficient for recessive alleles. For example, a sample of 100 egg masses has a precision of 0.005 for dominant alleles and 0.1 for recessive alleles. Leaving aside the issue of accuracy, this means that the theoretically best resolution of allele frequency is ±0.0025 for dominant alleles and ±0.05 for recessive alleles.

A screen of field-collected larvae could involve collecting the larvae in the field (presumably 3rd and 4th instar larvae- a discriminating dose assay for 5th instar larvae does not exist), transporting them to the lab, and conducting individual discriminating dose assays on the collected larvae. The costs for conducting this assay are not known, but assuming typical larval density in field corn during the second generation and death of some of the collected larvae, I estimate about $2.67 per larva to collect it and $0.98 to assay the larvae. In addition, travel time to and from the field site will add an additional $80 to collect the egg masses. The sample unit is the larva, because it is screened directly. If the larvae must be reared and mated before screening, the costs are quite a bit higher (Bolin et al. 1998). Statistical analysis for the larval screen is similar to the egg mass screen, and it too is inefficient for recessive alleles (Roush and Miller 1986). Because the method determines phenotype, when resistance is rare, its statistical precision is $1/2N$ for dominant alleles and $1/N^{1/2}$ for recessive alleles (leaving aside issues of accuracy). If the larvae are reared and mated, the statistical analysis is more complicated, and the precision is much less.

An in-field sweet corn screen involves planting Bt sweet corn (about 1 acre) and nearby unsprayed non-Bt sweet corn (about 1000 plants) late in the growing season and examining ear tips of the sweet corn at the end of the season (Venette et al., submitted). The costs for this method are not well known, so I will use estimates that are probably higher than average. Because sampling ear tips can be done quickly, the cost per larva is about $0.24, and the cost for plot rental, planting and maintenance is about $400. The sample unit is the larva, and although statistical analysis is very complicated (Andow, unpublished data), a rough estimate of statistical precision can be derived when the number of larvae screened is known (i.e., does not need to be estimated). Under this condition, the statistical precision converges to that of the previous two methods, because this method, like the previous two methods, is also a phenotype screen. Its statistical precision for large samples converges to $1/2N$ for dominant alleles and $1/N^{1/2}$ for recessive alleles (leaving aside issues of accuracy).

An F_2 screen involves collecting mated adult females from the field, transporting them to the lab, collecting their eggs, rearing the F_1 larvae, sib-mating the F_1 families, collecting

egg masses, and exposing neonates to *Bt* corn in the field (Andow and Alstad 1998). This is the most labor intensive monitoring method, and costs $14.90 per female line (Andow et al., submitted) plus $200 for collecting. This method, unlike the previous ones is a genic screen and will be able to detect each allele that is present in the collected female, including those of her mates. Leaving aside the issue of accuracy (Andow and Alstad 1998, 1999, Schneider 1999), the statistical precision of this method is $1/4N$ for dominant alleles and $1/4N$ for recessive alleles. This screen is particularly efficient for recessive alleles. For example, a sample of 100 female lines has a precision of 0.0025 for dominant alleles and 0.0025 for recessive resistance alleles.

Using the cost estimates, it is possible to estimate the cost per sample for each of the methods (Figure 2). As expected, the cost of the F_2 screen is much higher than any of the other methods, and the cost of the in-field *Bt* sweet corn screen is much lower than any of the other methods.

A more informative analysis is to evaluate the cost of each monitoring method in relation to its statistical precision. For dominant alleles, there is an intriguing cost-precision cross-over among the monitoring methods (Figure 3a). For low precision (>0.01) the least expensive methods are the two discriminating dose assays, and the in-field screen is the most expensive. At finer levels of precision (<0.01) the in-field screen is the least expensive and the F_2 screen is the most expensive. This occurs because the costs of planting and maintaining the *Bt* sweet corn make the in-field screen more expensive than the other methods until >50 larvae must be sampled to achieve the needed precision. For under $2000 the in-field screen can estimate dominant allele frequencies to a precision of better than 0.0001. With this information, there would be 3-4 years to respond with alternative resistance management tactics.

For recessive alleles, the least expensive method for all levels of precision is an F_2 screen (Figure 3b). This occurs because the phenotype methods require much larger sample sizes than the F_2 screen to attain similar levels of precision. For under $5000 the F2 screen can estimate recessive allele frequencies to a precision of better than 0.001. This will provide 7-12 years to respond with alternative resistance management tactics (Figure 1b). To provide ~5 years response time, a precision of 0.01 is needed (Figure 1b). The F2 screen can provide that level of precision for ~$500, which is between 2-4 times less expensive than the in-field screen, and nearly 80 times less expensive than the discriminating dose assays (Figure 3b).

Possible Management Responses

Potential responses to increases in the frequency of resistance in corn borer can be classified into two types. The first are those that focus on reducing the selection differential between resistant and susceptible individuals either by increasing survival of susceptible individuals or reducing survival of the resistant ones. Examples include spraying *Bt* corn with insecticides, creating super-refuges for susceptible individuals, factors that could affect fecundity and mating success, and release of natural enemies in

Bt corn. These ideas have received increasing attention during the past 6 months. The second are those that focus on the mating system. For example, it is well understood that mating must be random for the high-dose plus refuge strategy to be effective, and that slight deviations toward assortative mating greatly accelerate the evolution of resistance. Less appreciated is the fact that disassortative mating could greatly decelerate the evolution of resistance, and under some conditions can even reverse it. This can happen when resistant individuals are more likely to mate with susceptible ones than at random, producing greater than expected numbers of *RS* heterozygotes, which can be killed by *Bt* corn. One potential method for accomplishing this is to plant small aggregation sites inside *Bt* corn fields. These sites can accumulate large numbers of susceptible moths, which might then be more likely to mate with any resistant ones emerging from the *Bt* corn. We have observed that a 1/8 mile x 10 ft strip of sorghum-sudan grass accumulated extremely high densities of moths during the second generation in a field in central Minnesota. I believe that similar plantings can be made to be very attractive to moths in much of the central Corn Belt. Clearly more research and additional ideas are needed to elaborate an effective system of resistance management response.

Number of Sites to Sample

The previous discussion has been focused on how to sample a single location. Another critical question is how many sites need to be sampled. A complete answer to this question is beyond the scope of this paper. One approach is to use scientific information to put an upper and lower bound on the number of sample locations needed, but this has not been done. The unpublished results of Alstad et al. on the genetic structure of European corn borer can be used to determine the minimal number of populations that should be sampled to cover the entire range of European corn borer. These results indicate that at spatial scales of 300 km, there is significant genetic differentiation among corn borer populations, suggesting that monitoring should be done at least every 300 km, suggesting at least 15 locations to cover the geographic range of European corn borer in the United States. Because resistance is expected to arise at a local scale, an upper bound on the number of sample locations can be estimated from specification of a local unit at risk of resistance evolution. Because most adult movement is occurring within spatial scales of 1.5 km, an evolutionarily reasonable and convenient local unit is the township. If townships with >2500 acres of corn and >25% market penetration of *Bt* corn are at risk, then there may be as many as 2000 townships at risk, implying that <2000 locations would be sufficient for monitoring. Clearly, a range of 15-2000 is much too large on which to base sound public policy, and additional research will be necessary to efficiently allocate monitoring efforts.

Conclusions

Present scientific information indicates that the high-dose plus refuge strategy with 20% refuge has no safety margin to hedge against uncertainties in assumptions, implementation, and compliance. The present strategy is risky unless pro-active monitoring enables resistance management to adapt to the evolution of resistance as it

occurs but before control failures are observed. The time frame to respond before control failures occur depends on the precision of monitoring and the recessivity/dominance of resistance. It varies from 2 to 4 years for a dominant allele and from 2 to 7 or 12 years for a recessive allele. The in-field *Bt* sweet corn screen is the most cost-effective method for monitoring dominant resistance alleles, and the F2 screen is the most cost-effective method for monitoring recessive resistance alleles. Both methods can be conducted to provide >3 years response time. Two types of management responses can be developed, but both require additional research to evaluate effectiveness and efficacy.

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Figure 1. Number of years from detection of a given allele frequency to control failures for (a) a dominant allele and (b) a recessive allele. For the recessive allele, the time to control failures is given for fixed levels of inbreeding that correspond to known levels of inbreeding in European corn borer. These calculations assume that the estimate of allele frequency becomes available 1 generation after the insects are collected.

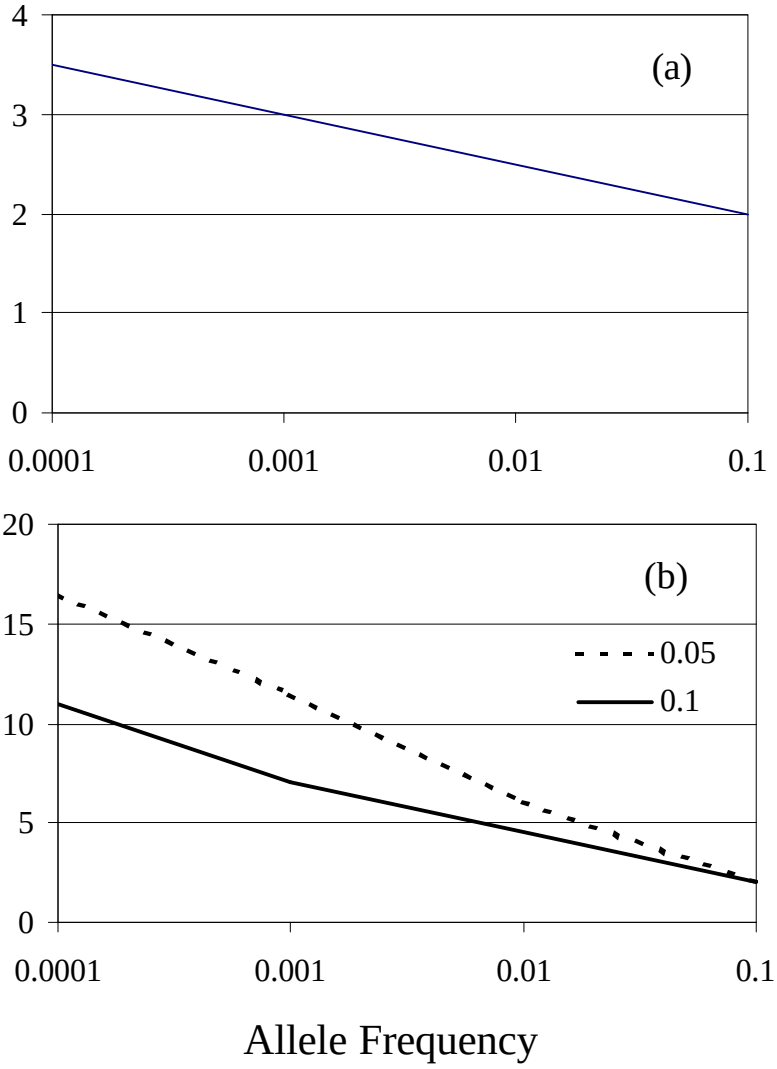

Figure 2. Total direct variable costs for conducting a given monitoring method for different numbers of European corn borers sampled. Sampling methods are F2 screen (F2), discriminating dose assay on field collected larvae (Larvae), discriminating dose assay on neonates from field collected egg masses (Egg Mass), and in-field *Bt* sweet corn screen (In-field).

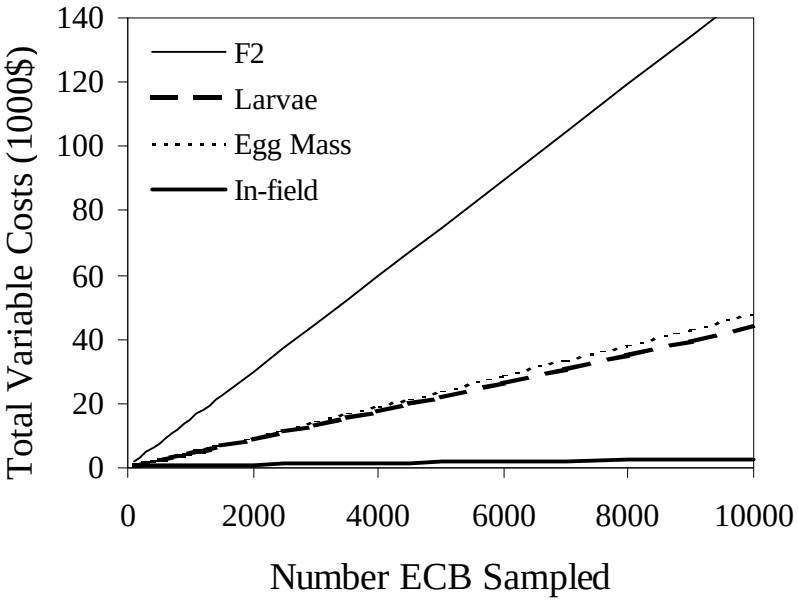

Figure 3. Total direct variable costs of four monitoring methods to achieve the given degree of precision (a) dominant allele, (b) recessive allele. Sampling methods are F2 screen (F2), discriminating dose assay on field collected larvae (Larvae), discriminating dose assay on neonates from field collected egg masses (Egg Mass), and in-field *Bt* sweet corn screen (In-field). In both panels, the lines for the Larvae and Egg Mass methods are overlapping and difficult to distinguish. The light dashed line associated with the in-field *Bt* sweet corn screen (In-field) represents costs under high infestation years (a best case scenario).