

Resistance Risk Assessment for Single and Multiple Insecticides: Responses of Indianmeal Moth (Lepidoptera: Pyralidae) to *Bacillus thuringiensis*

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J. Econ. Entomol. 87(4): 834-841 (1994)

ABSTRACT Criteria for comparing the risk of resistance development among single insecticides and between mixtures and sequences of two insecticides are described. The rate of development of resistance to an insecticide is proportional to the population's heritability (h^2) of resistance to that insecticide. When cross-resistance is absent, a sequence of two insecticides is expected to be more durable than a mixture unless the population's h^2 of resistance to the mixture is less than half of the mean of the population's h^2 of resistance to the two individual components of the mixture. We applied these criteria to 11 previously reported selection experiments with the biopesticide *Bacillus thuringiensis* and Indianmeal moth, *Plodia interpunctella* (Hübner), a major pest of stored grain. The risk of resistance development did not differ significantly between the HD-1 strain of *B. thuringiensis* ssp. *kurstaki* and three other strains (HD-112, HD-133, and HD-198) of *B. thuringiensis*. Significant declines in realized h^2 of resistance during individual selection experiments suggest that the initial frequency of resistance alleles was much higher than previously assumed. Our analysis also suggests that a mixture of HD-1 + HD-133 would not slow resistance development compared with a sequence of HD-1 followed by HD-133. Rapid evolution of resistance to the mixture of HD-1 + HD-133, which contained at least six different toxins, contradicts the claim that multiple toxins prevent or greatly retard resistance development.

KEY WORDS *Bacillus thuringiensis*, insecticide resistance, heritability

INCREASING PROBLEMS CAUSED by pesticide resistance have focused attention on tactics for slowing evolution of resistance in pests (National Research Council 1986, Roush & Tabashnik 1990, Denholm & Rowland 1992). In particular, the potential for resistance development threatens the continued success of environmentally safe insecticides derived from *Bacillus thuringiensis* Berliner (Gould 1988, Georgioui 1990, Tabashnik et al. 1990, McGaughey & Whalon 1992, Tabashnik 1994). One encouraging result is that resistance to the toxins in one strain of *B. thuringiensis* does not necessarily confer cross-resistance to all other *B. thuringiensis* strains or toxins (McGaughey & Johnson 1987, 1993; van Rie et al. 1990, Ferré et al. 1991, Tabashnik et al. 1993). In some cases, however, selection for resistance with a single *B. thuringiensis* toxin can produce resistance to several other *B. thuringiensis* toxins (Gould et al. 1992).

For situations in which cross-resistance is absent or minimal, what is the best way to use more than one toxin or set of toxins? Despite the lack of convincing evidence from experiments with

either conventional or biological pesticides (Tabashnik 1989, Denholm & Rowland 1992), mixtures often are mentioned as a "preferred approach for delaying development of insect resistance" (Stone et al. 1991; also see Georgioui 1990, van Rie 1991, Feitelson et al. 1992, Gill et al. 1992). Better understanding of the value of mixtures will require more empirical data and improved techniques for interpreting results.

In the first reported experimental assessment of the effect of the diversity of *B. thuringiensis* toxins on the rate of resistance development, McGaughey & Johnson (1992) compared responses of Indianmeal moth, *Plodia interpunctella* (Hübner), to various single strains and to a mixture of strains of *B. thuringiensis*. They found that this important pest of stored grain developed resistance to the mixture and each of the single strains tested in laboratory selection experiments. Although their results show that a mixture of strains did not preclude resistance, direct quantitative comparisons among treatments were problematic because of variation among treatments in selection intensity and the number of generations selected.

Quantitative genetic techniques (Via 1986, Falconer 1989) often can be used to reduce such

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problems. For example, estimation of realized heritability, the proportion of phenotypic variation in resistance caused by additive genetic variation, enables direct comparisons among selection experiments that differ in selection intensity and duration (Falconer 1989, Firko & Hayes 1990, Tabashnik 1992). This approach makes no assumptions beyond those used in probit analysis (Finney 1971) and requires no information about the mode of inheritance (Firko & Hayes 1990, Tabashnik 1992). Estimates of heritability and related parameters can be useful for understanding and managing evolution of resistance, particularly if one recognizes the limitations of such estimates (Tabashnik 1992).

We used quantitative genetic theory to derive criteria for comparing the risk of resistance development among single insecticides and between mixtures and sequences of two insecticides. These criteria were applied to previously reported data on responses of seven colonies of Indianmeal moth to selection with the subspecies *kurstaki*, *aizawai*, and *entomocidus* of *B. thuringiensis* (McGaughey & Beeman 1988, McGaughey & Johnson 1992). We also used estimates of realized heritability to determine if the rate of resistance development changed during individual selection experiments.

Materials and Methods

Selection Experiments. Data were obtained from selection experiments reported by McGaughey & Beeman (1988) and McGaughey & Johnson (1992). Six colonies of Indianmeal moth (each with an identification number in parentheses) were started from infestations in Nebraska (21), Iowa (37-6), Illinois (45-2 and 50-2), Oklahoma (343), and Kansas (688). The first five colonies had been reared in the laboratory for 18–26 generations before selection began (McGaughey & Beeman 1988). Colony 688 had been reared for eight generations before selection began (McGaughey & Johnson 1992). Colonies 21, 45-2, and 343 were started from individuals collected in grain bins that had been treated with *B. thuringiensis*; the other colonies were started from individuals collected from untreated bins. Each colony was started from 10–100 field-collected individuals.

The first five colonies were selected with Dipel, a wettable powder formulation of the HD-1 strain of *B. thuringiensis* ssp. *kurstaki* containing 16,000 IU of potency per mg of formulation (McGaughey & Beeman 1988). Colony 688 was split into five subsets that were selected with either Dipel, isolates HD-112 or HD-133 of *B. thuringiensis* ssp. *aizawai*, HD-198 of ssp. *entomocidus*, or a 1:1 mixture of Dipel and HD-133 (McGaughey & Johnson 1992). Colony 343R was a subcolony of 343 that had been selected with Dipel for 92 generations and showed >250-

fold resistance to Dipel when selection with HD-133 was started (McGaughey & Johnson 1992).

All four isolates of *B. thuringiensis* tested (HD-1, HD-112, HD-133, and HD-198) contained the toxins CryIA(a), CryIA(b), and at least two additional toxins (McGaughey & Johnson 1994). All isolates except HD-1 contained CryIC and CryID; HD-1 contained CryIA(c) and CryIIA; HD-112 contained CryIG and CryII (McGaughey & Johnson 1994).

Estimation of Realized Heritability. Heritability was estimated as described by Tabashnik (1992), with some minor modifications, as detailed below:

$$h^2 = R/S, \quad (1)$$

where R is the response to selection and S is the selection differential (Hartl 1988, Falconer 1989). We estimated the response to selection (R), the difference in mean phenotype between the offspring of the selected parents and the whole parental generation before selection (Falconer 1989), as

$$R = \frac{\log(\text{final LC}_{50}) - \log(\text{initial LC}_{50})}{n}, \quad (2)$$

where final LC_{50} is the LC_{50} of the offspring after n generations of selection. We estimated h^2 based on responses to 11–22 generations (mean = 18.6) of selection in 11 different experiments.

Initial LC_{50} was estimated as the LC_{50} of the parental generation before selection (generation 0) from the data reported in Tables 2–4 of McGaughey & Johnson (1992). Using the data of McGaughey & Beeman (1988), initial LC_{50} was estimated as the mean LC_{50} of the unselected control colony reared in parallel with each selected colony. Five to 18 estimates of LC_{50} per colony were used to estimate the mean LC_{50} for each unselected colony. These two methods of estimating initial LC_{50} produce similar results when the LC_{50} of the unselected colony is relatively constant. If the LC_{50} s of a control colony vary substantially, in an apparently random fashion, then the mean LC_{50} of a control colony is more appropriate than the initial parental LC_{50} for estimating R . If the LC_{50} s of an unselected colony show a strong directional trend through time, then one should use the final LC_{50} of the unselected colony (measured simultaneously with the final LC_{50} of the selected colony) rather than the initial LC_{50} of the selected line for calculating R .

The selection differential (S), the difference in mean phenotype between the selected parents and the entire parental generation (Hartl 1988, Falconer 1989), was estimated as

$$S = i\sigma_p, \quad (3)$$

where i is the intensity of selection and σ_p is the phenotypic standard deviation. Intensity of selection (i) was estimated as p , the percentage of the population with values above the selection threshold (i.e., the percentage surviving selection), divided by z , the height of the ordinate of a normal distribution at the selection threshold (Falconer 1989). Values of i for each p are tabulated by Falconer (1989). Polynomial regression analysis (SAS 1985) of tabulated values showed that for p between 10 and 80%, i can be estimated as

$$i = 1.583 - 0.0193336p + 0.0000428p^2 + 3.65194/p. \quad (4)$$

Mean total mortality per generation was estimated separately for each selected colony. For 180 of the 205 selected generations included in the analysis, mortality data were available. For the other 25 generations, mortality was estimated as the mean of the mortality in the two generations immediately preceding and following the data gap. In all such cases, the same concentration of *B. thuringiensis* had been used in the generations before, during, and after the gap. The number of generations with missing data ranged from 0 (colony 343R versus HD-133) to 6 (colony 50-2 versus HD-1) (mean = 2.3) per selected colony. Because mortality per generation was consistent within colonies and a minimum of eight estimates of mortality was available for each colony, it is unlikely that the data gaps introduced substantial error into the estimates of h^2 .

Mortality caused by insecticide was estimated by adjusting mean total mortality per generation for 10.5% mortality observed in untreated controls (McGaughey & Johnson 1992) with Abbott's (1925) method. The percentage of the population surviving selection (p), was estimated as 100% - adjusted mean % mortality.

The phenotypic standard deviation (σ_p) was estimated as the reciprocal of the mean of the estimated slopes of the concentration-mortality lines from probit analysis (Finney 1971) for each selected colony. Mean slope for each colony was calculated from 3-19 (mean = 11.5) independent estimates of slope. This approach may provide a more reliable estimate of mean slope than does simply averaging the initial and final slopes (Tabashnik 1992).

Criteria for Evaluating Durability of Single Insecticides. When using quantitative genetic techniques to evaluate selection experiments, one must recognize that estimates of heritability and related parameters are subject to experimental error and are specific to the populations and environments in which they are measured. Although the effects of these limitations can be reduced by replicating experiments, examining many conspecific populations, and using environments that are similar to the field, the cor-

respondence between laboratory-derived estimates and field outcomes has not been examined (Tabashnik 1992).

Response to selection (R) is the product of heritability (h^2) and selection differential (S) (Falconer 1989):

$$R = h^2S. \quad (5)$$

For any particular value of S , lower h^2 will produce slower resistance development. Thus, assuming that S is constant across insecticides, the insecticide for which the population has the lowest h^2 of resistance will have the greatest durability. Because S is the product of i and σ_p (equation 3), S is constant across insecticides for a particular percentage mortality only if the slope of the probit regression line (and thus σ_p) is constant across insecticides.

In practice, however, one would like to compare durability of insecticides when the mean percentage mortality per generation is the same across insecticides, but slope is not necessarily constant across insecticides. Thus, we define the response quotient (Q) as the response (R) divided by selection intensity (i)

$$Q = R/i. \quad (6)$$

This enables calculation of response to selection (R) without reference to slope

$$R = Qi. \quad (7)$$

For any particular mean percentage mortality, i is the same across insecticides (regardless of slope). Thus, assuming that the mean percentage mortality is constant across insecticides, the insecticide for which the population has the lowest Q will have the greatest durability. If the slopes of the concentration-mortality lines for a population's responses are similar for different insecticides, then evaluations of the population based on h^2 or Q will produce similar conclusions.

To test the hypothesis that the risk of resistance development is greater for strain HD-1 than strains HD-112, HD-133, and HD-198, we compared estimates of h^2 and Q obtained from 10 selection experiments previously reported by McGaughey & Beeman (1988) and McGaughey & Johnson (1992). We used a Mann-Whitney U -test (Sokal & Rohlf 1981) to evaluate differences in h^2 and Q between HD-1 and the three other strains.

Criteria for Evaluating Mixtures Versus Sequences of Insecticides. Assume that two insecticides, A and B, are available. One can use estimates of h^2 or Q to compare the expected useful life of A and B used sequentially versus A and B combined in a mixture. In the sequence, A is used repeatedly until resistance to A occurs, then B is used repeatedly until resistance to B occurs. In the mixture, A and B are combined and used

simultaneously until resistance occurs to the combination.

We first assume that no cross-resistance occurs; resistance to one insecticide (i.e., B) does not increase or decrease while the other one (i.e., A) is used (relaxation of this assumption is considered below). We also assume that the criterion for resistance is a 10-fold increase in LC_{50} . (The relative merits of mixtures versus sequences will be similar regardless of the level of increase designated as the criterion for resistance. Both tactics will have greater durability as the extent of increase in LC_{50} defined as resistance increases.) In general, the number of generations required for a 10-fold increase in LC_{50} , G , can be estimated (Tabashnik 1992) as

$$G = R^{-1} = (h^2 S)^{-1}. \quad (8)$$

The h^2 of resistance to A, B, and the mixture of A and B are defined as h_A^2 , h_B^2 , and h_{AB}^2 , respectively. Assume that the selection differential (S) is the same for the sequence and the mixture. The useful life of the sequence ($G_A + G_B$) can be estimated as

$$G_A + G_B = (h_A^2 S)^{-1} + (h_B^2 S)^{-1}. \quad (9)$$

The estimated useful life of the mixture (G_{AB}) is

$$G_{AB} = (h_{AB}^2 S)^{-1}. \quad (10)$$

By arithmetic rearrangement, the expected useful life of the mixture is greater than that of the sequence ($G_{AB} > G_A + G_B$) if

$$h_{AB}^2 < (h_A^2 h_B^2) / (h_A^2 + h_B^2). \quad (11)$$

If $h_A^2 = h_B^2$, then equation 11 simplifies to

$$h_{AB}^2 < h_A^2 / 2. \quad (12)$$

This shows that the mixture has greater durability than the sequence if heritability of resistance to the mixture is less than half of the heritability of resistance to the two individual components of the mixture.

When h_A^2 is not equal to h_B^2 , the insecticide with lower h^2 (e.g., A) will be used for more generations in the sequence than the insecticide with higher h^2 (i.e., B) ($G_A > G_B$). Thus, the overall mean h^2 per generation for the sequence of A and B will be lower than the mean of h_A^2 and h_B^2 . It follows that, in general, the sequence lasts longer than the mixture if the heritability of resistance to the mixture is not less than half of the mean heritability of resistance to the two individual components of the mixture. (This analysis can be extended to any number of insecticides; a sequence lasts longer than a mixture unless the heritability of resistance to the mixture is less than the mean heritability of resistance to the individual insecticides divided by the number of insecticides.)

As the discrepancy between h_A^2 and h_B^2 increases, the mixture is not favored unless h_{AB}^2 is an increasingly smaller proportion of the mean of h_A^2 and h_B^2 . For example, if $h_A^2 = 0.1$ and $h_B^2 = 0.4$, the mean h^2 for A and B = 0.25. Using equation 11, the mixture of A and B will last longer than the sequence of A and B if $h_{AB}^2 < 0.08$, which is less than one third of the mean of h_A^2 and h_B^2 ($0.08/0.25 = 0.32$).

As described for single insecticides, one would like a criterion for comparing mixtures versus sequences when the percentage mortality per generation (and thus i) is the same for both tactics, but the slope is not necessarily the same for both tactics. To derive this criterion, we define Q_A , Q_B , and Q_{AB} as the response quotients for insecticides A, B, and the mixture of A and B, respectively. Because $R = Qi$ (equation 7), we can use the approach illustrated in equations 8–12 to show that durability is greater for the mixture than the sequence if

$$Q_{AB} < (Q_A Q_B) / (Q_A + Q_B). \quad (13)$$

As described above, the sequence lasts longer than the mixture if the response quotient of the population to the mixture (Q_{AB}) is not less than half of the mean response quotient of the population to the two individual components of the mixture ($0.25 [Q_A + Q_B]$).

Thus far, we have assumed that selection with one insecticide (A) has no effect on resistance to the other insecticide (B). If, however, selection with A causes complete cross-resistance to B, only A will be useful in the sequence (10-fold resistance to A will confer 10-fold resistance to B). In this case, the durability of a mixture versus a sequence can be evaluated simply by comparing response quotients (Q) between the mixture and the first insecticide in the sequence; a lower value of Q indicates greater durability. This adjustment of the criterion reduces the expected durability of a sequence compared with a mixture. Conversely, if selection with A increases susceptibility to B (negative cross-resistance), the durability of a sequence relative to a mixture will be greater than expected on the basis of equations 11–13.

Temporal Changes within Colonies. To determine if R , S , or h^2 changed during the course of individual experiments, we calculated each of these parameters for the first and second parts of each experiment separately. The split between the two parts was as close to half as allowed by data. We used a sign test to examine the statistical significance of differences between the values for the first and second halves.

Results

Resistance to Single Strains of *B. thuringiensis*. For six colonies of Indianmeal moth (*Mc-*

Table 1. Realized heritability (h^2) and response quotient (Q) for resistance to Dipel (HD-1 strain of *B. thuringiensis* sp. *kurstaki*) in colonies of *P. interpunctella*

Colony ^a	Estimate of mean response per generation				Estimate of mean selection differential per generation				h^2	Q
	n^b	Initial LC ₅₀	Final LC ₅₀	R	p	i	Mean slope	S		
21	22	27.4	941	0.0698	76.2	0.406	1.33	0.305	0.23	0.17
37-6	20	18.8	451	0.0690	70.8	0.480	1.96	0.245	0.28	0.14
45-2	12	18.6	649	0.129	66.8	0.538	1.50	0.358	0.36	0.24
50-2	20	17.1	140	0.0457	74.7	0.426	2.53	0.168	0.27	0.11
343	20	12.9	1460	0.103	81.9	0.331	1.96	0.169	0.61	0.31
Means	19	19.0	728	0.0833	74.1	0.436	1.86	0.249	0.35	0.19

Estimated from laboratory selection experiments of McGaughey & Beeman (1988).

^a Origins of each colony of *P. interpunctella* are described in *Materials and Methods*.

^b Number of generations selected.

Gaughey & Beeman 1988, McGaughey & Johnson 1992), estimates of realized heritability (h^2) of resistance to the HD-1 strain of *B. thuringiensis* subsp. *kurstaki* (Dipel) ranged from 0.23–0.61 (mean = 0.35) (Tables 1 and 2). The response quotient (Q) for resistance to HD-1 ranged from 0.11–0.31 (mean = 0.19) (Tables 1 and 2).

The six h^2 estimates for resistance to strain HD-1 did not differ significantly from the four h^2 estimates for resistance to strains HD-112, HD-133, and HD-198 of *B. thuringiensis* (range, 0.22–0.43; mean = 0.32) (Mann–Whitney, $U = 11.5$, $P > 0.20$). Likewise, the response quotient (Q) of resistance did not differ significantly between the six estimates for HD-1 and the four estimates for HD-112, HD-133, and HD-198 (range, 0.10–0.24; mean = 0.16) (Table 2) (Mann–Whitney, $U = 15.5$, $P > 0.20$).

Resistance to a Mixture of Strains. The h^2 of resistance to the mixture of strains HD-1 and HD-133 was 0.23 for colony 688 (Table 2). Although this value is lower than the h^2 for colony 688 to either HD-1 ($h^2 = 0.34$) or HD-133 ($h^2 = 0.36$), h^2 of resistance to the mixture was greater than half of the mean h^2 of the two components of the mixture ($0.5 \times \text{mean } h^2 = 0.175$).

The same pattern occurred for the response quotients (Q). Q was less for the mixture ($Q = 0.10$) than for HD-1 ($Q = 0.18$) or HD-133 ($Q = 0.18$), but Q for the mixture was not less than half of the mean Q for the two components ($0.5 \times 0.18 = 0.09$) (Table 2). Thus, assuming no cross-resistance, a sequence of HD-1 and HD-133 would have greater expected durability than a mixture (see *Materials and Methods*).

Temporal Changes Within Colonies. R , S , and h^2 declined within colonies as selection progressed (Table 3). For all 11 experiments, R and S were higher in the first half than in the second half of the experiment (sign test, $P < 0.0001$). In 10 of the 11 experiments, h^2 was higher in the first half than in the second half of the experiment (sign test, $P = 0.01$). Mean h^2 in the first half (0.46) was more than double mean h^2 in the second half (0.17) of experiments (Table 3).

The higher initial value of S simply reflects greater mortality during initial generations of selection (i.e., more intense selection). The higher initial h^2 shows, however, that the proportion of phenotypic variation accounted for by additive genetic variation decreased during the course of experiments.

Table 2. Realized heritability (h^2) and response quotient (Q) for resistance to *B. thuringiensis* in *P. interpunctella*

Strain	Estimate of mean response per generation				Estimate of mean selection differential per generation				h^2	Q
	n^a	Initial LC ₅₀	Final LC ₅₀	R	p	i	Mean slope	S		
Colony 688										
HD-1 ^b	23	13.4	1880	0.0933	69.0	0.506	1.82	0.278	0.34	0.18
HD-112 ^c	21	3.9	112	0.0694	57.4	0.678	2.13	0.318	0.22	0.10
HD-133 ^c	22	5.1	314	0.0813	72.3	0.459	2.03	0.226	0.36	0.18
HD-198 ^d	18	8.4	176	0.0734	58.9	0.655	2.61	0.251	0.29	0.11
HD-1+133	16	8.5	126	0.0732	56.1	0.699	2.20	0.317	0.23	0.10
Colony 343R										
HD-133 ^c	11	44.3	949	0.121	69.6	0.497	1.78	0.279	0.43	0.24

Estimated from laboratory selection experiments of McGaughey & Johnson (1992).

^a Number of generations selected.

^b *Ssp. kurstaki*.

^c *Ssp. aizawai*.

^d *Ssp. entomocidus*.

Table 3. Estimates of response to selection (R), selection differential (S), and realized heritability (h^2) from the first versus second half of selection experiments with colonies of *P. interpunctella*

Colony	Strain ^a	First half				Second half			
		n^b	R	S	h^2	n^b	R	S	h^2
21	HD-1	8	0.110	0.361	0.30	14	0.047	0.274	0.17
37-6	HD-1	11	0.095	0.288	0.33	9	0.037	0.195	0.19
45-2	HD-1	5	0.277	0.386	0.72	7	0.022	0.399	0.07
50-2	HD-1	7	0.110	0.228	0.48	13	0.011	0.138	0.08
343	HD-1	10	0.155	0.202	0.76	10	0.051	0.137	0.37
343R	HD-133	6	0.175	0.349	0.50	5	0.056	0.202	0.28
688	HD-1	11	0.144	0.293	0.49	12	0.047	0.264	0.18
688	HD-112	13	0.070	0.323	0.22	8	0.068	0.311	0.22
688	HD-133	10	0.142	0.237	0.60	12	0.031	0.217	0.14
688	HD-198	11	0.163	0.264	0.39	7	0.027	0.230	0.12
688	HD-1+133	10	0.106	0.354	0.30	6	0.018	0.260	0.07

Data from McGaughey & Beeman 1988, McGaughey & Johnson 1992.

^a *B. thuringiensis* ssp. *kurstaki* (HD-1), *aizawai* (HD-112 and HD-133), and *entomocidus* (HD-198).

^b Number of generations selected.

Discussion

Interpretation of the results is complicated because each of the single strains of *B. thuringiensis* tested contained a mixture of at least four insecticidal crystal proteins (McGaughey & Johnson 1994). Further, the abundance of the toxins in each strain, the extent of interactions among toxins, and the relative potencies of some of the toxins are not known. Nonetheless, estimation of h^2 and related parameters provided a means for systematic comparisons between experiments.

McGaughey & Johnson (1992) hypothesized that the toxins in *B. thuringiensis* strains HD-112, HD-133, and HD-198 may be more diverse than those in HD-1 and, thus, might cause a slower progression of resistance than HD-1. Our analysis showed that for the colonies of Indianmeal moth examined, the mean values for h^2 (0.35) and Q (0.19) for resistance to HD-1 were not significantly higher than the respective means for resistance to HD-112, HD-133, and HD-198 ($h^2 = 0.32$, $Q = 0.16$). These results imply that if S or mean percentage mortality per generation were the same for HD-1 and the three other strains of *B. thuringiensis*, one would not expect Indianmeal moth to evolve resistance faster to HD-1 than to the three other strains.

Assuming no cross-resistance between HD-1 and HD-133, our analysis suggests that for Indianmeal moth, these strains would be no more durable in a mixture than in a sequence. Because selection with HD-1 did cause minimal cross-resistance to HD-133 (McGaughey & Beeman 1988, 1993), the durability of a sequence of HD-1 followed by HD-133 would be slightly less than expected on the basis of equation 9 (see *Material and Methods*). Even so, the mixture would have little or no advantage compared with a sequence of HD-1 followed by HD-133. In contrast, because selection with HD-133 caused complete cross-resistance to HD-1 (McGaughey & Johnson 1994), HD-1 would be useless in a

sequence after HD-133. For colony 688, the response quotient was about half for the mixture of HD-1 + HD-133 ($Q = 0.10$) compared with HD-133 ($Q = 0.18$), which suggests that the mixture would be approximately twice as durable as HD-133. Therefore, we conclude that the worst strategy would be a sequence of HD-133 followed by HD-1; a sequence of HD-1 followed by HD-133 or a mixture of HD-1 + HD-133 would be substantially better.

The asymmetrical pattern of cross-resistance between HD-1 and HD-133 reflects differences in the toxin composition of the two strains. CryIA(b) and CryIC are highly toxic to Indianmeal moth (van Rie et al. 1990). CryIA(b) is found in both strains; CryIC is found in HD-133 but not in HD-1 (McGaughey & Johnson 1994). Selection with HD-1 did not cause cross-resistance to CryIC in Indianmeal moth (van Rie et al. 1990, McGaughey & Johnson 1994); thus, selection with HD-1 caused limited cross-resistance to HD-133 (van Rie et al. 1990, McGaughey & Johnson 1994). Selection with HD-133 causes resistance to CryIA(b), a major component of HD-1 (McGaughey & Johnson 1994).

The finding that a mixture of strains HD-1 and HD-133 would not slow development of resistance compared with sequential use of HD-1 followed by HD-133 contradicts the claim that mixtures of insecticides prevent or greatly retard resistance development. Theoretical models that show advantages of mixtures compared with sequences of insecticides are based on many assumptions, including absence of cross-resistance, recessive inheritance, low initial frequency of resistance alleles, and untreated refuges for a portion of the population (Mani 1985, Curtis 1985, Tabashnik 1989). Available evidence suggests that these assumptions were not valid for the experiments that examined resistance of Indianmeal moth to *B. thuringiensis*.

If resistance to each component of a mixture is not completely recessive, heterozygotes survive, and the expected advantage of the mixture is greatly diminished or eliminated (Mani 1985, Curtis 1985). Inheritance of resistance to the HD-1 isolate of *B. thuringiensis* was not completely recessive in Indianmeal moth (McGaughey & Beeman 1988).

Unless a portion of the population is allowed to escape exposure in refuges, treatment with mixtures is expected to cause local extinction (if no doubly resistant individuals are present) or rapid resistance development (Tabashnik 1989). Because Indianmeal moth larvae ate treated artificial diet, variation in dose among larvae was likely, but complete refuge from exposure to *B. thuringiensis* was unlikely. Survival was relatively high (19%) in the first generation of selection with the mixture, indicating the presence of doubly resistant individuals. Without refuges, resistance to the mixture increased quickly, as expected.

In most models of resistance development, including those that show advantages of mixtures (Mani 1985), alleles conferring resistance are assumed to be rare, with a frequency typically ranging from 10^{-5} to 10^{-2} (Tabashnik 1990). Results with Indianmeal moth, however, suggest that alleles for resistance to *B. thuringiensis* are more common than previously assumed. The colonies used in the selection experiments were started with 10–100 individuals and maintained without exposure to *B. thuringiensis* for at least 8 generations. Assuming that the effects of mutation after colonization were negligible, no response to selection would have occurred unless at least one allele for resistance was present in the diploid individuals used to start each colony. This sets a range for the lower limit for the frequency of one or more resistance alleles of $0.005 - 0.05$ ($1/[2 \times 100] - 1/[2 \times 10]$).

The finding that h^2 declined during selection experiments (Table 3) suggests that the initial frequency of one or more resistance alleles was higher than the range mentioned. With partially recessive inheritance, as seen in Indianmeal moth resistance to HD-1 (McGaughey 1985, McGaughey & Beeman 1988), additive genetic variance and h^2 peak at resistance allele frequencies between 50 and 75%; they approach zero as allele frequencies approach either 0 or 100% (Falconer 1989). High initial levels of h^2 followed by declines after 5–11 generations of selection suggest that alleles for resistance were at intermediate levels (>10%) initially, then approached fixation as selection progressed.

Estimates of h^2 of resistance to *B. thuringiensis* were significantly higher for Indianmeal moth (range, 0.23–0.61; $n = 11$) than for seven other species of moths (range, 0.04–0.20; $n = 10$) (Tabashnik 1994) (Mann-Whitney $U = 110$, $P < 0.001$). Although only three of the colonies of

Indianmeal moth were started from grain bins that had been treated commercially with *B. thuringiensis*, periodic exposure to natural infestations of *B. thuringiensis* (Burgess & Hurst 1977) may have increased the frequency of alleles conferring resistance to *B. thuringiensis* in this pest.

In summary, we evaluated responses to four strains of *B. thuringiensis* by seven laboratory colonies of Indianmeal moth. Although this represents one of the most extensive examinations of resistance to *B. thuringiensis* in any insect, estimates of heritability and related parameters are specific to the populations and environments in which they are measured. Thus, we do not know if our conclusions apply in the field to Indianmeal moth or other pests. The initial frequency of alleles conferring resistance to *B. thuringiensis* may be higher in Indianmeal moth than in other pests. If so, experiments with Indianmeal moth may underestimate the potential of mixtures to slow resistance development in other pests. The selection experiments analyzed in our study did not incorporate refuges, yet refuges are likely to be essential for successful implementation of mixtures in the field.

Temporal changes in h^2 of resistance to *B. thuringiensis* within single laboratory colonies of Indianmeal moth highlight the fact that h^2 of resistance to a particular insecticide is not a fixed property, even for a single population. Because h^2 can change through time as evolution of resistance progresses, extrapolation of results substantially beyond the number of generations studied may be misleading.

Additional data from laboratory and field experiments are needed to clarify the issues considered here. In particular, direct experimental comparisons of responses to single toxins versus mixtures of toxins from *B. thuringiensis* have not been reported. When such data are obtained, the criteria described here can be used for systematic comparisons between replicated experiments. The available evidence suggests that Indianmeal moth and other pests can evolve resistance to mixtures of *B. thuringiensis* toxins (Tabashnik 1994). Until more data are available, one cannot assume that mixtures of toxins will prevent or greatly retard development of resistance.

Acknowledgments

We are grateful to D. Heckel (Department of Biological Sciences, Clemson University), C. Boake (Department of Zoology, University of Tennessee), S. Via (Department of Entomology, Cornell University), and G. Roderick (Hawaiian Evolutionary Biology Program, University of Hawaii) for their thoughtful comments. This research was supported by USDA grant HAW00947H, USDA-CSRS Special Grant in Tropical/Subtropical Agriculture 92-34135-7314 and the USDA Western Regional Integrated Pest Management Program. This is paper 3889 of the Hawaii Institute of

Tropical Agriculture and Human Resources Journal Series. University of Hawaii, Honolulu.

References Cited

- Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265-267.
- Burges, H. D. & J. A. Hurst. 1977. Ecology of *Bacillus thuringiensis* in storage moths. *J. Invertebr. Pathol.* 30: 131-139.
- Curtis, C. F. 1985. Theoretical models of the use of insecticide mixtures for the management of resistance. *Bull. Entomol. Res.* 75: 259-265.
- Denholm, I. & M. W. Rowland. 1992. Tactics for managing pesticide resistance in arthropods: theory and practice. *Annu. Rev. Entomol.* 37: 91-112.
- Falconer, D. S. 1989. Introduction to quantitative genetics, 3rd ed. Longman, New York.
- Feitelson, J. S., J. Payne & L. Kim. 1992. *Bacillus thuringiensis*: insects and beyond. *Bio/Technology* 10: 271-275.
- Ferré, J., M. D. Real, J. van Rie, S. Jansens & M. Peferoen. 1991. Resistance to the *Bacillus thuringiensis* bioinsecticide in a field population of *Plutella xylostella* is due to a change in a midgut membrane receptor. *Proc. Natl. Acad. Sci. U.S.A.* 88: 5119-5123.
- Finney, D. J. 1971. Probit analysis, 3rd ed. Cambridge University, London.
- Firko, M. J. & J. L. Hayes. 1990. Quantitative genetic tools for insecticide resistance risk assessment: estimating the heritability of resistance. *J. Econ. Entomol.* 83: 647-654.
- Georghiou, G. P. 1990. Resistance potential to biopesticides and consideration of countermeasures, pp. 409-420. *In* J. E. Casida [ed.], *Pesticides and alternatives*. Elsevier, New York.
- Gill, S. S., E. A. Cowles & P. V. Pietrantonio. 1992. The mode of action of *Bacillus thuringiensis* endotoxins. *Annu. Rev. Entomol.* 37: 615-636.
- Gould, F. 1988. Genetic engineering, integrated pest management and the evolution of pests. *Trends Ecol. Evol.* 3: 515-518.
- Gould, F., A. Martinez-Ramirez, A. Anderson, J. Ferré, F. J. Silva & W. J. Moar. 1992. Broad-spectrum resistance to *Bacillus thuringiensis* toxins in *Heliothis virescens*. *Proc. Natl. Acad. Sci. U.S.A.* 89: 7986-7988.
- Hartl, D. L. 1988. A primer of population genetics, 2nd ed. Sinauer, Sunderland, MA.
- Mani, G. S. 1985. Evolution of resistance in the presence of two insecticides. *Genetics* 109: 761-783.
- McGaughey, W. H. 1985. Insect resistance to the biological insecticide *Bacillus thuringiensis*. *Science* (Washington, DC) 229: 193-195.
- McGaughey, W. H. & R. W. Beeman. 1988. Resistance to *Bacillus thuringiensis* in colonies of Indianmeal moth and almond moth (Lepidoptera: Pyralidae). *J. Econ. Entomol.* 81: 28-33.
- McGaughey, W. H. & D. E. Johnson. 1987. Toxicity of different serotypes and toxins of *Bacillus thuringiensis* to resistant and susceptible Indianmeal moth (Lepidoptera: Pyralidae). *J. Econ. Entomol.* 80: 1122-1126.
1992. Indianmeal moth (Lepidoptera: Pyralidae) resistance to different strains and mixtures of *Bacillus thuringiensis*. *J. Econ. Entomol.* 85: 1594-1600.
1994. Influence of crystal protein composition of *Bacillus thuringiensis* strains on cross-resistance in Indianmeal moths (Lepidoptera: Pyralidae). *J. Econ. Entomol.* 87: 535-540.
- McGaughey, W. H. & M. E. Whalon. 1992. Managing insect resistance to *Bacillus thuringiensis* toxins. *Science* (Washington, DC) 258: 1451-1455.
- National Research Council. 1986. Pesticide resistance: strategies and tactics for management. National Academy of Sciences, Washington, DC.
- Roush, R. T. & B. E. Tabashnik [eds.]. 1990. Pesticide resistance in arthropods. Chapman & Hall, New York.
- SAS Institute. 1985. SAS user's guide: statistics, 5th ed. SAS Institute, Cary, NC.
- Sokal, R. R. & R. L. Rohlf. 1981. Biometry, 2nd ed. Freeman, San Francisco.
- Stone, T. B., S. R. Sims, S. C. MacIntosh, R. L. Fuchs & P. G. Marrone. 1991. Insect resistance to *Bacillus thuringiensis*, pp. 53-66. *In* K. Maramorosch [ed.], *Biotechnology for biological control of pests and vectors*. CRC, Boca Raton, FL.
- Tabashnik, B. E. 1989. Managing resistance with multiple pesticide tactics: theory, evidence and recommendations. *J. Econ. Entomol.* 82: 1263-1269.
1990. Modeling and evaluation of resistance management tactics, pp. 153-182. *In* R. T. Roush & B. E. Tabashnik [eds.], *Pesticide resistance in arthropods*. Chapman & Hall, New York.
1992. Resistance risk assessment: realized heritability of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae), tobacco budworm (Lepidoptera: Noctuidae), and Colorado potato beetle (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* 85: 1551-1559.
1994. Evolution of resistance to *Bacillus thuringiensis*. *Annu. Rev. Entomol.* 39: 47-79.
- Tabashnik, B. E., N. L. Cushing, N. Finson & M. W. Johnson. 1990. Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). *J. Econ. Entomol.* 83: 1671-1676.
- Tabashnik, B. E., N. Finson, M. W. Johnson & W. J. Moar. 1993. Resistance to toxins from *Bacillus thuringiensis* subsp. *kurstaki* causes minimal cross-resistance to *B. thuringiensis* subsp. *aizawai* in the diamondback moth (Lepidoptera: Plutellidae). *Appl. Environ. Microbiol.* 59: 1332-1335.
- Van Rie, J. 1991. Insect control with transgenic plants: resistance proof? *Trends Biotechnol.* 9: 177-179.
- Van Rie, J., W. H. McGaughey, D. E. Johnson, B. D. Barnett & H. Van Mellaert. 1990. Mechanism of insect resistance to the microbial insecticide *Bacillus thuringiensis*. *Science* (Washington, DC) 247: 72-74.
- Via, S. 1986. Quantitative genetic models and the evolution of pesticide resistance, pp. 222-235. *Pesticide resistance: strategies and tactics for management*. National Academy of Sciences, Washington, DC.

Received for publication 23 September 1993; accepted 1 March 1994.