

INSECTICIDE RESISTANCE AND RESISTANCE MANAGEMENT

Indianmeal Moth (Lepidoptera: Pyralidae) Resistance to Different Strains and Mixtures of *Bacillus thuringiensis*

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ABSTRACT The potential for Indianmeal moths, *Plodia interpunctella* (Hübner), to evolve resistance to isolates of *Bacillus thuringiensis* other than subsp. *kurstaki* HD-1 was determined through laboratory selection experiments. Resistance developed quickly toward isolates HD-112 and HD-133 of subsp. *aizawai* and HD-198 of subsp. *entomocidus*, reaching levels of 28, 61, and 21, respectively, after \approx 20 generations of selection. However, the rates of progression were somewhat slower toward these isolates than toward HD-1, probably because of the different toxin composition of these other isolates. Simultaneous selection for resistance to a mixture of HD-1 and HD-133 resulted in resistance to both isolates, but the rate toward the mixture was somewhat slower than that to the individual isolates. An insect population that was already resistant to isolate HD-1 readily developed resistance to isolate HD-133 as well. The results demonstrate that mixtures of strains do not preclude the possibility of resistance and that their value in slowing resistance development may not be large.

KEY WORDS *Plodia interpunctella*, *Bacillus thuringiensis*, insecticide resistance

INSECT RESISTANCE to the insecticidal crystal proteins (ICPs) or δ -endotoxins of the bacterium *Bacillus thuringiensis* is a relatively recent phenomenon that has the potential for severely limiting their usefulness in pest control. These environmentally safe insect toxins have wide applications in the control of lepidopteran pests of crops, ornamentals, forests, and stored products; at least one coleopteran pest of crops; and several dipteran pests that are of public health significance. Currently, they are the subject of intense research and development efforts directed toward incorporation of the genes that encode for their production into crop plants to enable the plants to produce the insecticidal proteins within their own tissues. (For recent reviews, see Gasser & Fraley 1989, Boulter et al. 1990.) This gene transfer approach using foreign genes represents a major technological advance in pest control that would eliminate the need for conventional application of insecticides and would overcome some of the stability and coverage problems associated with conventional application. Unfortunately, the gene transfer approach may also increase the likelihood of insect resistance by providing continuous, long-term exposure of pests to the toxins.

No cases have been reported that describe insects evolving resistance to toxins in genetically transformed plants, but no such plants are yet in commercial use. However, there have been at least five cases of insects being selected for re-

sistance to these ICPs under laboratory conditions or when applied by conventional means to crop plants. The Indianmeal moth, *Plodia interpunctella* (Hübner), and the almond moth, *Cadra cautella* (Walker), both pests of stored food products, were the first to be selected for resistance in the laboratory (McGaughey 1985, McGaughey & Beeman 1988). A colony of the tobacco budworm, *Heliothis virescens* (F.), was selected in the laboratory for resistance to a genetically transformed *Pseudomonas fluorescens* strain that expresses endotoxin protein from *B. thuringiensis* subsp. *kurstaki* (Stone et al. 1989). A colony of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), was selected in the laboratory for resistance to a strain of *B. thuringiensis* active against Coleoptera (Miller et al. 1990). Tabashnik et al. (1990) have recently reported field development of resistance in the diamondback moth, *Plutella xylostella* (L.), a major lepidopteran pest of vegetables. Low levels of resistance have been reported in two mosquito species, *Aedes aegypti* (L.) and *Culex quinquefasciatus* (Say), but the practical implications in these species is not apparent (Georghiou et al. 1983, Goldman et al. 1986).

With the recognition that insect resistance is a serious threat to the future use of *B. thuringiensis*, either as a conventionally applied insecticide or in crop plant transformation, research is now beginning to focus on strategies for minimizing or avoiding resistance development. Current

strategies seem to be based upon the belief that there is a virtually unlimited variety of *B. thuringiensis* ICPs that differ in structure and insecticidal spectrum (see Höfte & Whiteley [1989] for classification), which can be deployed using various rotational, sequential, or mixture strategies either to prevent resistance development or to reestablish control once resistance has occurred. The theoretical basis of these approaches and some of the biological factors influencing their success have been reviewed and partially evaluated by Gould (1986a,b, 1988), Roush (1989), Tabashnik (1989), Georghiou (1990), Gould & Anderson (1991), Gould et al. (1991a,b), and Tabashnik et al. (1991). The success of these approaches ultimately may require detailed knowledge of pest genetics and population ecology and behavior under field conditions. At the very least, these approaches will require knowledge of the extent to which insects are capable of evolving resistance to various different ICPs and an understanding of the cross-resistance patterns among the various kinds of ICPs.

The purpose of our study was to determine whether, and to what extent, Indianmeal moths are capable of evolving resistance to several strains of *B. thuringiensis* other than the HD-1 isolate of subsp. *kurstaki*, which was used in the previously reported studies. The studies focused on *B. thuringiensis* isolates to which the HD-1-resistant Indianmeal moths remained partially or completely susceptible, including isolates of subspp. *aizawai* and *entomocidus*. We attempted to select for resistance (1) to these additional isolates individually; (2) to a second isolate in a colony already resistant to HD-1 (sequentially); and (3) to two different isolates simultaneously.

Materials and Methods

The potential for resistance to four different *B. thuringiensis* isolates was investigated. Although the capacity for resistance to subsp. *kurstaki* isolate HD-1 has already been reported in the Indianmeal moth (McGaughey 1985, McGaughey & Beeman 1988), a formulation containing this isolate was included as a standard in the experiments (Dipel wettable powder [WP], 18,000 IU/mg, Abbott Laboratories, North Chicago, IL). Two isolates of subsp. *aizawai*, HD-112 and HD-133, and one of subsp. *entomocidus*, HD-198, were included because previous studies had shown that these strains were still toxic toward HD-1-resistant Indianmeal moths (McGaughey & Johnson 1987).

Each isolate was cultured in Fernbach flasks with YEG medium consisting of the following: yeast extract, 15 g/liter; glucose, 2 g/liter; K_2PO_4 , 3 g/liter. The pH was adjusted to 7.3 with 1 M KOH. The cultures were incubated for 4 to 5 d at 30°C with agitation until sporulation was complete. Spores and crystals were harvested and

washed initially by centrifugation with 1 M NaCl, followed by three to four washes with 0.02 M Tris·HCl, 0.05 M NaCl (pH 8.0). Spore-crystal preparations from each isolate were lyophilized and stored at -20°C.

Indianmeal moths used in the studies were from a newly established *B. thuringiensis* susceptible colony (RC-688; eight generations in the laboratory) started with ≈100 adults collected during June 1988 from a farm grain storage bin in Riley County, KS, and from a laboratory colony already selected for very high resistance to subsp. *kurstaki* isolate HD-1 (343R, selected for 92 generations; >250-fold resistance to Dipel) (McGaughey 1985, McGaughey & Beeman 1988). Colony RC-688 had no known previous exposure to *B. thuringiensis*. This susceptible colony was used in experiments to determine the progression of resistance toward the four isolates individually and toward isolates HD-133 and Dipel simultaneously. The HD-1-resistant colony (343R) was used to test the capacity of already resistant insects to evolve sequential resistance to a second isolate, HD-133. The insects were reared on an enriched ground wheat larval diet and the selection experiments were done by rearing them on diet in which the appropriate *B. thuringiensis* isolate had been incorporated using the methods reported in earlier studies (McGaughey 1985, McGaughey & Beeman 1988). All rearing, selection, and bioassay procedures were done at 25°C and 60–70% RH. Under these conditions, generation time was ≈22 d on untreated diet.

Before beginning selection, bioassays were conducted to estimate baseline levels of susceptibility of the insect colonies to each of the *B. thuringiensis* isolates. Data from these preliminary bioassays were used to estimate doses to be used initially in the rearing medium that would cause ≈70% mortality. As resistance progressed and survival on the treated diet increased, the doses used in the diet were increased in subsequent generations. The schedule followed in selecting each line is shown in Tables 1, 2, and 3 along with the data on the levels of survival in each generation. For several of the selected lines, low survival early in the selection process necessitated skipping a generation of selection and rearing the insects on untreated diet to restore the size of the colony. These instances are noted in the tables. In testing the potential for resistance to evolve toward two isolates simultaneously, a 50:50 mixture of Dipel and isolate HD-133 was used. As with the selection experiments on individual isolates, preliminary bioassays were used to estimate the dose of this mixture to use in the rearing medium to produce ≈70% mortality in the initial generation.

Survival levels in each generation were monitored by counting the adults that emerged from small (30-g) samples of treated diet that were

Table 1. Selection schedule and survival rates of *B. thuringiensis*-susceptible Indianmeal moth colony RC-688 selected for resistance to different isolates of *B. thuringiensis*

<i>kurstaki</i> (Dipel)			<i>aizawai</i> (HD-112)			<i>aizawai</i> (HD-133)			<i>entomocidus</i> (HD-198)		
Generation selected	Rate (mg/kg)	% Survival ±SEM	Generation selected	Rate (mg/kg)	% Survival ±SEM	Generation selected	Rate (mg/kg)	% Survival ±SEM	Generation selected	Rate (mg/kg)	% Survival ±SEM
1	31.25	20 ± 2.3	1	8	27 ± 4.3	1	11	31 ± 1.8	1	18	20 ± 3.1
2	31.25	—	2	8	—	2	11	—	2	18	—
	0 ^a			0 ^a			0 ^a			0 ^a	
3	31.25	40 ± 3.1	3	8	29 ± 5.2	3	11	57 ± 9.0	3	18	19 ± 6.6
4	31.25	55 ± 6.6		0 ^a	—	4	11	57 ± 4.7		0 ^a	—
5	31.25	81 ± 1.8	4	8	35 ± 3.5	5	11	—	4	18	33 ± 2.9
6	31.25	85 ± 3.5	5	8	40 ± 4.0	6	11	67 ± 5.3	5	18	46 ± 8.1
7	31.25	71 ± 5.2	6	8	49 ± 3.5	7	11	72 ± 2.0		0 ^a	—
8	31.25	64 ± 4.0	7	8	61 ± 3.5	8	11	80 ± 5.0	6	18	52 ± 8.3
9	31.25	70 ± 4.6	8	8	63 ± 2.9	9	11	85 ± 3.7	7	18	71 ± 2.4
10	31.25	77 ± 4.8	9	8	65 ± 1.8	10	11	—	8	18	70 ± 3.1
11	31.25	67 ± 1.3	10	8	71 ± 5.9	11	11	70 ± 3.1	9	18	77 ± 2.7
12	125	68 ± 6.4	11	8	73 ± 2.9	12	62.5	71 ± 5.7	10	18	78 ± 5.3
13	125	80 ± 4.6	12	8	65 ± 4.7	13	62.5	68 ± 5.0	11	18	72 ± 6.0
14	125	62 ± 3.1	13	8	55 ± 4.4	14	62.5	73 ± 3.7	12	62.5	50 ± 2.0
15	125	59 ± 1.3	14	31.25	49 ± 3.7	15	62.5	78 ± 7.6	13	62.5	53 ± 3.7
16	250	60 ± 1.2	15	31.25	53 ± 4.7	16	125	55 ± 4.8	14	62.5	63 ± 3.7
17	250	78 ± 5.0	16	31.25	53 ± 1.8	17	125	35 ± 1.8	15	62.5	45 ± 4.7
18	250	65 ± 3.7	17	31.25	58 ± 2.0	18	125	71 ± 4.0	16	62.5	67 ± 11.6
19	250	53 ± 5.7	18	31.25	51 ± 2.4	19	125	60 ± 4.2	17	62.5	60 ± 6.1
20	250	65 ± 2.9	19	31.25	53 ± 2.4	20	125	69 ± 2.4	18	62.5	53 ± 5.5
21	250	55 ± 6.6	20	31.25	43 ± 2.4	21	125	70 ± 3.5	19	125	41 ± 2.7
22	250	62 ± 5.3	21	31.25	58 ± 3.5	22	125	71 ± 0.7			
23	250	53 ± 4.3	22	62.5	41 ± 1.8	23	1000	11 ± 0.7			
24	500	67 ± 2.4									

^a Reared on untreated diet for a generation because survival was very low in the preceding generation.

infested with 50 eggs at the same time that cultures were set up for each selected colony. Comparative survival rates were determined for an unselected colony reared on untreated diet. In addition, each colony was tested periodically to monitor for changes in LC₅₀ or slope of the concentration-mortality regression using the standard multiple-larvae ground wheat diet assay techniques previously reported (McGaughey 1985, McGaughey & Beeman 1988, Johnson et al. 1991). In this bioassay procedure, mortality is based upon comparison of adult emergence in treated and untreated samples of diet. Dry spore-crystal preparations of the isolates were suspended in water and serial 1:2 dilutions were

prepared to provide 7–10 rates for application to diet at a rate of 3 ml/30 g. Each sample was infested with 50 eggs. Typically, three replicate

Table 3. Selection schedule and survival rates of *B. thuringiensis*-susceptible Indianmeal moth colony RC-688 selected simultaneously for resistance to Dipel (HD-1) and subsp. *aizawai* (HD-133)^a

Generation selected	Rate (mg/kg)	% Survival ± SEM		
		Dipel	Mixture	HD-133
1	24	—	19 ± 2.4	—
2	24	—	—	—
	0 ^b	—	—	—
3	24	—	30 ± 4.0	—
4	24	—	51 ± 0.7	—
5	24	76 ± 4.6	33 ± 1.3	50 ± 2.3
6	24	81 ± 1.8	61 ± 7.7	61 ± 6.4
7	24	—	50 ± 3.5	—
8	24	71 ± 7.5	55 ± 6.4	57 ± 2.9
9	24	—	75 ± 4.1	—
10	24	—	59 ± 1.8	—
11	24	—	—	—
12	24	—	68 ± 4.2	—
13	24	—	78 ± 4.6	—
14	24	—	63 ± 3.7	—
15	125	—	26 ± 3.1	—
16	125	—	47 ± 9.0	—
17	125	85 ± 5.2	43 ± 4.8	71 ± 1.8

^a A 50:50 mixture of the two strains of *B. thuringiensis* was used for selection. Percent survival was determined in separate jars of diet treated with the mixture, Dipel alone, or HD-133 alone. Survival on diet treated with Dipel or HD-133 was measured for generations 5, 6, 8, and 17 only.

^b Reared on untreated diet for a generation because survival was very low in the preceding generation.

Table 2. Selection schedule and survival rates of HD-1 resistant Indianmeal moth colony 343R selected for additional resistance to *B. thuringiensis* subsp. *aizawai* (HD-133)

Generation selected	Rate (mg/kg)	% Survival ±SEM
1	55	38 ± 4.6
2	55	48 ± 2.3
3	100	56 ± 2.0
4	100	73 ± 2.9
5	250	56 ± 2.0
6	250	57 ± 4.0
7	250	68 ± 8.3
8	250	74 ± 2.0
9	250	71 ± 0.7
10	250	71 ± 1.3
11	250	73 ± 2.9
12	1000	47 ± 1.8

Table 4. Selection for resistance in Indianmeal moths to individual isolates of *B. thuringiensis* subsp. *kurstaki*, *aizawai*, and *entomocidus*

Isolate	Generation	n	Slope ± SEM	LC ₅₀ (mg/kg diet)	95% FL (mg/kg diet)	RF ^a
HD-1 subsp. <i>kurstaki</i> (Dipel)	0	1,350	1.74 ± 0.08	13.4	12.0– 15.1	—
	4	1,200	1.59 ± 0.18	51.1	36.2– 75.8	3.8
	12	900	1.77 ± 0.12	516.1	447.8– 606.9	38.5
	16	1,050	1.97 ± 0.27	723.1	503.5– 1,145.3	54.0
	17	500	2.36 ± 0.20	1,003.7	877.1– 1,168.0	74.9
	18	1,050	1.50 ± 0.20	964.4	643.4– 1,748.2	72.0
	24	1,200	1.82 ± 0.18	1,877.5	1,510.5– 2,423.4	140.1
HD-112 subsp. <i>aizawai</i>	0	1,050	2.10 ± 0.11	3.9	3.5– 4.3	—
	5	1,350	2.25 ± 0.15	8.5	7.1– 10.2	2.1
	14	1,500	1.45 ± 0.25	31.8	16.6– 61.3	8.2
	15	1,200	2.19 ± 0.10	37.1	33.5– 41.1	9.5
	16	1,200	2.48 ± 0.12	58.4	53.0– 64.3	15.0
	22	1,050	2.32 ± 0.27	111.6	82.7– 150.5	28.6
	23	1,050	2.23 ± 0.23	5.1	3.9– 6.7	—
HD-133 subsp. <i>aizawai</i>	0	1,350	2.10 ± 0.15	23.9	19.5– 29.2	4.7
	11	1,350	1.51 ± 0.25	132.8	79.5– 264.4	26.0
	15	2,100	2.12 ± 0.11	202.1	173.8– 234.7	39.6
	16	2,100	2.06 ± 0.29	269.5	183.5– 421.5	52.8
	18	1,050	2.34 ± 0.30	250.4	181.3– 356.1	49.1
	23	1,950	1.83 ± 0.24	313.6	217.1– 472.3	61.5
	23	1,950	1.83 ± 0.24	313.6	217.1– 472.3	61.5
HD-198 subsp. <i>entomocidus</i>	0	1,350	1.84 ± 0.21	8.4	5.8– 11.8	—
	12	900	3.46 ± 0.20	113.9	104.9– 123.6	13.6
	13	1,050	2.64 ± 0.13	126.4	115.2– 138.8	15.0
	14	1,050	2.73 ± 0.34	143.8	105.8– 195.9	17.1
	19	1,050	2.40 ± 0.33	176.1	121.4– 259.1	21.0

^a Ratio of LC₅₀ to LC₅₀ before selection (0 generation).

bioassays were done for each generation tested. Mortality data from the replicate bioassays were pooled (150 insects per concentration) and used to estimate probit regressions (Finney 1971). In the colony simultaneously selected for resistance to Dipel and HD-133, bioassays were done on the individual *B. thuringiensis* strains as well as the 50:50 mixture.

Results and Discussion

As shown in Tables 1, 2, and 3, survival in the first generation in the selected lines was between 19 and 38%. In each case, survival steadily increased in subsequent generations and selection pressure was increased by periodically increasing the amount of *B. thuringiensis* in the larval diet. Despite these periodic increases in concentration, selection pressure was relatively low, with survival rates in the range of 50 to 75% after only three to six generations. Despite some fluctuations in survival rates from generation to generation, selection pressures for the four *B. thuringiensis* isolates were generally equivalent. The rate of survival of unselected insects on untreated diet over several generations was 89.5% (SD = 5.2).

Periodic bioassays confirmed that susceptibility of the Indianmeal moths to each of the isolates declined quickly and steadily under selection (Table 4). Resistance to Dipel progressed most rapidly, reaching 140-fold by 24 generations. Resistance to HD-133 reached 60-fold in 23 generations. Resistance to HD-112 and HD-

198 was considerably slower, reaching only 28- and 21-fold by the twenty-second and nineteenth generations, respectively. Thus, Indianmeal moths have the capacity to evolve resistance quickly to a variety of *B. thuringiensis* isolates. However, the sizeable differences in the rates of resistance progression could have practical implications.

Resistance also evolved quickly (both sequentially and simultaneously) toward multiple isolates. The Indianmeal moth colony that was already resistant to isolate HD-1 of subsp. *kurstaki* readily evolved resistance to a second strain, isolate HD-133 of subsp. *aizawai* (Table 5). Thus, the fact that an insect colony was already highly resistant to one type of toxin was no obstacle to the development of resistance to another type. Our attempt to select simultaneously for resistance to isolates HD-1 of subsp. *kurstaki* and HD-133 of subsp. *aizawai* also resulted in the rapid evolution of resistance toward both strains

Table 5. Sequential selection of HD-1-resistant Indianmeal moths for additional resistance to HD-133 subsp. *aizawai*

Generation	n	Slope ± SEM	LC ₅₀ (mg/kg diet)	95% FL (mg/kg diet)	RF ^a
0	1,500	1.88 ± 0.27	44.3	28.6– 69.6	—
4	1,050	2.07 ± 0.12	382.0	340.3– 432.5	8.6
5	1,050	1.82 ± 0.31	342.0	213.9– 651.7	7.7
7	750	1.81 ± 0.24	496.6	344.0– 856.9	11.2
12	1,200	1.34 ± 0.19	948.7	597.2– 1,718.2	21.4

^a Ratio of LC₅₀ to LC₅₀ before selection (0 generation).

Table 6. Simultaneous selection for resistance in Indianmeal moths to isolates HD-1 subsp. *kurstaki* (Dipel) and HD-133 subsp. *aizawai*

Generation	Formulation	n	Slope \pm SEM	LC ₅₀ (mg/kg diet)	95% FL (mg/kg diet)	RF ^a
0	Dipel	1,350	1.74 \pm 0.08	13.4	12.0- 15.1	—
	HD-133	1,350	2.23 \pm 0.23	5.1	3.9- 6.7	—
	Mixture	1,350	2.34 \pm 0.11	8.5	7.7- 9.4	—
11	Dipel	1,050	2.06 \pm 0.28	190.4	128.8-289.9	14.2
	HD-133	900	2.03 \pm 0.22	72.8	53.1-100.5	14.3
	Mixture	900	2.41 \pm 0.20	98.6	78.9-123.7	11.6
17	Dipel	900	2.32 \pm 0.13	280.3	252.9-311.7	20.9
	HD-133	1,350	2.12 \pm 0.16	123.6	102.0-151.7	24.2
	Mixture	900	1.85 \pm 0.20	126.2	92.8-177.3	14.8

^a Ratio of LC₅₀ to LC₅₀ before selection (0 generation).

of *B. thuringiensis* (Table 6). The rate of resistance progression was generally the same toward the two components of the mixture, but somewhat slower toward the mixture. A mixture would be expected to provide a large advantage if one assumes that the frequency of genes for resistance to both toxins is a product of the frequency for the toxins individually. In our experiment, a mixture apparently improved durability of the toxins, but only to a small extent. However, our experimental procedures were not adequate to determine precisely how much durability was improved or to compare the progress of resistance using pyramided (simultaneous) versus sequential deployment as in the simulation studies by Gould (1986a,b).

The different rates of resistance progression toward the four individual isolates of *B. thuringiensis* (Table 4) are potentially of considerable practical and scientific interest. Although resistance can likely evolve more readily toward some toxins than to others because of differences in gene frequency in the insect populations, the progression of resistance would also be expected to vary with the number or complexity of ICPs produced by the strains. If a particular isolate produces two or more distinct ICPs that do not cause cross-resistance to one another, resistance would probably proceed more slowly than toward a single toxin, just as a mixture prepared by combining two or more ICPs would delay resistance.

The rapid progression of resistance toward HD-1 that was observed in our study is due primarily to resistance to CryIA toxins (Van Rie et al. 1990). The three CryIA proteins [CryIA(a), CryIA(b), and CryIA(c)] found in the HD-1 isolate are structurally very closely related and also show largely overlapping activity spectra (Whiteley et al. 1985, Höfte & Whiteley 1989). Evidence in certain insect species suggests variable toxicity among these three ICPs (Höfte et al. 1988, Van Frankenhuyzen et al. 1991), but Van Rie et al. (1989) found a degree of binding site cross-reactivity among the three toxins in *H. virescens*. The rapid progression of resistance to

HD-1 by Indianmeal moths suggests that, in this species, the three types of CryIA toxins could share the same binding sites and function as a single toxin, or that the insects may not be susceptible to all three toxins. Strain HD-1 also produces CryIIA and CryIIB toxins (Höfte & Whiteley 1989), but preliminary data indicate little or no toxicity of these toxins toward Indianmeal moths (unpublished data). In fact, CryIIA and CryIIB exhibit 30- to 50-fold lower toxicity than CryIA toxins toward *Manduca sexta* (L.) (Widner & Whiteley 1989, Höfte & Whiteley 1989), which is much more susceptible to *B. thuringiensis* than the Indianmeal moth (Schesser & Bulla 1979).

However, HD-133 produces three structurally distinct ICPs, CryIA(b), CryIC, and CryID, to which Indianmeal moths are susceptible and that do not cause cross-resistance to one another (Van Rie et al. 1990, Aronson et al. 1991). Thus, this isolate may function like a mixture of toxins of different specificities and exhibit a somewhat slower progression of resistance, and the data confirm that assumption. Biochemical data on the toxins produced by isolates HD-112 and HD-198 are lacking. However, isolate HD-112, a member of subsp. *aizawai*, might be expected to produce toxins similar to HD-133 and to elicit a similar resistance response. Isolate HD-198 is a member of subsp. *entomocidus*, some of which are known to produce at least three distinct toxins, CryIA, CryIB, and CryIC (Höfte & Whiteley 1989). If HD-198 also produces all three types, this could account for the slower progression of resistance toward it.

Although definitive biochemical data are not available, results from our study are consistent with the assumption that *B. thuringiensis* strains that produce a mixture of toxin types will be somewhat more durable than strains producing only one type of toxin. Studies with pure, single-gene toxins are needed to determine the extent to which certain toxin types are more likely to elicit resistance in insects. At present the availability of such toxins is too limited to undertake long-term selection studies.

In summary, our results demonstrate that insect resistance to isolate HD-1 of *B. thuringiensis* subsp. *kurstaki* is not an anomaly. Indianmeal moths have the capacity to evolve resistance to several different strains of *B. thuringiensis* very quickly. Our results further suggest that there probably is some value in using multiple toxins to prolong the usefulness of *B. thuringiensis* against resistance-prone pest species, although that value may not be large. Indianmeal moths quickly evolved resistance to two unrelated *B. thuringiensis* strains deployed sequentially and simultaneously. The value of using multiple toxins to avoid resistance obviously depends upon the extent to which the toxins employed cause cross resistance in the target pest. Thus, in developing multiple-toxin deployment strategies, we need a detailed understanding of the cross-resistance patterns for all of the available toxins for each pest insect species. Resistance management strategies that limit selection pressure, such as providing untreated refugia, controlling doses, or using tissue-specific expression in transgenic plants, have been proposed (Mani 1985, Gould 1986b, Roush 1989, Van Rie 1991), but these also require further experimental validation. The success of any of these approaches undoubtedly will vary among different insect species and cropping systems.

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