Inheritance of Resistance to the *Bacillus thuringiensis* Toxin Cry1C in the Diamondback Moth

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Laboratory selection increased resistance to the *Bacillus thuringiensis* toxin Cry1C in a strain of diamondback moth (*Plutella xylostella*). The selected strain was derived from a field population that had evolved high levels of resistance to *Bacillus thuringiensis* subsp. *kurstaki* and moderate resistance to Cry1C. Relative to the responses of a susceptible strain of diamondback moth, the resistance to Cry1C of the selected strain increased to 62-fold after six generations of selection. The realized heritability of resistance was 0.10. Analysis of F₁ hybrid progeny from reciprocal crosses between the selected strain and a susceptible strain showed that resistance to Cry1C was autosomally inherited. The dominance of resistance to Cry1C depended on the concentration; inheritance was increasingly dominant as the concentration decreased. Responses of progeny from single-pair families showed that resistance to Cry1C and resistance to Cry1Ab were inherited independently, which enhances opportunities for managing resistance. However, compared with projections based on previously reported recessive inheritance of resistance to Cry1A toxins, the potentially dominant inheritance of resistance to Cry1C observed here could accelerate evolution of resistance.

Insecticides derived from the soil bacterium *Bacillus thuringiensis* are the most widely used biological pesticides (4). However, evolution of resistance by insect pests threatens the continued success of these environmentally benign insecticides (11, 23). Widespread use in the United States of transgenic corn, cotton, and potato varieties that express toxins from *B. thuringiensis* has increased the likelihood of resistance. Laboratory selection has produced resistance to *B. thuringiensis* in many insects, but so far, only the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae), has evolved resistance to *B. thuringiensis* in open-field populations (39).

Understanding the genetic basis of resistance in pests such as the diamondback moth is essential for devising strategies to delay resistance (27). In particular, some management strategies are especially effective when resistance is inherited as a recessive trait. For example, one of the most promising tactics is to provide a spatial refuge from exposure to *B. thuringiensis* to increase survival of susceptible pests and slow evolution of resistance (11, 17, 23, 39, 40); refuges are required in conjunction with plantings of *B. thuringiensis* toxin-expressing transgenic cotton in the United States. If resistance is recessive, heterozygous offspring produced by mating between resistant and susceptible individuals can be killed by toxin, which delays the evolution of resistance (11, 23, 39, 40). The ability of combinations of toxins to delay resistance is also greatly enhanced if the inheritance of resistance to each toxin is recessive (20, 30, 37). Further, attempts to thwart resistance with groups of toxins used simultaneously or sequentially are more likely to succeed if the gene or genes conferring resistance to one toxin segregate independently of those conferring resistance to other toxins (20, 30, 37).

Most previous studies of resistance to *B. thuringiensis* in the diamondback moth have focused on resistance to *Bacillus thuringiensis* subsp. *kurstaki*. Resistance to *B. thuringiensis* subsp.

kurstaki in the diamondback moth is autosomally inherited and partially to completely recessive (8, 12, 42). In a resistant strain of diamondback moth from Hawaii, a single recessive gene conferred resistance to the three Cry1A toxins in *B. thuringiensis* subsp. *kurstaki* (Cry1Aa, Cry1Ab, and Cry1Ac) as well as cross-resistance to Cry1F (46).

Although laboratory selection has produced resistance to Cry1C in several insects (9, 22, 25, 26), relatively little is known about the genetics of resistance to Cry1C in the diamondback moth or in other insects. Cry1C is produced by some strains of *Bacillus thuringiensis* subsp. *aizawai* but not by *B. thuringiensis* subsp. *kurstaki* (15). Extremely high resistance to *B. thuringiensis* subsp. *kurstaki* and Cry1A toxins confers little or no cross-resistance to Cry1C in the diamondback moth (7, 43, 47). Although low levels of resistance to *B. thuringiensis* subsp. *aizawai* have been found in several field populations (28, 32, 36, 39), we know of only one documented case of field-evolved resistance to Cry1C (19). The lack of cross-resistance to Cry1C in strains resistant to Cry1A toxins and the relatively low levels of resistance to Cry1Ab in a Cry1C-resistant strain suggest that the gene(s) conferring resistance to Cry1C segregates independently of the gene conferring resistance to Cry1Ab in the diamondback moth (19).

In the present study, we analyzed a strain of diamondback moth derived from a field population that had evolved a high level of resistance to *B. thuringiensis* subsp. *kurstaki* and moderate resistance to Cry1C (19). First, we selected this strain with Cry1C in the laboratory to increase the proportion of resistant individuals. Second, we evaluated maternal effects, sex linkage, and dominance by measuring the responses to Cry1C by hybrid progeny from crosses between the selected strain and a susceptible strain. We also used bioassays of progeny from single-pair crosses to test for genetic variation within the selected and susceptible strains. Finally, we tested progeny from single-pair crosses to estimate the genetic correlation between resistance to Cry1C and resistance to Cry1Ab. The results show that resistance to Cry1C is inherited independently of and differently from resistance to Cry1Ab.

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MATERIALS AND METHODS

Insects. We started with a susceptible strain (LAB-P) and a resistant strain (NO-95) of diamondback moth. LAB-P had been maintained for >150 generations without exposure to insecticide. In May 1995, NO-95 was established from >600 larvae (>400 adults emerged) from a field population (NO) that had high resistance to *B. thuringiensis* subsp. *kurstaki*, low resistance to *B. thuringiensis* subsp. *aizawai*, and moderate resistance to Cry1C (19). NO-95C was started from strain NO-95 after NO-95 had been reared for three generations in the laboratory without exposure to insecticide. To increase genetic diversity, we infused NO-95C with a second group of field-collected individuals as described in detail below. All insects were reared on cabbage (41).

***B. thuringiensis* toxins.** We used liquid formulations containing Cry1C (MYX833-4C1) or Cry1Ab (MYX03604) expressed in and encapsulated by transgenic *Pseudomonas fluorescens* (Mycogen, San Diego, Calif.). Toxins were diluted with distilled water containing 0.2% Triton AG-98 (a surfactant; Rohm & Haas, Philadelphia, Pa.). Concentrations were measured as milliliters of formulated Cry1C or Cry1Ab per liter. For brevity, we use the phrases milliliters of Cry1C per liter and milliliters of Cry1Ab per liter to signify milliliters of formulated Cry1C per liter and milliliters of formulated Cry1Ab per liter, respectively.

Bioassays. We used leaf-residue bioassays (18, 44). Third-instar larvae in groups of 10 were placed on toxin-treated leaf disks. After 2 days, fresh untreated cabbage leaves were added. Mortality was recorded after 5 days. Bioassays and rearing were conducted at 28°C and with a photoperiod of 14:10 (light-dark) h. To estimate background mortality, all bioassays included groups of control larvae that were fed leaf disks that had been treated with distilled water containing 0.2% Triton AG-98 with no toxin.

Selection. The method of exposing larvae to Cry1C in selection experiments was similar to the bioassay method described above; third-instar larvae were fed leaf disks that had been treated with Cry1C. In each selected generation, 300 to 1,050 larvae were exposed to Cry1C. Because of their low numbers, generations 2, 5, and 8 of strain NO-95C were reared without selection. Adult survivors from treated leaf disks in each selection were pooled to produce progeny for the next generation.

NO-95C was selected initially by exposing larvae to leaf disks that had been treated with 5 ml of Cry1C per liter. To increase genetic diversity, we collected a second set of insects (more than 100 larvae; 38 adults emerged) from the NO field population in October 1995. This second group of field-collected individuals was reared to the next generation and selected with 5 ml of Cry1C per liter. We added the survivors from this second group to the NO-95C colony after the NO-95C colony had been selected twice. After the second group of individuals had been added to NO-95C, we performed four additional selections. The concentration was raised to 10 ml of Cry1C per liter for the final selection.

Evaluation of maternal effects, sex linkage, dominance, and genetic variation within strains. We analyzed responses to Cry1C of hybrid F_1 progeny from mass crosses and single-pair crosses as well as F_2 progeny from single-pair crosses. We tested F_1 progeny to evaluate maternal effects, sex linkage, and dominance. Mass crosses provided the large numbers of offspring needed for multiple-concentration tests, which are required to estimate the LC_{50} s (concentrations lethal to 50% of the larvae tested). Tests of F_1 and F_2 progeny from single-pair crosses enabled detection of genetic variation within the parental strains, which is not possible with mass crosses (46). Detection of genetic variation within parental strains is important because standard methods for estimating dominance assume that the susceptible and resistant parental strains are homozygous (13, 35; see also descriptions below). If the parental strains are genetically variable at the locus or loci controlling resistance, estimates of dominance may be biased (46).

After the sixth selection, we produced hybrid F_1 progeny from reciprocal mass crosses between NO-95C and LAB-P. We determined the sex of fourth-instar larvae based on the color of the fifth abdominal segment (16). For one cross, we pooled 30 LAB-P females with 30 NO-95C males. For the other cross, we pooled 30 NO-95C females with 30 LAB-P males. Larvae from the two parental colonies (NO-95C and LAB-P) and from the F_1 progeny of the two reciprocal crosses were tested with bioassays at six concentrations (0, 0.016, 0.08, 0.4, 2.0, and 10 ml of Cry1C per liter).

We also tested F_1 progeny from five single-pair crosses between LAB-P and NO-95C. Pupae from each parental strain were isolated individually in gelatin capsules. Single pairs consisting of a virgin male LAB-P moth and a virgin female NO-95C moth or vice versa were caged together for mating and egg production. F_1 progeny from each of five single-pair families were reared separately on cabbage plants. We then tested the F_1 larvae with 0.4 or 2 ml of Cry1C per liter.

To obtain F_2 progeny, we used single-pair crosses between F_1 progeny from the two reciprocal mass crosses and either LAB-P or NO-95C. F_2 progeny from four single-pair crosses between F_1 and LAB-P were tested with 0.4 ml of Cry1C per liter. F_2 progeny from three single-pair crosses between F_1 and NO-95C were tested with 2 ml of Cry1C per liter.

F_1 and F_2 progeny from single-pair crosses were tested simultaneously. As internal standards, 30 larvae from the parental strains LAB-P and NO-95C were also tested simultaneously. For each parental strain or single-pair family, we exposed 30 larvae (three replicates of 10 larvae) to each concentration of Cry1C and used 10 additional larvae as controls.

Test for linkage between Cry1C and Cry1Ab resistance. To test for genetic linkage between resistance to Cry1C and resistance to Cry1Ab, we analyzed

responses of F_3 progeny from single-pair crosses as follows. Male F_1 (NO-95C $\times$ LAB-P) larvae were pooled with female LAB-P larvae, and female F_1 larvae were pooled with male LAB-P larvae. The resulting F_2 backcross progeny were pooled. Single pairs consisting of a virgin F_2 male moth and a virgin F_2 female moth were caged together for mating and egg production. F_3 progeny from 16 single-pair families were reared separately on cabbage plants. For each F_3 family, approximately 30 larvae were tested with Cry1C (0.4 ml/liter), 30 larvae were tested with Cry1Ab (10 ml/liter), and 10 larvae were used as controls.

Data analysis. For bioassays with multiple concentrations, we used probit analysis to estimate the LC_{50} s and their 95% fiducial limits as well as the slopes of the concentration-mortality lines and their standard errors (31, 44). LC_{50} s were considered significantly different if their fiducial limits did not overlap. Resistance ratios were calculated by dividing the LC_{50} for a strain by the LC_{50} for the susceptible strain, LAB-P. To provide a standardized measure of the results of selection with Cry1C, we calculated the realized heritability of resistance to Cry1C as the response to selection divided by the intensity of selection, as described by Tabashnik (38). Heritability is the proportion of total phenotypic variation that is accounted for by additive genetic variation; as heritability increases, so does the response to selection (6).

We used two methods to estimate dominance: Stone's (35) method, which is based on LC_{50} s, and a single-concentration method, which is based on Hartl's (13) definition of dominance and on survival at any single concentration. Stone's (35) method estimates degree of dominance (D) at the LC_{50} as follows: $D = (2X_2 - X_1 - X_3)/(X_1 - X_3)$, where X_1 , X_2 , and X_3 are the logarithms of the LC_{50} s for the resistant homozygotes, heterozygotes, and susceptible homozygotes, respectively. We estimated X_1 , X_2 , and X_3 as the LC_{50} s for NO-95C, F_1 progeny, and LAB-P, respectively. Because this method requires LC_{50} s, D could be estimated only from the LC_{50} s of the parental strains and the LC_{50} s of the F_1 progeny from mass crosses. D values range from -1 (completely recessive resistance) to 1 (completely dominant resistance) (35).

The single-concentration method (13, 40) estimates dominance (h) as follows: $h = (w_{12} - w_{22})/(w_{11} - w_{22})$, where w_{11} , w_{12} , and w_{22} are the fitnesses at a particular toxin concentration for resistant homozygotes, heterozygotes, and susceptible homozygotes, respectively. The fitness of treated resistant homozygotes is defined as 1. We estimated the fitness of susceptible homozygotes treated with Cry1C as the survival rate of treated LAB-P larvae divided by the survival rate of treated NO-95C larvae. Likewise, fitness of heterozygotes treated with Cry1C was estimated as the survival rate of treated F_1 progeny divided by the survival rate of treated NO-95C larvae. In each case, we first estimated mortality caused by Cry1C by adjusting total mortality for control mortality using Abbott's correction (1). The survival rate was estimated as $100\% - \text{mortality}$ (expressed as a percentage). Values of h range from 0 (completely recessive resistance) to 1 (completely dominant resistance). When h is 0.5, resistance is called codominant or additive. We use the terms partially recessive ($0 < h < 0.5$) and partially dominant ($0.5 < h < 1$).

We used the single-concentration method to estimate dominance at four of the concentrations of Cry1C tested against F_1 progeny from the mass cross and at the two concentrations tested against F_1 progeny from single-pair crosses. We did not estimate dominance at the lowest concentration of Cry1C tested (0.016 ml/liter) because this concentration killed only 7.5% of the susceptible LAB-P larvae. To enable direct comparison of dominance estimates from the two methods we transformed the estimate of D to the same scale as that for h as follows: $h = (D + 1)/2$.

To test for genetic variation within parental strains, we used three separate analyses of variance (ANOVA) to test for significant variation in mortality among families produced by the single-pair crosses LAB-P $\times$ NO-95C, LAB-P $\times$ F_1 , and NO-95C $\times$ F_1 . Mortality data were transformed by $\arcsin \sqrt{p}$ (where p is the proportion of mortality) before each ANOVA was performed. We also combined probabilities from the three independent ANOVA to test the overall null hypothesis that the parental strains did not contain genetic variation (34). To test for genetic linkage between resistance to Cry1C and resistance to Cry1Ab, mortality data for Cry1C and Cry1Ab from split broods of single-pair families were analyzed by correlation analysis (31).

RESULTS

Response to selection. Selection increased the resistance to Cry1C of the NO-95C colony of diamondback moth. Initially, only 2 to 6% of treated individuals survived exposure to 5 ml of Cry1C per liter (Table 1). After six selections, the survival rate of individuals treated with 10 ml of Cry1C per liter was 12.0% (Table 1). Selection increased the LC_{50} of Cry1C for NO-95C and produced a final resistance ratio of 62 (Table 2).

The realized heritability of resistance to Cry1C was 0.10, which is based on the average response to selection per generation (0.150) divided by the average selection differential (1.46). These estimates were calculated by applying the equations detailed elsewhere (6, 38) to the following values: average

TABLE 1. Selection of diamondback moth strain NO-95C with the *B. thuringiensis* toxin Cry1C

Generation	Cry1C concn (ml/liter)	No. of larvae	% of larvae surviving to adulthood
1	5	300	2.0
3	5	600	5.2
3 ^a	5	450	5.8
4	5	445	4.9
6	5	610	9.2
7	5	598	9.7
9	10	600	12.0

^a A second group of larvae were collected from the NO field population, reared for one generation without selection, and selected with Cry1C. To increase genetic diversity, the offspring of the survivors from this group were added to NO-95C to begin generation 4 (see Materials and Methods).

survival, 0.072; intensity of selection, 1.91; and phenotypic standard deviation, 0.77.

Evaluation of maternal effects and sex linkage. Resistance to Cry1C was autosomally inherited. Bioassays of the F₁ progeny from mass crosses between NO-95C and LAB-P showed no significant difference in LC₅₀ between the two reciprocal crosses (Table 2). Thus, neither maternal effects nor sex linkage was evident.

Dominance. Bioassays of F₁ progeny from mass crosses and single-pair crosses between NO-95C and LAB-P showed that the dominance of resistance to Cry1C depended on the concentration (Tables 2 to 4). At the LC₅₀, F₁ progeny from the mass crosses (reciprocal crosses pooled) were significantly less susceptible than LAB-P but did not differ significantly from NO-95C (Table 2). The LC₅₀s for the progeny from the mass crosses (Table 2) yield a *D* value of 0.26, which is equivalent to an *h* value of 0.63 (see Materials and Methods). These estimates indicate that the resistance was partially dominant at the LC₅₀.

Evaluation of dominance at each of four concentrations (Tables 3 and 4) showed that resistance tended to be more dominant as the concentration decreased. In the F₁ progeny from mass crosses, resistance was recessive at 10 ml of Cry1C per liter (*h* = 0), partially recessive at 2 ml of Cry1C per liter (*h* = 0.44), and almost completely dominant at either 0.08 or 0.4 ml of Cry1C per liter (*h* = 0.88 and 0.98, respectively)

TABLE 2. Responses of susceptible (LAB-P), resistant (NO-95 and NO-95C), and hybrid F₁ progeny of diamondback moth to Cry1C

Strain or generation	No. of larvae	Slope ± SE	LC ₅₀ (95% fiducial limits)	RR ^a
LAB-P ^b	400	2.3 ± 0.9	0.03 (0.004–0.2)	
NO-95 ^b	390	1.5 ± 0.3	0.7 (0.4–1.3)	22
LAB-P	240	1.5 ± 0.1	0.09 (0.06–0.13)	
NO-95C ^c	240	1.1 ± 0.4	5.5 (1.4–539)	62
F ₁ (L × C) ^d	240	1.7 ± 0.5	0.9 (0.3–3.3)	10
F ₁ (C × L) ^e	240	1.8 ± 0.4	1.5 (0.7–3.5)	17
F ₁ (pooled) ^f	480	1.8 ± 0.4	1.2 (0.6–2.6)	13

^a RR, resistance ratio (the LC₅₀ for the strain divided by the LC₅₀ for LAB-P in the same set of tests).

^b Data obtained from Liu et al. (19); resistance to Cry1C in NO-95 was based entirely on field exposure to *B. thuringiensis*.

^c NO-95C was derived from NO-95; NO-95C was at generation 10 and had been selected with Cry1C six times when these data were obtained.

^d Progeny of mass cross between 30 LAB-P females and 30 NO-95C males.

^e Progeny of mass cross between 30 NO-95C females and 30 LAB-P males.

^f Pool of F₁ (L × C) and F₁ (C × L) progeny.

TABLE 3. Dominance (*h*) of resistance to Cry1C in the diamondback moth as a function of the concentration of Cry1C

Concn (ml/liter)	Strain or generation ^a	No. of larvae	Mortality (%) ^b	Fitness ^c	<i>h</i> ^d
0.08	LAB-P	40	65.0	0.35	
	NO-95C	40	0	1	
	F ₁	80	7.5	0.93	0.88
0.4	LAB-P	40	70.0	0.31	
	NO-95C	40	2.5	1	
	F ₁	80	3.8	0.99	0.98
2.0	LAB-P	40	100	0	
	NO-95C	40	20.0	1	
	F ₁	80	65.0	0.44	0.44
10	LAB-P	40	100	0	
	NO-95C	40	75.0	1	
	F ₁	80	100	0	0

^a LAB-P is the susceptible strain, NO-95C is the resistant strain, and F₁ are hybrid progeny pooled from the two reciprocal mass crosses between the two parental strains.

^b Adjusted for control mortality by Abbott's method (1).

^c Fitness is the survival rate of the larvae divided by the survival rate of NO-95C larvae (see Materials and Methods).

^d *h* can vary from 0 (completely recessive resistance) to 1 (completely dominant resistance) (see Materials and Methods).

(Table 3). For the five families of F₁ progeny from single-pair crosses, the mean *h* value was 0.19 at 2 ml of Cry1C per liter and 0.58 at 0.4 ml of Cry1C per liter (Table 4).

Evaluation of genetic variation within strains by single-pair crosses. Among the five families of F₁ progeny from single-pair crosses between LAB-P and NO-95C, the variation in larval mortality caused by Cry1C was not significant (df = 4, 20; *F* = 1.51; *P* = 0.24) (Table 4). Among the three families of F₂ progeny from single-pair crosses between NO-95C and F₁, mortality ranged from 48.1 to 81.4%, but this variation was not significant (df = 2, 6; *F* = 1.78; *P* = 0.25) (Table 5). Among the four families of F₂ progeny obtained from single-pair crosses between LAB-P and F₁, mortality varied significantly (df = 3, 8; *F* = 5.57; *P* = 0.02; range, 66.7 to 96.3%) (Table 5). An overall test combining probabilities from each of the three separate tests described above revealed significant variation in mortality within the sets of single-pair families (df = 6, χ^2 = 13.2, *P* < 0.05).

Test for linkage to Cry1Ab resistance. Resistance to Cry1C was not genetically linked with resistance to Cry1Ab. Mortality caused by Cry1C was not correlated with mortality caused by Cry1Ab in split-brood bioassays of F₃ progeny from single-pair crosses of F₂s from mass crosses between F₁ and LAB-P (Fig. 1). This result indicates that the gene or genes conferring resistance to Cry1C segregate independently of the gene that confers resistance to Cry1Ab.

DISCUSSION

Laboratory selection increased resistance to Cry1C in the NO-95C strain, which was derived from a diamondback moth population that had already evolved moderate resistance to Cry1C in the field. This result indicates that the field population had genetic variation for resistance to Cry1C and would be expected to attain higher levels of resistance if exposure to Cry1C continued. The realized heritability of resistance to Cry1C in strain NO-95C (0.10) was intermediate between the low values for resistance to *B. thuringiensis* subsp. *aizawai*

TABLE 4. Dominance (h) of resistance to Cry1C in the diamondback moth as a function of the concentration of Cry1C for single-pair hybrid F_1 families

Strain or family ^a	Characteristics of larvae at Cry1C concn ^b					
	0.4 ml/liter			2.0 ml/liter		
	Mortality (%)	Fitness	h	Mortality (%)	Fitness	h
Strains						
LAB-P	92.6	0.08		100	0	
NO-95C	3.3	1		55.5	1	
Single-pair F_1 families						
CL11	37.0	0.65	0.62	77.8	0.49	0.49
CL12	29.7	0.73	0.71	100	0	0
LC13	48.1	0.54	0.50	100	0	0
LC14	33.3	0.69	0.66	100	0	0
LC15	55.5	0.46	0.41	78.4	0.47	0.47
Mean	40.7	0.61	0.58	91.2	0.19	0.19

^a LAB-P is the susceptible strain, NO-95C is the resistant strain, and single-pair F_1 families were produced by crosses between a single NO-95C female and a single LAB-P male (CL11 and CL12) or vice versa (LC13 through LC15). Thirty larvae per concentration per each strain or family were tested.

^b Mortality is adjusted for control mortality by Abbott's method (1). Fitness is the survival rate of the larvae divided by the survival rate of NO-95C larvae (see Materials and Methods). h can vary from 0 (completely recessive resistance) to 1 (completely dominant resistance) (see Materials and Methods).

(0.022 and 0.069 for strains SN1 and SN2, respectively [17]) and the higher values for resistance to *B. thuringiensis* subsp. *kurstaki* (0.14, 0.17, and 0.18 for strains NO-P, NO-Q, and NO-R, respectively [38]).

Like the other cases of resistance to *B. thuringiensis* analyzed so far (39), resistance to Cry1C was autosomally inherited. However, unlike resistance to *B. thuringiensis* subsp. *kurstaki*, Cry1A toxins, and Cry1F toxin in the diamondback moth (8, 12, 24, 42, 46), resistance to Cry1C in strain NO-95C was not recessive. Similar to the resistance to Cry1Ac in the CP73-3 strain of *Heliothis virescens* (9), the extent of dominance of resistance to Cry1C depended on the concentration of toxin. However, in strain CP73-3, resistance was more recessive at low concentrations (9), whereas in NO-95C, resistance was more recessive at high concentrations.

In the present study, the mortality of treated larvae and estimated dominance at a given concentration also varied between experiments conducted on different dates. In particular, mortality was higher in the experiments with single-pair families (Tables 4 and 5) than in the earlier test of hybrid F_1

progeny from mass crosses (Table 3). For example, at 0.4 ml of Cry1C per liter, the mean mortality of F_1 larvae was 40.7% for five single-pair families ($n = 150$) (Table 4) compared with 3.8% for pooled progeny from mass crosses ($n = 80$) (Table 3). Similar increases in mortality across experimental dates were also observed for the LAB-P strain (at 0.4 ml of Cry1C per liter; 92.6 [Table 4] compared to 70.0% [Table 3]) and the NO-95C strain (at 2.0 ml Cry1C per liter; 55.5 [Table 4] compared to 20.0% [Table 3]). Because the same trend was observed for all three strains, the increases in mortality were probably caused by environmental variation between experiments conducted on different dates. Although we tried to maintain a consistent environment, variation in plant quality, humidity, or other factors might have affected mortality. This type of variation is typical in bioassays with *B. thuringiensis* (29, 44) and reinforces the need to make simultaneous comparisons among strains when estimating resistance levels or analyzing inheritance. Furthermore, when dominance varies as a function of mortality (as observed here with resistance to Cry1C in the diamondback moth), this kind of environmental influence on mortality will also cause variation in dominance (2).

Our numerical estimates of dominance are based on the

TABLE 5. Responses to Cry1C of diamondback moth larvae from single-pair F_2 families

Backcross and F_2 family ^a	Cry1C concn (ml/liter)	Mortality (%) ^b
Backcross to LAB-P		
FL21	0.4	66.7
LF22	0.4	74.1
FL23	0.4	92.6
LF24	0.4	96.3
Backcross to NO-95C		
CF25	2	48.1
FC26	2	66.7
FC27	2	81.4

^a F_1 adults were obtained from pooled progeny of the two reciprocal mass crosses between the NO-95C and LAB-P strains. Each F_2 family was produced by a single-pair cross between an F_1 adult and either a LAB-P adult or an NO-95C adult. The first letter in each family name denotes the female parent's strain and the second letter denotes the male parent's strain (e.g., FL21 was derived from an F_1 female and a LAB-P male, and CF25 was derived from an NO-95C female and an F_1 male). Thirty larvae per family were tested.

^b Adjusted for control mortality by Abbott's method (1).

FIG. 1. Relationship between mortality caused by Cry1Ab and that caused by Cry1C in split broods from 16 families of F_3 progeny from single-pair crosses ($df = 14$, $r = -0.15$, $P = 0.58$).

assumption that the resistant strain NO-95C and the susceptible strain LAB-P were completely homozygous when F_1 progeny were produced. The presence of heterozygotes in NO-95C would tend to lower the survival rate of F_1 progeny and thus lead to underestimates of dominance. Heterozygotes in the LAB-P strain would have the opposite effect (46). The significant variation in response to Cry1C within sets of single-pair families suggests that NO-95C was not homozygous for resistance at the time of the crosses. The frequency of heterozygotes was probably higher for NO-95C than for LAB-P, which suggests that our estimates of dominance may be lower than the true values. It is also important to note that our dominance estimates reflect the cumulative effect of all loci influencing resistance to Cry1C; we do not know how many loci are involved.

Bioassays of progeny from split broods of single-pair families (Fig. 1) confirmed previous suspicions that the gene or genes conferring resistance to Cry1C segregate independently of the gene or genes conferring resistance to Cry1Ab in the diamondback moth (19). Previous results showed that diamondback moth resistance to Cry1Ab did not confer cross-resistance to Cry1C (7, 19, 43, 47). The results reported here provide direct evidence that resistance to Cry1C does not confer cross-resistance to Cry1Ab in the NO-95C strain of diamondback moth. A multitoxin resistance gene in the diamondback moth confers resistance to Cry1Aa, Cry1Ab, Cry1Ac, and Cry1F (46). Thus, independent segregation of the gene for resistance to Cry1Ab implies independent segregation of the multitoxin resistance gene.

Despite some evidence to the contrary (5, 21), the most likely cause of resistance to Cry1A toxins in the diamondback moth is reduced binding of toxins to midgut membranes (3, 7, 45, 47). The recessive inheritance of resistance to Cry1A toxins is consistent with expectations for an altered target site (14). In contrast, we hypothesize that resistance to Cry1C, which is not recessive, is not based on an altered target site. If this hypothesis is correct, the mechanism of resistance to Cry1C might be similar to that of resistance to Cry1Ac in the *H. virescens* strain CP73-3, which also was less than 100-fold, not recessive, and not based on reduced binding (9).

Our results have implications for management of resistance to *B. thuringiensis*. Independent segregation of genes conferring resistance to Cry1C and Cry1A toxins enhances opportunities for using these two groups of toxins sequentially or simultaneously. The partially dominant resistance to Cry1C in the diamondback moth shows that it is not safe to assume that resistance to *B. thuringiensis* toxins is recessive, despite many examples of partially or completely recessive resistance in moths (39). Further, the results show that one cannot reliably predict the mode of inheritance across groups of *B. thuringiensis* toxins, even for a single insect population. The NO field population of diamondback moth harbors at least one partially dominant mutation that confers resistance to Cry1C as well as at least one recessive mutation conferring resistance to Cry1A and Cry1F toxins (46). In a related example, the dominance of resistance to *B. thuringiensis* toxins in laboratory-selected strains of *H. virescens* depended on the toxin and the particular strain (9, 10, 33).

Variation in dominance of resistance as a function of the toxin concentration and perhaps other environmental factors complicates resistance management. Simulation models show that refuges are likely to work best when resistance is recessive, i.e., when heterozygotes are killed (11, 23, 40). In principle, one could render resistance recessive simply by spraying sufficiently high concentrations of formulations or ensuring sufficiently high expression of toxins in transgenic plants. However,

achieving functional recessivity by increasing the toxin concentration is not without risk. First, toxin concentrations vary in space and time, with either foliar applications or transgenic plants. Second, increasing the concentration of Cry1C might increase the cost of foliar applications or reduce yields of transgenic plants. Perhaps more importantly, if the optimistic assumptions of some models are violated and mating between resistant and susceptible insects is limited, increasing the toxin concentration could quickly eliminate susceptible individuals and thus greatly accelerate evolution of resistance.

One alternative to dealing with potentially dominant resistance is to increase the sizes of refuges. Another option is to use toxin concentrations that are low enough to enable survival of substantial numbers of treated susceptible insects (39). These two approaches will almost certainly delay resistance but may require integration with other tactics, such as biological control, to achieve adequate suppression of pests.

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