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Cross-Resistance to *Bacillus thuringiensis* Toxin CryIF in the Diamondback Moth (*Plutella xylostella*)

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Selection with *Bacillus thuringiensis* subsp. *kurstaki*, which contains CryIA and CryII toxins, caused a >200-fold cross-resistance to CryIF toxin from *B. thuringiensis* subsp. *aizawai* in the diamondback moth, *Plutella xylostella*. CryIE was not toxic, but CryIB was highly toxic to both selected and unselected larvae. The results show that extremely high levels of cross-resistance can be conferred across classes of CryI toxins of *B. thuringiensis*.

Insecticidal proteins from *Bacillus thuringiensis* are gaining importance in pest control, but their continued success is jeopardized by the potential for evolution of resistance in pests (12, 14). Because many *B. thuringiensis* toxins are available (6, 9), one might be able to counter insect resistance simply by switching to a new toxin (19). However, the usefulness of this approach will be limited if resistance to some toxins confers cross-resistance to other toxins.

Studies of resistant strains of three species of moths show variation in patterns of cross-resistance (8, 12, 14). Laboratory selection produced limited cross-resistance in several strains of the Indianmeal moth, *Plodia interpunctella* (10, 11, 19), and broad cross-resistance in one strain of the tobacco budworm, *Heliothis virescens* (8). Reduced binding of toxin to receptors in the larval midgut was associated with the relatively high and specific resistance in the Indianmeal moth (19) but not with the moderate, broad resistance in the tobacco budworm (8).

For the diamondback moth, *Plutella xylostella*, the first insect with field populations resistant to *B. thuringiensis*, extremely high and specific resistance has been associated with reduced binding of toxins to midgut receptors (2, 4, 7, 15, 17). Laboratory strains of the diamondback moth derived from field populations in the Philippines had a >200-fold resistance to CryIA(b) but were not resistant to CryIA(a), CryIA(c), CryIB, or CryIC (2, 7). Strains of the diamondback moth from the Philippines also did not show resistance to Dipel, a commercial formulation of the HD-1 strain of *B. thuringiensis* subsp. *kurstaki* that contains spores, formulation ingredients, and toxins CryIA(a), CryIA(b), CryIA(c), CryIIA, and CryIIB (1).

In Hawaii, repeated field exposure to various formulations of *B. thuringiensis* subsp. *kurstaki* followed by laboratory selection with Dipel produced extremely high resistance to Dipel in the NO-QA strain of the diamondback moth (15, 17). The NO-QA strain was also extremely resistant to CryIA(a), CryIA(b), and CryIA(c) and moderately resistant to CryIIA, all of which occur in Dipel (17, 18). NO-QA showed little or no cross-resistance to CryIC, which occurs in *B. thuringiensis* subsp. *aizawai* but not in *B. thuringiensis* subsp. *kurstaki* (17, 18).

In the present study, we assessed cross-resistance to CryIB, CryIE, and CryIF in the NO-QA strain of the diamondback moth. We tested larvae from NO-QA simultaneously with larvae from the unselected, susceptible LAB-P strain from Hawaii (15, 16). As far as we know, the NO-QA strain had not been exposed to CryIB, CryIE, or CryIF. Thus, decreased susceptibility to these toxins in the NO-QA strain relative to that in the LAB-P strain would indicate cross-resistance.

Insects were maintained and bioassays were performed as described previously (15, 18). Third-instar larvae were fed leaf disks of cabbage that had been dipped in dilutions containing various concentrations of the toxins. Controls in which the concentration of toxin was zero were included in all tests. Mortality was recorded at 2 and 5 days after treatment. Data were analyzed with the PROC PROBIT program of the Statistical Analysis System as described previously (15).

Douglas Bradley and August Zitzka, University of Washington, provided CryIB from the HD-290-1 strain of *B. thuringiensis* subsp. *thuringiensis* (3). Luke Masson, National Research Council of Canada, Montreal, provided CryIE from an acrystalliferous strain of *B. thuringiensis* that had been transformed with the toxin gene by Roger Frutos at the Centre de Cooperation Internationale en Recherche Agronomique pour le Developpement, Montpellier, France. Wendy Gelernter and Brian Stockhoff, Mycogen Corporation, provided formulations containing transgenic *Pseudomonas fluorescens* that had expressed and encapsulated CryIA(b) derived from *B. thuringiensis* subsp. *kurstaki* and CryIF derived from *B. thuringiensis* subsp. *aizawai* (13).

NO-QA larvae were extremely resistant to CryIF (Table 1). The highest concentration of CryIF tested (10,600 mg/liter) caused no mortality to NO-QA larvae at 5 days after treatment. In contrast, LAB-P larvae exposed to a concentration that was 100-fold lower (106 mg/liter) showed 89% mortality at 2 days and 100% mortality at 5 days. Because the NO-QA strain was so resistant to CryIF, we could only estimate a lower limit for the concentration needed to kill 50% of the larvae (LC_{50}). On the basis of this estimate, the resistance ratio (LC_{50} for NO-QA/LC_{50} for LAB-P) for CryIF was >240 (Table 1).

NO-QA larvae were also extremely resistant to CryIA(b) (Table 1), which confirms our previous results (18). In response to CryIB, the LC_{50} for NO-QA was about five times greater than the LC_{50} for LAB-P, but the 95% fiducial limits of the two estimates overlapped (Table 1). After 2 days, the mortalities of the larvae exposed to the highest concentration of CryIE tested (100 mg/liter) were 0% for NO-QA larvae and 2% for LAB-P larvae. After 5 days, the mortality increased slightly but was still not significantly greater than the control

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TABLE 1. Responses of diamondback moth larvae to *B. thuringiensis* toxins

Toxin	Insect strain[a]	n^b	Slope[c]	LC_{50} (mg/liter) at 2 days	95% Fiducial limit		Resistance ratio[d]
					Lower	Upper	
CryIA(b)[e]	NO-QA	196	NA[f]	>10,600[g]	NA	NA	>750
	LAB-P	198	0.6 ± 0.2	14.0	0.49	90	
CryIB	NO-QA	234	1.0 ± 0.3	33.5	13	250	5
	LAB-P	239	0.9 ± 0.2	6.2	2.6	28	
CryIF[h]	NO-QA	232	NA	>10,600[g]	NA	NA	>240
	LAB-P	242	3.2 ± 0.8	43.3	21	63	

[a] The selected strain was NO-QA; the unselected strain was LAB-P.
[b] Number of larvae tested.
[c] Estimated slope of the probit regression line ± the standard error.
[d] Calculated as LC_{50} for NO-QA/LC_{50} for LAB-P.
[e] Mycogen formulation MYX03604.
[f] NA, not available.
[g] The highest concentration tested; caused 0% mortality.
[h] Mycogen formulation MYX837-446.

mortality of either strain. Thus, we found little or no cross-resistance to CryIB and no evidence of toxicity of CryIE to selected or unselected larvae. Our results with CryIA(b), CryIB, and CryIE are similar to results with diamondback moth strains from the Philippines (2, 7).

The results presented here show that extremely high levels of cross-resistance can be conferred across classes of CryI toxins from *B. thuringiensis*. The CryIF toxin isolated from *B. thuringiensis* subsp. *aizawai* is distinct from CryIA toxins in its spectrum of insecticidal activity against lepidopteran larvae and in its amino acid sequence (5). Amino acid sequence similarity to CryIA(a), CryIA(b), and CryIA(c) ranges from 70 to 72% overall and from 49 to 52% in the N-terminal region (amino acids 1 to 602), which determines the specificity of some CryI toxins (5). This level of similarity is intermediate between the high percentage of overall amino acid identity among CryIA toxins (82 to 90%) and the lower overall correspondence between CryIA toxins and CryIB (55 to 56%) or CryIC (58 to 67%) (9).

Although thousands of strains of *B. thuringiensis* have been collected and dozens of δ-endotoxins have been identified (6, 9), relatively few toxins may be useful against any particular pest. Of the δ-endotoxins tested against susceptible strains of the diamondback moth, seven have been reported to be highly or moderately toxic [CryIA(a), CryIA(b), CryIA(c), CryIB, CryIC, CryIF, and CryIIA], whereas three had little or no toxicity (CryID, CryIE, and CryIIIA) (2, 4, 7, 17) (Table 1). Extensive use of *B. thuringiensis* subsp. *kurstaki* in the field in Hawaii followed by laboratory selection has produced cross-resistance to CryIF as well as resistance to at least four other toxins, leaving only CryIB and CryIC, which are known to be highly toxic to the selected larvae. These results underline the importance of using *B. thuringiensis* toxins wisely to prolong their efficacy.

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