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Cross-Resistance of Pink Bollworm (*Pectinophora gossypiella*) to *Bacillus thuringiensis* Toxins

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Two strains of pink bollworm (*Pectinophora gossypiella*) selected in the laboratory for resistance to *Bacillus thuringiensis* toxin Cry1Ac had substantial cross-resistance to Cry1Aa and Cry1Ab but not to Cry1Bb, Cry1Ca, Cry1Da, Cry1Ea, Cry1Ja, Cry2Aa, Cry9Ca, H04, or H205. The narrow spectrum of resistance and the cross-resistance to activated toxin Cry1Ab suggest that reduced binding of toxin to midgut target sites could be an important mechanism of resistance.

Evolution of resistance threatens the continued efficacy of insecticidal crystal proteins (ICPs) from *Bacillus thuringiensis* for insect pest control (6, 7, 9, 18, 20). In particular, widespread adoption of transgenic cotton that produces Cry1Ac could cause resistance to Cry1Ac in pink bollworm (*Pectinophora gossypiella*), the major lepidopteran pest of cotton in the southwestern United States (12, 14). The usefulness of switching toxins or using combinations of toxins to combat this potential resistance depends on the extent to which selection with Cry1Ac causes cross-resistance to other toxins. Field- and laboratory-selected strains of other insects show narrow to broad cross-resistance to ICPs (2, 10, 11, 15–17, 21–23, 25). Here we tested 13 ICPs against a susceptible strain (APHIS-S) of pink bollworm and two strains (APHIS-98R and AZP-R) selected in the laboratory for resistance to Cry1Ac.

The susceptible APHIS-S strain had been reared in the laboratory for many years without exposure to insecticides. The resistant APHIS-98R strain (14) was derived from APHIS-S and had been exposed repeatedly to leaf powder from Cry1Ac-producing transgenic cotton (1) and to MVP II (Dow Agrosciences, San Diego, Calif.) in an artificial diet. MVP II is a liquid formulation containing a hybrid protoxin (GenBank accession number I06283) expressed in and encapsulated by transgenic *Pseudomonas fluorescens* (8). A portion of the carboxyl-terminal region of the MVP II protoxin, which is cleaved during activation, is from Cry1Ab, and the active toxin portion is from Cry1Ac derived from HD-73 (8, 18). The resistant AZP-R strain was derived from individuals collected from the field in 1997 and exposed repeatedly to MVP II in an artificial diet (19). All larvae were reared on an artificial diet (19).

We obtained powders of the protoxin form of the following ICPs from Ecogen: Cry1Aa, Cry1Ab, Cry1Ac, Cry1Bb, Cry1Ca, Cry1Da, Cry1Fa, Cry1Ja, and Cry2Aa (4, 21). Powder containing Cry9Ca protoxin (13) was obtained from Plant Genetic Systems. We tested the activated toxin forms of Cry1Ab, Cry1Ca, Cry1E, H04, and H205. These ICPs were expressed in and purified from *Escherichia coli* (3, 5). H04 has domains I and II from Cry1Ab and domain III from Cry1C, whereas the reciprocal hybrid, H205, has domains I and II from Cry1C and domain III from Cry1Ab (3).

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Larvae were tested for susceptibility to ICPs with artificial diet bioassays. Each ICP was diluted in distilled water and blended into the diet thoroughly with a food processor to achieve the appropriate concentration. We tested groups of five neonates by using 10 to 12 g of diet per cup in sealed 37.5-ml plastic cups (Bio-Serv, Frenchtown, N.J.). For each strain and each ICP concentration tested, eight cups with five neonates per cup were held in an environmental chamber for 21 days at 27°C with a photoperiod consisting of 14 h of light and 10 h of darkness. After 1 week, the cups were put in a sealed plastic box with a small screened window in the lid for ventilation and a cup of water to maintain moisture. At 21 days, the numbers of survivors and their stages of development were recorded. Pupae and live fourth-instar larvae were counted as survivors. For each ICP, the susceptible APHIS-S strain and a resistant strain (APHIS-98R or AZP-R) were tested simultaneously. For activated toxins Cry1Ab, Cry1Ca, H04, and H205 two independent tests were done. Each trial was done on a separate date with an independently prepared batch of each toxin. The adjusted survival value for each strain was calculated by dividing the percent survival on the ICP-treated diet by the percent survival on the untreated diet.

Of the 10 protoxins tested against the susceptible APHIS-S strain of pink bollworm, all except Cry1Fa were highly toxic (Table 1). As expected, APHIS-98R was highly resistant to Cry1Ac. APHIS-98R had strong cross-resistance to Cry1Aa and Cry1Ab but little or no cross-resistance to Cry1Ca, Cry1Da, Cry2Aa, and Cry9Ca. APHIS-98R showed weak, concentration-dependent cross-resistance to Cry1Bb and Cry1Ja (Table 1).

The patterns of resistance and cross-resistance for AZP-R were similar to those of APHIS-98R (Table 1). AZP-R was resistant to Cry1Ac, with 53% survival in response to 10 μg of Cry1Ac powder per g of diet and 90% survival in response to 10 μg of MVP II per g of diet (Table 1). In the active toxin portion of the ICP, the powder obtained from Ecogen (strain EG11771, derived from HD-263) differs from MVP II (derived from HD-73) by only one amino acid (24). Thus, the apparently greater resistance to MVP II (against which AZP-R had been selected) may be related to differences in the carboxyl-terminal region or to other components of the MVP II formulation that differ from the components of the powder. Like APHIS-98R, AZP-R had cross-resistance to Cry1Aa and Cry1Ab as well as little or no cross-resistance to Cry1Ca, Cry1Da, and Cry2Aa (Table 1). Also like APHIS-98R, AZP-R had limited cross-resistance to Cry1Bb at 10 μg per g of diet

TABLE 1. Responses to *B. thuringiensis* proteins by pink bollworm larvae of susceptible (APHIS-S) and resistant (APHIS-98R and AZP-R) strains

ICP[a]	Concn (μg/g of diet)	% Survival[b] Susceptible strain	% Survival[b] Resistant strain
Tests of protoxins with APHIS-98R as the resistant strain			
Cry1Aa	1	0	100
	10	0	86
Cry1Ab	1	0	100
	10	0	79
Cry1Ac	1	0	97
	10	0	86
Cry1Bb	1	72	72
	10	17	48
Cry1Ca	1	34	38
	10	0	0
Cry1Da	1	0	0
	10	0	0
Cry1Fa	1	100	100
	10	97	90
Cry1Ja	1	6	58
	10	0	0
Cry2Aa	1	0	0
	10	0	0
Cry9Ca[c]	1	0	0
	10	0	0
Tests of protoxins with AZP-R as the resistant strain			
Cry1Aa	10	0	30
Cry1Ab	10	0	70
Cry1Ac	10	0	53
MVP II[d]	10	0	90
Cry1Bb	10	0	20
Cry1Ca	10	0	0
Cry1Da	10	0	0
Cry1Fa	10	94	93
Cry1Ja	10	0	0
Cry2Aa	10	0	0
Tests of activated toxins with AZP-R as the resistant strain			
Trial 1			
Cry1Ab	1	0	81
	10	0	73
Cry1Ca	1	0	0
	10	0	0
H04	1	0	0
	10	0	0
H205	1	67	69
	10	13	0
Trial 2			
Cry1Ab	1	0	83
	10	0	48
Cry1Ca	1	0	0
	10	0	0
H04	1	0	0
	10	0	0
H205	1	3	0
	10	0	0
Cry1Ea	1	0	0
	10	0	0

[a] ICP protoxins were obtained as powders from Ecogen (see text) unless noted otherwise.
[b] Adjusted survival (see the text).
[c] Protoxin was obtained as a powder from Plant Genetic Systems.
[d] MVP II contains a hybrid protoxin in which the active toxin fragment is from Cry1Ac (see text).

(Table 1). AZP-R was tested against only 10 μg of Cry1Ja per g of diet, and at this concentration, as with APHIS-98R, no cross-resistance occurred (Table 1).

All five of the activated toxins tested (Cry1Ab, Cry1Ca, H04, H205, Cry1Ea) were potent against the susceptible APHIS-S strain. In both trials, Cry1Ca and H04 killed all larvae tested. Although the absolute levels of mortality in response to Cry1Ab and H205 varied between trials 1 and 2, the mortality of AZP-R relative to the mortality of APHIS-S was consistent, as were the implications for cross-resistance. In both trials, AZP-R showed strong cross-resistance to activated Cry1Ab toxin and no cross-resistance to Cry1Ca, H04, H205, or Cry1Ea.

The strong cross-resistance of AZP-R to activated Cry1Ab toxin implies that reduced activation of protoxin to toxin is not an important mechanism of resistance in this strain of pink bollworm. This result, along with the narrow spectrum of cross-resistance observed in both AZP-R and APHIS-98R, is consistent with the hypothesis that reduced binding is a primary mechanism of resistance to Cry1A toxins in pink bollworm. Direct tests of this hypothesis require comparisons of toxin binding in susceptible and resistant strains.

Unlike the Cry1A-resistant NO-QA strain of diamondback moth (22), the resistant AZP-R strain of pink bollworm was not cross-resistant to hybrid toxin H04 (Table 1). Because H04 has domains I and II from Cry1Ab and domain III from Cry1C (3), we concluded that domain III from Cry1C was sufficient to counter the resistance of AZP-R. Therefore, in contrast to the pattern seen in diamondback moth, altered interactions with domain II alone apparently do not account for pink bollworm resistance to Cry1A toxins. Neither the NO-QA strain of diamondback moth (unpublished data) nor the AZP-R strain of pink bollworm was cross-resistant to hybrid toxin H205, which has domains I and II from Cry1C and domain III from Cry1Ab (3). These results imply that for both species of moths, altered interactions with domain III alone do not account for resistance to Cry1A toxins.

In summary, the narrow spectrum of cross-resistance of pink bollworm fits the general pattern of "mode 1" resistance observed in one or more strains of at least three other species of moths (21). Unlike diamondback moth, however, pink bollworm was not susceptible to Cry1Fa, had limited cross-resistance to Cry1Ja, and did not show cross-resistance to H04. This suggests that general trends in cross-resistance may be predicted across species of moths, but detailed knowledge requires experiments with each pest. The practical implication of our results is that switching toxins may have some utility against pink bollworm. Notably, the second generation of transgenic cotton produces a Cry2A toxin along with Cry1Ac. The lack of cross-resistance to Cry2Aa bodes well for the efficacy of Cry2A-producing transgenic cotton against Cry1Ac-resistant pink bollworm.

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