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Modified *Bacillus thuringiensis* Toxins and a Hybrid *B. thuringiensis* Strain Counter Greenhouse-Selected Resistance in *Trichoplusia ni*▽

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Resistance of greenhouse-selected strains of the cabbage looper, *Trichoplusia ni*, to *Bacillus thuringiensis* subsp. *kurstaki* was countered by a hybrid strain of *B. thuringiensis* and genetically modified toxins Cry1AbMod and Cry1AcMod, which lack helix α-1. Resistance to Cry1AbMod and Cry1AcMod was >100-fold less than resistance to native toxins Cry1Ab and Cry1Ac.

Insecticidal proteins from *Bacillus thuringiensis* are used widely for pest control, but evolution of resistance by pests can reduce their efficacy (3, 4, 6, 14). Resistance to *B. thuringiensis* toxins has been reported in field populations of four species of Lepidoptera, one species in response to sprays (3, 14) and three species in response to transgenic crops (10, 15, 16). Here, we focus on understanding and countering resistance to sprays of *Bacillus thuringiensis* subsp. *kurstaki* that evolved in commercial greenhouse populations of the cabbage looper, *Trichoplusia ni* (7, 17).

We compared responses to single toxins and formulations of *B. thuringiensis* by two resistant strains (GipBtR and GlenBtR) and two related susceptible strains (GipS and GlenS) of *T. ni*. All four strains were started by the collection of larvae in 2001 from commercial greenhouses near Vancouver in British Columbia, Canada (7). Resistance evolved in the greenhouses in response to repeated sprays of DiPel (7), a formulation of *B. thuringiensis* subsp. *kurstaki* strain HD1 containing Cry1Aa, Cry1Ab, Cry1Ac, and Cry2Aa (9). Previously reported concentrations required to kill 50% of larvae (LC_{50}s) indicated that, relative to a susceptible laboratory strain, initial resistance to DiPel was 113-fold in the Gip population (labeled T2c in reference 7) and 24-fold in the Glen population (labeled P5 in reference 7).

We reared larvae on a wheat germ diet (5) at 26°C on a light-to-dark schedule of 16 h:8 h. GipS and GlenS were reared on diet without *B. thuringiensis* toxins, which allowed resistance to decline (7). To maintain resistance, GipBtR and GlenBtR were reared each generation on a diet treated with 5 or 10 mg of DiPel WP (Abbott Laboratories, Ontario, Canada) per milliliter of diet (7). In bioassays, groups of five third-instar larvae were put in 60-ml plastic cups containing diet, and mortality was assessed after 3 days by gently probing larvae for movement.

We used diet overlay bioassays to evaluate the toxicity to GipBtR and GipS of the protoxin forms of Cry1Ab, Cry1Ac, Cry1AbMod, and Cry1AcMod produced in *B. thuringiensis* strains (12). Cry1AbMod and Cry1AcMod are genetically engineered variants of Cry1Ab and Cry1Ac, respectively, each lacking 56 amino acids from the amino-terminal region, including helix α-1 (12). An 80-μl aliquot containing distilled water and toxin was dispensed evenly over the surfaces of 2 ml of diet (a mean surface area of 7.1 cm^2) and allowed to dry. Fifty to 200 larvae from each strain were tested at five to eight concentrations of each toxin.

We used diet incorporation bioassays (7) to evaluate the toxicities of DiPel and Agree WG (Certis, Columbia, MD) to GipS, GipBtR, GlenS, and GlenBtR. Agree is a formulation of hybrid strain GC91, which was created from the conjugation-like transfer of a plasmid from *B. thuringiensis* subsp. *kurstaki* strain HD191 into *B. thuringiensis* subsp. *aizawai* strain HD135, and it contains Cry1Ac, Cry1C, and Cry1D (1, 8). DiPel and Agree were diluted in distilled water and mixed into diet (7). Twenty-five to 50 larvae from each strain were tested at six to seven concentrations of DiPel and Agree.

We used probit analysis (13) to estimate the LC_{50}s and their 95% fiducial limits (FL), as well as the slopes of concentration-mortality lines and their standard errors. The mortality of larvae fed treated diet was not adjusted for the mortality of control larvae on untreated diet, because the control mortality was low (mean, 3.6%; range, 0 to 16%). LC_{50}s with nonoverlapping 95% FL are significantly different. Resistance ratios were calculated as the LC_{50} of a resistant strain (GipBtR or GlenBtR) divided by the LC_{50} of its susceptible counterpart (GipS or GlenS).

The genetically modified toxins Cry1AbMod and Cry1AcMod were much more effective than the native toxins Cry1Ab and Cry1Ac against larvae of *T. ni* from the resistant GipBtR strain (Table 1). Resistance ratios of GipBtR were 580 for Cry1Ab and 1,400 for Cry1Ac but only 5.5 for Cry1AbMod and 9.3 for Cry1AcMod (Table 1). Against GipBtR, the LC_{50} was 53-fold

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TABLE 1. Responses of resistant (GipBtR and GlenBtR) and susceptible (GipS and GlenS) strains of *T. ni* to native toxins (Cry1Ab and Cry1Ac), modified toxins (Cry1AbMod and Cry1AcMod), and formulations (DiPel and Agree)

Toxin or formulation	Strain	No. of larvae	LC_{50} (95% FL)[a]	Slope ± SE	Resistance ratio[b]
Cry1Ab	GipBtR	400	180 (59–2,900)[c]	0.41 ± 0.09	580
	GipS	376	0.30 (0.21–0.41)	0.56 ± 0.06	
Cry1AbMod	GipBtR	400	3.4 (2.6–4.6)	0.52 ± 0.05	5.5
	GipS	375	0.62 (0.51–0.75)	0.99 ± 0.09	
Cry1Ac	GipBtR	600	54 (35–110)[d]	0.50 ± 0.07	1,400
	GipS	1,450	0.038 (0.031–0.046)	0.44 ± 0.02	
Cry1AcMod	GipBtR	600	5.1 (4.4–5.8)	0.85 ± 0.06	9.3
	GipS	1,145	0.55 (0.47–0.64)	0.60 ± 0.03	
DiPel	GipBtR	125	66 (21–420,000)[e]	0.43 ± 0.17	370
	GipS	125	0.18 (0.08–0.27)	0.73 ± 0.16	
Agree	GipBtR	300	4.9 (3.6–7.7)	0.81 ± 0.12	9.9
	GipS	300	0.49 (0.42–0.57)	1.4 ± 0.14	
DiPel	GlenBtR	150	3.2 (2.7–3.9)	1.9 ± 0.27	26
	GlenS	125	0.13 (0.05–0.17)	1.5 ± 0.44	
Agree	GlenBtR	300	2.0 (1.7–2.4)	1.2 ± 0.12	5.9
	GlenS	295	0.34 (0.29–0.39)	1.4 ± 0.17	

[a] Concentration that killed 50% and its 95% FL in mg protoxin per cm^2 diet for toxins and mg formulation per ml of diet for DiPel and Agree.
[b] LC_{50} of the resistant strain divided by the LC_{50} of the related susceptible strain for each toxin or formulation.
[c] Total of 17% mortality at the highest toxin concentration tested (17 mg protoxin/cm^2 diet).
[d] Total of 35% mortality at the highest toxin concentration tested (23 mg protoxin/cm^2 diet).
[e] Total of 24% mortality at the highest toxin concentration tested (15 mg DiPel/ml diet).

higher for Cry1Ab than for Cry1AbMod and 11-fold higher for Cry1Ac than for Cry1AcMod (Table 1). Against GipS, however, the LC_{50} was 2-fold higher for Cry1AbMod than for Cry1Ab and 14-fold higher for Cry1AcMod than for Cry1Ac (Table 1).

Agree was more effective than DiPel against the two resistant strains GipBtR and GlenBtR (Table 1). Resistance ratios for DiPel were 370 for GipBtR and 26 for GlenBtR compared to resistance ratios for Agree, which were 9.9 for GipBtR and 5.9 for GlenBtR (Table 1). For the two resistant strains, LC_{50}s were higher for DiPel than for Agree (13-fold higher against GipBtR and 1.6-fold higher against GlenBtR) (Table 1). Conversely, against the two susceptible strains, the LC_{50}s were higher for Agree than for DiPel (2.7-fold higher against GipBtR and 2.6-fold higher against GlenBtR).

The resistant GipBtR strain examined here (Table 1) and the resistant GLEN-Cry1Ac-BCS strain of *T. ni* studied by Wang et al. (17) had >500-fold resistance to Cry1Ab and Cry1Ac. Both GipBtR and GLEN-Cry1Ac-BCS were derived from greenhouse populations of *T. ni* that had been sprayed repeatedly with DiPel (7, 17), which contains Cry1Ab and Cry1Ac but not Cry1C or Cry1D (9). The GLEN-Cry1Ac-BCS strain had cross-resistance of only 2.5-fold to Cry1C and 2.4-fold to Cry1D (17). Agree contains Cry1C and Cry1D (8), which probably boosted its efficacy against GipBtR and GlenBtR (Table 1).

The results here with Cry1AbMod and Cry1AcMod extend those of previous work indicating that modified toxins killed larvae of *Manduca sexta* in which susceptibility to Cry1Ab was decreased via RNA interference and also killed larvae of *Pectinophora gossypiella* that had laboratory-selected, genetically based resistance to Cry1Ab and Cry1Ac (12). The efficacy of Cry1AbMod and Cry1AcMod against greenhouse-selected *T. ni* suggests that the modified toxins may be useful against resistance that evolves in commercial agricultural settings. The results here also increase the number of lepidopteran species against which the modified toxins were effective to three, with each species representing a different family (Sphingidae, Gelechiidae, and Noctuidae). In the two other species, decreased susceptibility to native Cry1A toxins was mediated by alterations in a cadherin protein that binds Cry1Ac (2, 11, 12), whereas the role of cadherin in *T. ni* resistance has not been demonstrated or excluded.

Similar to patterns observed with *P. gossypiella* (12), modified toxins were more effective than native toxins against resistant *T. ni* larvae, but native toxins were more effective than modified toxins against susceptible *T. ni* larvae (Table 1). This raises the intriguing possibility that combinations of native and modified toxins might be especially effective against populations with a mixture of susceptible and resistant individuals. In any case, the Cry1AMod toxins and hybrid *B. thuringiensis* products applied either jointly or separately may be useful for countering or delaying evolution of resistance in *T. ni.* However, further work is needed to determine how native and modified toxins interact when used in combination and how modified toxins perform in the greenhouse and field.

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