Different Cross-Resistance Patterns in the Diamondback Moth (Lepidoptera: Plutellidae) Resistant to *Bacillus thuringiensis* Toxin Cry1C

JIAN-ZHOU ZHAO,[1] YA-XIN LI,[1] HILDA L. COLLINS,[1] JUN CAO,[2] ELIZABETH D. EARLE,[2] AND ANTHONY M. SHELTON[1, 3]

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ABSTRACT Two strains of the diamondback moth, *Plutella xylostella* (L.), were selected using Cry1C protoxin and transgenic broccoli plants expressing a Cry1C toxin of *Bacillus thuringiensis* (Bt). Both strains were resistant to Cry1C but had different cross-resistance patterns. We used 12 Bt protoxins for cross-resistance tests, including Cry1Aa, Cry1Ab, Cry1Ac, Cry1Bb, Cry1C, Cry1D, Cry1E, Cry1F, Cry1J, Cry2Ab, Cry9Aa, and Cry9C. Compared with the unselected sister strain (BCS), the resistance ratio (RR) of one strain (BCS-Cry1C-1) to the Cry1C protoxin was 1,090-fold with high level of cross-resistance to Cry1Aa, Cry1Ab, Cry1Ac, Cry1F, and Cry1J (RR > 390-fold). The cross-resistance to Cry1A, Cry1F, and Cry1J in this strain was probably related to the Cry1A resistance gene(s) that came from the initial field population and was caused by intensive sprayings of Bt products containing Cry1A protoxins. The neonates of this strain can survive on transgenic broccoli plants expressing either Cry1Ac or Cry1C toxins. The other strain (BCS-Cry1C-2) was highly resistant to Cry1C but not cross-resistant to other Bt protoxins. The neonates of this strain can survive on transgenic broccoli expressing Cry1C toxin but not Cry1Ac toxin. The gene(s) conferring resistance to Cry1C segregates independently from Cry1Ac resistance in these strains. The toxicity of Cry1E and Cry2Ab protoxins was low to all of the three strains. The overall progress of all work has resulted in a unique model system to test the stacked genes strategy for resistance management of Bt transgenic crops.

KEY WORDS *Plutella xylostella*, *Bacillus thuringiensis*, Cry1C, resistance, transgenic plants

INSECT RESISTANCE TO *Bacillus thuringiensis* (Bt) has immediate and widespread significance because of increasing reliance on Bt toxins in genetically engineered crops and conventional sprays in pest management (Tabashnik et al. 1998). Most studies on Bt resistance have emphasized resistance to Bt formulations or Cry1A toxins. The most well-documented mode of resistance to Bt is characterized by >500-fold resistance to at least one Cry1A toxin, recessive inheritance, little or no cross-resistance to Cry1C, and reduced binding of at least one Cry1A toxin (Tabashnik et al. 1998).

A few cases of Cry1C resistance have been reported. High level of Cry1C resistance (>500-fold) developed in *Spodoptera exigua* (Moar et al. 1995) and *S. littoralis* (Muller-Cohn et al. 1996) after selection in laboratories. The *S. exigua* insects resistant to Cry1C were also resistant to Cry1Ab, Cry2A, and Cry9C (=CryIH) (Moar et al. 1995). For *S. littoralis*, partial cross-resistance was exhibited to Cry1D and Cry1E but not to Cry1F (Muller-Cohn et al. 1996).

The diamondback moth, *Plutella xylostella* (L.), is the only insect species to develop resistance to Bt toxins in open field populations (Tabashnik et al. 1998). An autosomal recessive gene in DBM was shown to confer high levels of resistance to four Bt toxins: Cry1Aa, Cry1Ab, Cry1Ac, and Cry1F (Tabashnik et al. 1997a, 1997b). Laboratory populations of Cry1A-resistant *P. xylostella* can also survive on transgenic crucifers expressing a high level of Cry1Ac (Metz et al. 1995; Ramachandran et al. 1998; Tang et al. 1999). Low to moderate levels of resistance to Cry1C developed either in the field (23-fold) or the laboratory (62-fold) has been reported (Liu et al. 1996, Liu and Tabashnik 1997). The resistance to Cry1C and resistance to Cry1Ab were inherited independently (Liu & Tabashnik 1997).

In the earlier period of our study, we used a field-collected *P. xylostella* population which had developed 31-fold resistance to Cry1C for continued selection with Cry1C protoxin and transgenic broccoli expressing a Cry1C protein. The resistance developed was high enough that neonates of the resistant strain could complete their entire life cycle on transgenic broccoli expressing high levels of Cry1C (Zhao et al. 2000). The Cry1C resistance in this strain was autosomally inherited and incompletely recessive when evaluated using a leaf dip assay, and recessive when

[1] Department of Entomology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456.
[2] Department of Plant Breeding, Cornell University, Ithaca, NY 14853.
[3] E-mail: ams5@cornell.edu.

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using Cry1C transgenic broccoli. The results from binding studies suggest that reduced binding is not the major mechanism of resistance to Cry1C in this population (Zhao et al. 2000). Additional bioassays proved that this Cry1C resistant strain was also highly resistant to Cry1Ac (J.-Z.Z. and A.M.S., unpublished data).

The objective of this study was to develop a Cry1Ac susceptible *P. xylostella* strain that could survive on transgenic broccoli expressing a high level of Cry1C toxin. Once we succeeded, we examined the cross-resistance patterns for the Cry1C resistant populations.

Materials and Methods

Transgenic Broccoli Expressing Cry1Ac or Cry1C Toxin. Cytoplasmic male sterile broccoli (*Brassica oleracea* ssp. *italica*) was transformed with a full length synthetic *cry1Ac* gene of Bt (Metz et al. 1995). Progeny were produced by pollinating transformed plants with Green Comet hybrid broccoli. Toxin expression in the progeny was verified by screening the plants with *P. xylostella* neonates when plants were 4–5 wk old (Tang et al. 2001). About 30 eggs from the susceptible colony (Geneva 88) were placed onto each plant. Only those plants on which the neonates showed 0% survival were categorized as high expressing and were used as Bt plants in the tests. These Bt plants could also kill 100% of the neonates of RS F_1 heterozygotes (susceptible × Cry1Ac resistant strain) (Tang et al. 2001).

A synthetic truncated *cry1C* gene (1.9 kb in length) was introduced into broccoli (Green Comet hybrid) by Cao et al. (1999). The Cry1C protein in the leaves of transgenic broccoli plants used (lines H12 and H14) was ≈0.4% of total soluble protein (Cao et al. 1999).

***Bacillus thuringiensis* Toxins.** Liquid formulations of Cry1Ac (MVP, 10%), Cry1C (M-C, 15%), and Cry1F (M-press, 20%) protoxins expressed in and encapsulated by transgenic *Pseudomonas fluorescens* (Mycogen, San Diego, CA), were used for bioassays with Cry1Ac, Cry1C and Cry1F, respectively. A blend of Cry1Ac and Cry1C (Mattch, 12%, including 10% of Cry1Ac and 2% of Cry1C) of similar formulation was also used. Cry1Aa, Cry1Ab, Cry1D and Cry1E protoxins (4 mg/ml) were obtained from Luke Masson (National Research Council of Canada, Montreal). Cry1Bb (EG7283, 0.94 mg/ml), Cry2Ab (EG7699, 0.6 mg/ml), and Cry9C (ET59, 1.3 mg/ml) protoxins were obtained from Monsanto (St. Louis, MO). Cry1Ja protoxin (EG7279, 3.6% with spores) was obtained from Ecogen (Langhorne, PA). Cry9Aa protoxin (0.15 mg/ml) was obtained from Eija Pehu (UniCrop, Helsinki, Finland).

Insects. Two strains of *P. xylostella* were used to develop a strain resistant to Cry1C but susceptible to Cry1Ac. The susceptible Geneva 88 strain was collected in 1988 from cabbage at the New York State Agricultural Experiment Station, Robbins Farm, Geneva, NY, and has been maintained on a wheat germ-casein artificial diet (Shelton et al. 1991) for over 200 generations. While on diet, the strain was kept in an environmental chamber at 27 ± 1°C, 50 ± 2% RH, and photoperiod of 16:8 (L:D) h. The Cry1C-Sel resistant strain was originally collected from a collard field in Lexington, SC, where *B. thuringiensis* subsp. *aizawai* and *kurstaki* products were reported as failing. Using a leaf dip bioassay, we determined that it had 31-fold resistance to Cry1C before the selection. The susceptibility of this colony to Cry1Ac was not tested before selection. After 26 generations of selection using Cry1C protoxin and transgenic broccoli expressing Cry1C toxin, the resistance ratio (RR) of neonates of this strain was 12,400-fold to Cry1C protoxin as previously described (Zhao et al. 2000). Further bioassays showed that this strain was also highly resistant to Cry1Ac. The RR was ≈200,000-fold compared with the susceptible strain, and the neonates could complete their entire life cycle on Bt broccoli expressing Cry1Ac toxin (J.-Z.Z. and A.M.S., unpublished data).

Crosses and Selection for Cry1C Resistance. The Cry1C-Sel resistant strain (Zhao et al. 2000) of *P. xylostella* was crossed with the susceptible (S) strain and then backcrossed with the S strain. The backcross offspring (BCS) were selected with Cry1C protoxin for two generations (F_1, F_2) using the cabbage leaf dip assay method (Shelton et al. 1993, Zhao et al. 2000). The concentrations were 8 mg (AI)/liter for F_1 larvae and 17 mg (AI)/liter for F_2 larvae, which could cause 100% mortality to susceptible individuals (SS) and > 50% mortality to RS F_1 heterozygotes (susceptible × Cry1C resistant strain). Groups of egg from F_{3-5} and F_{7-12} BCS colony were infested on Bt broccoli plants expressing Cry1C toxin for selection in the same way as previously reported (Zhao et al. 2000). After the selections we named this colony 'BCS-Cry1C-1' strain. A sister colony of BCS without any selection was reared on artificial diet by the same method as the Geneva 88 strain. Susceptibility of BCS and BCS-Cry1C-1 to Cry1Ac and Cry1C before selection (F_1) and after selection (F_5, F_6, and F_{10}) was tested using a cabbage leaf dip bioassay (Zhao et al. 2000). The F_{12} larvae of this strain were used for cross-resistance tests.

Twenty single-pairs were mated using the F_4 moths of the BCS-Cry1C-1 strain. A discriminating concentration of Cry1Ac protoxin ($LC_{99.5}$ of susceptible strain, 8.1 mg [AI]/liter) was used to test the susceptibility of the F_2 offspring from each pair. The offspring from pairs Nos. 7, 9, and 19 were most susceptible to Cry1Ac (97–100% in mortality). The nontested larvae of each of the three pairs were pooled to develop the 'BCS-Cry1C-2' strain. The F_1–F_3 offspring of this new strain were infested on Bt broccoli expressing Cry1C toxin for further selection before the cross-resistance tests using F_4 larvae.

Cross-Resistance Tests and Bioassays. Three sister strains of *P. xylostella* with the same genetic background, BCS, BCS-Cry1C-1 and BCS-Cry1C-2, were used to test cross-resistance patterns to 12 Bt protoxins. Cabbage leaf dip bioassays, as previously reported (Shelton et al. 1993; Zhao et al. 2000), were used for each strain of *P. xylostella* using second instars. Larvae of each strain were reared for the bioassay on oilseed

Table 1. Resistance development of the BCS-Cry1C-1 strain of *P. xylostella* after selection by Cry1C protoxin and Bt broccoli expressing a Cry1C toxin

Generation selected	Bt protoxin	*n*	Slope (SE)	LC_{50} (95% CI), mg (AI)/liter	χ^2 (df)[a]	RR (95% CI)[b]
0 (F_1)	Cry1C	180	1.26 (0.24)	1.62 (0.74–2.74)	1.98 (4)	1
	Cry1Ac	180	0.91 (0.13)	0.385 (0.036–2.36)	11.3 (4)*	1
5 (F_6)	Cry1C	150	1.63 (0.23)	714 (355–1,790)	3.97 (3)	440 (224–867)
	Cry1Ac	180	0.65 (0.12)	34.1 (17.1–838)	0.56 (4)	88.6 (32.8–240)
8 (F_{10})	Cry1C	150	1.58 (0.21)	1,200 (833–1,750)	1.48 (3)	740 (438–1,250)
	Cry1Ac	150	0.79 (0.17)	18,300 (6,140–687,000)	5.52 (3)	47,500 (14,700–154,000)

[a] A chi-square value followed by * was significantly different ($P < 0.05$).
[b] RR, resistance ratio = LC_{50}/LC_{50} of F_1 for each protoxin.

rape plants (*B. napus*, Dwarf Essex variety, LL Olds Seed, Madison, WI) in a greenhouse. Five to six concentrations plus a control and six disks for each concentration were included in each bioassay for most of the protoxins. Five second instars (0.2–0.3 mg/larva) were placed on each of the leaf disks inside 30-ml plastic cups. Only a single concentration of Cry1E or Cry2Ab protoxins was used for cross-resistance tests because of their low toxicity based on preliminary assays. There were four replicates for each treatment and 10 larvae for each replicates. Bond spreader/sticker (Loveland Industry, Loveland, CO) was added at 0.1% to all test concentrations and to the water control. Mortality was determined after 72 h at 27 ± 1°C. Larvae were considered dead if they did not move when prodded. The control mortality was 0–10% for BCS, BCS-Cry1C-1 and BCS-Cry1C-2 strains.

Survival of Different Strains on Transgenic Broccoli. Neonates of the Geneva 88, BCS, BCS-Cry1C-1, and BCS-Cry1C-2 strains were infested onto Cry1Ac and Cry1C transgenic broccoli leaves inside 30-ml plastic cups. Nontransgenic broccoli (Green Comet hybrid) was used as a control. There were four replicates for each treatment and 10 neonates for each replicate. Mortality was determined after 72 h at 27 ± 1°C.

Statistical Analysis. The POLO program (LeOra Software 1997) was used for probit analysis of dose–response data (Russell et al. 1977). Mortality was corrected using Abbott's formula (Abbott 1925) for each probit analysis. The 95% CI of the resistance ratios (RRs) were calculated using the method of Robertson and Preisler (1992). SAS programs were used for analysis of variance (ANOVA) (SAS Institute 1985). Mortality data were transformed to arcsine square-root value before each ANOVA was performed. Treatment means were compared and separated by Tukey studentized range test at $P = 0.05$ (SAS Institute 1985).

Results

Resistance Development after Selections with Cry1C. Compared with the BCS strain before selection, the RR of F_6 larvae of BCS-Cry1C-1 strain increased to 440- and 88.6-fold to Cry1C and Cry1Ac, respectively, after five generations of selection with Cry1C. After three more generations of selection using Cry1C broccoli, the RR to Cry1C increased slowly (740-fold) but quickly to Cry1Ac (47,500-fold) for the F_{10} larvae (Table 1).

Cross-resistance to Other Bt Protoxins. Based on the LC_{50}s of the three sister strains using 10 protoxins, the BCS-Cry1C-1 strain was resistant to Cry1C and highly cross-resistant to Cry1Aa, Cry1Ab, Cry1Ac, Cry1F, and Cry1J (RR > 390-fold). The BCS-Cry1C-2 strain was only highly resistant to Cry1C with minimal cross-resistance to other protoxins tested (RR < five-fold) (Table 2). A mixture of Cry1Ac and Cry1C (5:1 in Mattch) could overcome most of the Cry1C-resistance in the BCS-Cry1C-2 strain but had no significant effect on Cry1Ac or Cry1C resistance in BCS-Cry1C-1 strain. The protoxin of Cry1E and Cry2Ab needed much higher concentrations (100–500 mg [AI]/liter) than other protoxins to cause an evident mortality to the BCS and BCS-Cry1C-2 strains. The mortality of the BCS-Cry1C-1 strain was significantly lower than that of the BCS strain at such concentrations of Cry1E and Cry2Ab (Table 3).

Survival of Different Strains on Transgenic Broccoli. The neonates of the BCS-Cry1C-1 strain could survive on both Cry1Ac and Cry1C broccoli, whereas the BCS-Cry1C-2 neonates could only survive on Cry1C broccoli (Table 4). The Geneva 88 susceptible strain and BCS could not survive on either Cry1Ac or Cry1C broccoli plants.

Discussion

We developed a Cry1Ac susceptible *P. xylostella* strain (BCS-Cry1C-2) that can survive on transgenic broccoli expressing a high level of Cry1C toxin. This Cry1C-resistant strain was not cross-resistant to 11 other protoxins tested. Another sister strain, selected with Cry1C, BCS-Cry1C-1, was highly cross-resistant to Cry1Aa, Cry1Ab, Cry1Ac, Cry1F, and Cry1J. Our results suggested different cross-resistance patterns in *P. xylostella* resistant to Cry1C. Both cross-resistance patterns were different from two Cry1C-resistant Spodoptera species, *S. exigua* (Moar et al. 1995) and *S. littoralis* (Muller-Cohn et al. 1996).

The original Cry1C-Sel *P. xylostella* strain (Zhao et al. 2000) that we used for crosses was also highly resistant to Cry1Ac based on our preliminary tests. The larvae of the Cry1C-Sel strain showed absence of binding to Cry1Ac (Herrero et al. 2000). The Cry1Ac-resistance gene(s) in this strain probably came from

Table 2. Cross-resistance tests of different *P. xylostella* strains to Bt protoxins

Protoxin	Insect strain	*n*	Slope (SE)	LC_{50}, (95% CI), mg (AI)/liter	χ^2 (df)[a]	RR (95% CI)[b]
Cry1Aa	BCS	150	1.39 (0.25)	0.054 (0.032–0.088)	1.47 (3)	1
	BCS-Cry1C-1	150	1.45 (0.27)	21.4 (12.7–34.2)	1.62 (3)	395 (202–773)
	BCS-Cry1C-2	150	2.08 (0.41)	0.211 (0.132–0.302)	1.71 (3)	3.9 (2.1–7.1)
Cry1Ab	BCS	200	1.51 (0.22)	0.032 (0.021–0.047)	1.33 (3)	1
	BCS-Cry1C-1	180	0.85 (0.13)	19.3 (8.22–47.1)	4.21 (4)	595 (301–1,180)
	BCS-Cry1C-2	180	1.28 (0.19)	0.044 (0.030–0.065)	0.28 (3)	1.4 (0.7–2.7)
Cry1Ac	BCS	150	1.48 (0.25)	0.353 (0.208–0.551)	0.61 (3)	1
	BCS-Cry1C-1	150	1.02 (0.19)	15,400 (8,810–37,000)	2.61 (3)	43,600 (19,300–98,500)
	BCS-Cry1C-2	150	1.04 (0.29)	0.663 (0.218–1.41)	0.75 (3)	1.9 (0.7–5.3)
Cry1Bb	BCS	150	0.87 (0.19)	0.223 (0.111–0.504)	0.93 (3)	1
	BCS-Cry1C-1	150	1.47 (0.21)	0.507 (0.346–0.768)	2.18 (3)	2.3 (1.0–5.1)
	BCS-Cry1C-2	150	1.66 (0.36)	1.04 (0.490–1.64)	2.82 (3)	4.7 (2.4–9.1)
Cry1C	BCS	150	1.38 (0.22)	1.14 (0.454–2.56)	3.21 (3)	1
	BCS-Cry1C-1	150	1.48 (0.20)	1,240 (619–2,680)	3.40 (3)	1,090 (603–1,970)
	BCS-Cry1C-2	150	2.44 (0.55)	575 (328–826)	0.31 (3)	504 (287–885)
Cry1Ac+Cry1C (Mattch)	BCS	150	1.40 (0.32)	0.286 (0.110–0.497)	2.71 (3)	1
	BCS-Cry1C-1	150	1.47 (0.20)	4,140 (2,820–6,200)	1.09 (3)	14,500 (6,700–31,200)
	BCS-Cry1C-2	150	1.30 (0.21)	0.687 (0.396–1.09)	0.24 (3)	2.4 (1.3–4.5)
Cry1D	BCS	150	1.37 (0.36)	1.88 (1.05–3.06)	1.30 (3)	1
	BCS-Cry1C-1	180	1.40 (0.20)	2.80 (1.34–5.77)	3.85 (3)	1.5 (0.8–2.7)
	BCS-Cry1C-2	150	1.26 (0.22)	1.82 (0.901–3.07)	1.42 (3)	1.0 (0.5–1.9)
Cry1F	BCS	150	1.37 (0.25)	4.01 (0.482–10.3)	4.18 (3)	1
	BCS-Cry1C-1	150	0.63 (0.15)	31,600 (9,760–98,200)	3.40 (3)	7,890 (1,950–31,900)
	BCS-Cry1C-2	180	1.52 (0.32)	6.72 (1.75–12.2)	4.65 (4)	1.7 (0.4–6.6)
Cry1J	BCS	150	1.36 (0.21)	0.154 (0.044–0.345)	3.77 (3)	1
	BCS-Cry1C-1	150	0.70 (0.16)	2,020 (924–9,480)	0.50 (3)	13,100 (4,360–39,500)
	BCS-Cry1C-2	150	1.57 (0.35)	0.447 (0.205–0.714)	2.53 (3)	2.9 (0.9–9.1)
Cry9Aa	BCS	150	2.02 (0.37)	0.357 (0.221–0.504)	0.76 (3)	1
	BCS-Cry1C-1	200	2.44 (0.39)	1.05 (0.770–1.35)	0.90 (3)	3.0 (1.8–4.7)
	BCS-Cry1C-2	200	1.66 (0.26)	0.540 (0.145–1.05)	5.28 (3)	1.5 (1.0–2.3)
Cry9C	BCS	150	1.52 (0.24)	0.284 (0.173–0.433)	0.67 (3)	1
	BCS-Cry1C-1	210	2.28 (0.56)	0.981 (0.559–1.31)	3.85 (4)	3.5 (2.0–6.1)
	BCS-Cry1C-2	150	2.31 (0.44)	0.395 (0.242–0.551)	2.40 (3)	1.4 (0.8–2.4)

[a] No chi-square values were significant ($P > 0.05$).
[b] RR, resistance ratio = LC_{50}/LC_{50} of BCS for each protoxin.

the field population as a result of intensive sprayings of *B. thuringiensis* subsp. *kurstaki* products consisting primarily of spore and Cry1A proteins with some Cry2A protoxins (Koziel et al. 1993).

The allele frequency of Cry1Ac resistance gene(s) in the BCS strain from Cry1C-Sel strain after a backcross (RS $F_1 \times$ SS) was 25% if monogenically inherited (Tabashnik et al. 1997a, Tang et al. 1997). The exact mechanism causing the significant increase in RR to Cry1Ac in the BCS-Cry1C-1 strain after selection with Cry1C is uncertain. But the ability to isolate the BCS-Cry1C-2 strain from the BCS-Cry1C-1 indicates that the gene(s) conferring resistance to Cry1Ac or Cry1C segregates independently. This is similar to the results that resistance to Cry1C and resistance to Cry1Ab are inherited independently (Liu & Tabashnik 1997). Our on-going genetic mapping for Cry1C and Cry1Ac resistance in the BCS-Cry1C-1 strain using the mapping method of Heckel et al. (1999) will provide direct evidence on the linkage of Cry1C and Cry1Ac resistance in this strain.

The cross-resistance to Cry1Aa, Cry1Ab, Cry1Ac, Cry1F, and Cry1J in the BCS-Cry1C-1 strain was probably all related to the Cry1A resistance. Two *P. xylostella* strains from Hawaii and Pennsylvania were also extremely resistant to Cry1Aa, Cry1Ab, Cry1Ac, Cry1F, and Cry1J, but because neither strain had been exposed to Cry1F or Cry1J, the resistance to these toxins represented cross-resistance (Tabashnik et al. 1997b). One autosomal recessive gene conferred high

Table 3. Susceptibility of different strains of *P. xylostella* to a single concentration of Cry1E and Cry2Ab protoxins ($n = 40$)

Bt protoxin	Concn, mg (AI)/liter	% mortality (SEM) 3 d after treatment		
		BCS	BCS-Cry1C-1	BCS-Cry1C-2
Cry1E	500	40 (4) A	10 (0) B	33 (5) A
Cry2Ab	100	80 (6) A	10 (0) B	75 (3) A
Control	—	5 (3) A	8 (3) A	10 (0) A

Within a row means followed by different letters are significantly different ($P < 0.05$, HSD).

Table 4. Efficacy of Bt broccoli on neonates of different *P. xylostella* strains ($n = 40$)

Bt broccoli	% mortality (SEM) 3 d after treatment			
	Geneva 88	BCS	BCS-Cry1C-1	BCS-Cry1C-2
Cry1Ac	100 (0) A	100 (0) A	20 (9) B	100 (0) A
Cry1C	100 (0) A	100 (0) A	23 (5) C	43 (6) B
Non-transgenic	15 (3) A	25 (5) A	15 (3) A	20 (6) A

Within a row means followed by different letters are significantly different ($P < 0.05$, HSD).

resistance to four Bt toxins (Cry1Aa, Cry1Ab, Cry1Ac, and Cry1F) in both strains (Tabashnik et al. 1997a, b).

Our results proved that the neonates of the BCS-Cry1C-2 strain could survive on transgenic broccoli expressing a Cry1C but not a Cry1Ac toxin. We have previously shown that *P. xylostella* larvae resistant to Cry1A cannot survive on transgenic broccoli expressing a high level of Cry1C toxin (Cao et al. 1999). A combination of Cry1Ac and Cry1C toxin within one broccoli plant could overcome either Cry1Ac or Cry1C resistance, as performed by the mixture of Cry1Ac and Cry1C in Mattch (Table 2). We have developed such stacked-genes broccoli plants expressing both Cry1Ac and Cry1C toxins that caused 100% mortality of *P. xylostella* larvae resistant either to Cry1Ac or Cry1C toxins (J.C. and J.-Z.Z., unpublished data). The overall progress of all work has resulted in broccoli plants expressing either or both Cry1Ac and Cry1C toxins, and *P. xylostella* strains resistant to either or both Cry1Ac and Cry1C toxins (Cao et al. 1999, Metz et al. 1995; Zhao et al. 2000). This is a unique model system to study the interactions between Bt transgenic crops expressing single or two toxins and the target insect pest resistant to either or both toxins. It also can be used to test the stacked genes strategy as the next generation of Bt transgenic crops (Shelton et al. 2000) for resistance management.

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References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Cao, J., J. D. Tang, N. Strizhov, A. M. Shelton, and E. D. Earle. 1999. Transgenic broccoli with high level of *Bacillus thuringiensis* protein control diamondback moth larvae resistant to Cry1A or Cry1C. Mol. Breed. 5: 131–141.

Heckel, D. G., L. J. Gahan, Y.-B. Liu, and B. E. Tabashnik. 1999. Genetic mapping of resistance to *Bacillus thuringiensis* toxins in diamondback moth using biphasic linkage analysis. Proc. Natl. Acad. Sci. U.S.A. 96: 8373–8377.

Herrero, S., B. Escriche, J.-Z. Zhao, H. L. Collins, A. M. Shelton, and J. Ferré. 2000. Biochemical bases of the resistance selection of *Plutella xylostella* with Cry1Ac and Cry1C. (abstr.) for the Workshop on the Environmental Implications of Genetically Modified Plants with Insect Resistance Genes, 28–30 September 2000, Berne, Switzerland.

Koziel, M. G., N. B. Carozzi, T. C. Currier, G. W. Warren, and S. V. Evola. 1993. The insecticidal crystal protein of *Bacillus thuringiensis:* past, present, and future use. Biotechnol. Genet. Engl. Rev. 11: 171–228.

LeOra Software. 1997. POLI-PC: Probit and Logit analysis. LeOra Software, Berkeley, CA.

Liu, Y.-B., B. E. Tabashnik, and M. Pusztai-Carey. 1996. Field-evolved resistance to *Bacillus thuringiensis* toxin Cry1C in diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 89: 798–804.

Liu, Y.-B., and B. E. Tabashnik. 1997. Inheritance of resistance to *Bacillus thuringiensis* toxin Cry1C in the diamondback moth. Appl. Environ. Microbiol. 63: 2218–2223.

Metz, T. D., R. T. Roush, J. D. Tang, A. M. Shelton, and E. D. Earle. 1995. Transgenic broccoli expressing a *Bacillus thuringiensis* insecticidal crystal protein: implications for pest resistance management strategies. Mol. Breed. 1: 309–317.

Moar, J. W., M. Pusztai-Carey, H. V. Faassen, D. Bosch, R. Frutos, C. Rang, K. Luo, and M. J. Adang. 1995. Development of *Bacillus thuringiensis* CryIC resistance by *Spodoptera exigua* (Hubner) (Lepidoptera:Noctuidae). Appl. Environ. Microbiol. 61: 2086–2092.

Muller-Cohn, J., J. Chaufaux, C. Buisson, N. Gilois, V. Sanchis, and D. Lereclus. 1996. Spodoptera littoralis (Lepidoptera:Noctuidae) resistance to CryIC and cross-resistance to other *Bacillus thuringiensis* crystal toxins. J. Econ. Entomol. 89: 791–797.

Ramachandran, S., G. D. Buntin, J. N. All, B. E. Tabashnik, P. L. Raymer, M. J. Adang, D. A. Pulliam, and C. N. Stewart, Jr. 1998. Survival, development, and oviposition of resistant diamondback moth on transgenic canola producing a *Bacillus thuringiensis* toxin. J. Econ. Entomol. 91: 1239–1244.

Robertson, J. L., and H. K. Preisler. 1992. Pesticide bioassays with arthropods. CRC, Boca Raton, FL.

Russell, R. M., J. L. Robertson, and N. E. Savin. 1977. POLO: a new computer program for probit analysis. Bull. Entomol. Soc. Am. 23: 209–213.

SAS Institute. 1985. SAS user's guide: statistics, 5th ed. SAS Institute, Cary, NC.

Shelton, A. M., R. J. Cooley, M. K. Kroening, W. T. Wilsey, and S. D. Eigenbrode. 1991. Comparative analysis of two rearing procedures for diamondback moth. J. Entomol. Sci. 26: 17–26.

Shelton, A. M., J. L. Robertson, J. D. Tang, C. Perez, S. D. Eigenbrode, H. K. Preisler, W. T. Wilsey, and R. J. Cooley. 1993. Resistance of diamondback moth to *Bacillus thuringiensis* subspecies in the field. J. Econ. Entomol. 86: 697–705.

Shelton, A. M., J. D. Tang, R. T. Roush, T. D. Metz, and E. D. Earle. 2000. Field tests on managing resistance to Bt-engineered plants. Nature Biotech. 18: 339–342.

Tabashnik, B. C., Y.-B. Liu, N. Finson, L. Masson, and D. G. Heckel. 1997a. One gene in diamondback moth confers resistance to four *Bacillus thuringiensis* toxins. Proc. Natl. Acad. Sci. U.S.A. 94: 1640–164.

Tabashnik, B. C., Y.-B. Liu, T. Malvar, D. G. Heckel, L. Masson, V. Ballester, F. Granero, J. L. Mensua, and J. Ferré. 1997b. Global variation in the genetic and biochemical basis of diamondback moth resistance to *Bacillus thuringiensis.* Proc. Natl. Acad. Sci. U.S.A. 94: 12780–12785.

Tabashnik, B. E., Y. B. Liu, T. Malvar, D. G. Heckel, L. Masson, and J. Ferré. 1998. Insect resistance to *Bacillus thuringiensis:* uniform or diverse? Philos. Trans. R. Soc. Lond. B 353: 1751–1756.

Tang, J. D., S. Gilboa, R. T. Roush, and A. M. Shelton. 1997. Inheritance, stability and lack of fitness costs of field-selected resistance to *Bacillus thuringiensis* in diamondback moth from Florida. J. Econ. Entomol. 90: 732–741.

Tang, J. D., H. L. Collins, R. T. Roush, T. D. Metz, E. D. Earle, and A. M. Shelton. 1999. Survival, weight gain, and oviposition of resistant and susceptible *Plutella xylostella* (L.) on broccoli expressing Cry1Ac toxin of *Bacillus thuringiensis.* J. Econ. Entomol. 92: 47–55.

Tang, J. D., H. L. Collins, T. D. Metz, E. D. Earle, J. -Z. Zhao, R. T. Roush, and A. M. Shelton. 2001. Greenhouse tests

on resistance management of Bt transgenic plants using refuge strategies. J. Econ. Entomol. 94: 240–247.

Zhao, J.-Z., H. L. Collins, J. D. Tang, J. Cao, E. D. Earle, R. T. Roush, S. Herrero, B. Escariche, J. Ferre, and A. M. Shelton. 2000. Development and characterization of diamondback moth resistance to transgenic broccoli expressing high levels of Cry1C. Appl. Environ. Microbiol. 66: 3784–3789.

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