Introgression of a disrupted cadherin gene enables susceptible *Helicoverpa armigera* to obtain resistance to *Bacillus thuringiensis* toxin Cry1Ac

Y.-H. Yang, Y.-J. Yang, W.-Y. Gao, J.-J. Guo, Y.-H. Wu and Y.-D. Wu*

Key Lab of Monitoring and Management of Plant Diseases and Insects,
Ministry of Agriculture, College of Plant Protection,
Nanjing Agricultural University, Nanjing 210095, China

Abstract

A disrupted allele (r_1) of a cadherin gene (*Ha_BtR*) is genetically associated with incompletely recessive resistance to *Bacillus thuringiensis* toxin Cry1Ac in a Cry1Ac-selected strain (GYBT) of *Helicoverpa armigera*. The r_1 allele of *Ha_BtR* was introgressed into a susceptible SCD strain by crossing the GYBT strain to the SCD strain, followed by repeated backcrossing to the SCD strain and molecular marker assisted family selection. The introgressed strain (designated as SCD-r1, carrying homozygous r_1 allele) obtained 438-fold resistance to Cry1Ac, > 41-fold resistance to Cry1Aa and 31-fold resistance Cry1Ab compared with the SCD strain; however, there was no significant difference in susceptibility to Cry2Aa between the integrated and parent strains. It confirms that the loss of function mutation of *Ha_BtR* alone can confer medium to high levels of resistance to the three Cry1A toxins in *H. armigera*. Reciprocal crosses between the SCD and SCD-r1 strains showed that resistance to Cry1Ac in the SCD-r1 strain was completely recessive. Life tables of the SCD and SCD-r1 strains on artificial diet in the laboratory were constructed, and results showed that the net replacement rate (R_0) did not differ between the strains. The toxicity of two chemical insecticides, fenvalerate and monocrotophos, against the SCD-r1 strain was not significantly different from that to the SCD strain. However, larval development time of the SCD-r1 strain was significantly longer than that of the SCD strain, indicating a fitness cost of slower larval growth is associated with *Ha_BtR* disruption in *H. armigera*.

Keywords: *Helicoverpa armigera* (Hübner), *Bacillus thuringiensis*, Cry1Ac, resistance, cadherin, fitness costs

Introduction

Transgenic cotton expressing *Bacillus thuringiensis* (Bt) endotoxin Cry1Ac has been widely adopted in the United States, China, India, Australia and South Africa (James, 2006).

The major lepidopteran pests targeted by transgenic Bt cotton, such as *Helicoverpa armigera*, *Helicoverpa zea*, *Heliothis virescens* and *Petinophora gossypiella*, have been selected for high levels of resistance to Bt toxin Cry1Ac in the laboratory (Gould *et al.*, 1995; Liu *et al.*, 2001; Xu *et al.*, 2005; Anilkumar *et al.*, 2008). Although resistance to transgenic Bt cotton has not yet evolved in field populations of the target pests, it remains the major threat to the long-term efficacy of transgenic Bt cotton.

It is critical to understand the molecular mechanisms of insect Bt resistance if informed and proactive resistance

---

*Author for correspondence
Fax: +86 25 8439 6062
E-mail: wyd@njau.edu.cn

management tactics are to be developed. Although field populations of cotton pests with Bt resistance are not available, laboratory-selected Bt-resistant insect strains allow us to dissect possible resistance pathways. In three lepidopteran cotton pests, *H. virescens*, *P. gossypiella* and *H. armigera*, mutations in a cadherin gene are genetically linked to Cry1Ac resistance in the laboratory-selected strains (Gahan *et al.*, 2001; Morin *et al.*, 2003; Xu *et al.*, 2005). Recently, through a biphasic F_1 screening strategy, two resistance alleles of cadherin of *H. armigera* were identified from a field population collected from the Yellow River cotton area of China in 2005 (Yang *et al.*, 2007).

A disrupted allele (r_1) of a cadherin gene (Ha_BtR) is genetically associated with resistance to Cry1Ac in a Cry1Ac-selected strain GYBT of *H. armigera* (Xu *et al.*, 2005). In the present study, the r_1 allele from the GYBT strain was introgressed into a susceptible SCD strain by crossing between GYBT and SCD strains, followed by repeated backcrossing to the SCD strain and molecular marker assisted family selection. The cross-resistance pattern and inheritance mode of Cry1Ac resistance in the introgressed strain (SCD-r1) were characterized.

The SCD and SCD-r1 strains are near-isogenic lines, which are appropriate materials for studies on fitness costs associated with the Ha_BtR r_1 allele of *H. armigera*. Life history traits on the artificial diet and susceptibility to chemical insecticides of the susceptible SCD and resistant SCD-r1 strains were compared to determine whether there is a fitness cost associated with Cry1Ac resistance in *H. armigera*.

Materials and methods

Insect strains

The susceptible strain (SCD) originated from the Cote D'Ivoire in the 1970s and was passed to our laboratory by Bayer Crop Science in 2001. The SCD strain was maintained in the laboratory without exposure to insecticides or Bt toxins. The resistant GYBT strain was derived from a field-collected population (GY) from Hebei Province of China in 2001 by 28 generations of selection with activated Cry1Ac (Xu *et al.*, 2005). The GYBT strain has more than 500-fold resistance to activated Cry1Ac compared with the parental GY strain. The GY strain is homozygous for the wild type allele of Ha_BtR (*Helicoverpa armigera* cadherin-like gene), and the GYBT strain is homozygous for a truncated allele (r_1) of Ha_BtR (Xu *et al.*, 2005; Yang *et al.*, 2006).

Larvae of *H. armigera* were reared on an artificial diet based on wheat germ and soybean powder at $27 \pm 1°C$ with a 16:8 (L:D) light regime. Adults were held under the same temperature and light conditions at an RH of 60% and supplied with a 10% sugar solution.

Molecular detection of the r_1 allele of Ha_BtR

Individual adults (half of the abdomen tissue) were ground in 300 μl of hot DNA lysis buffer (100 mM Tris, 50 mM EDTA, 200 mM NaCl, 1% SDS, pH 8.0). After adding 2 μl Proteinase K (20 mg ml^{-1}), the homogenates were incubated at 56°C for 1–3 h. The homogenates were extracted twice with a solution of 150 μl phenol plus 150 μl chloroform-isoamylalchohol (24:1, v/v). After centrifugation at 12,000 rpm for 5 min, the supernatant was collected and DNA was precipitated using 900 μl 100% ethanol. The DNA
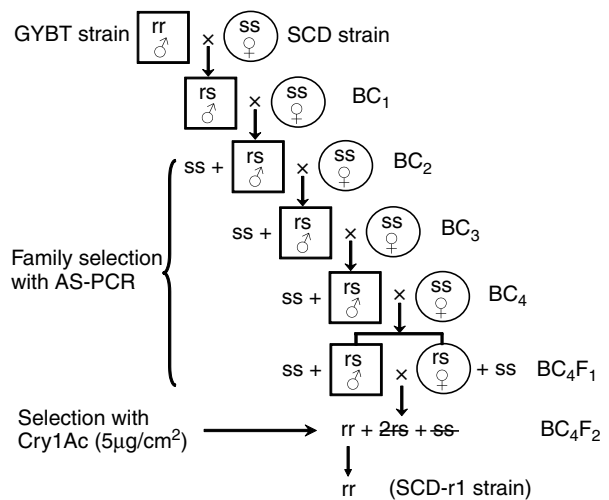

Fig. 1. Schematic program for introgression of a disrupted cadherin gene from the GKBT strain into the SCD strain of *H. armigera*.

pellet was recovered by centrifugation at 12,000 rpm, washed with 600 μl 70% ethanol and resuspended in 50-100 μl of TE buffer (1 mM EDTA, 10 mM Tris, pH 8.0) and stored at −20°C for use.

Allele specific PCR (AS-PCR) was used to amplify and discriminate between the wild type allele and the mutant allele (r_1) of Ha_BtR using the method of Yang *et al.* (2006). Briefly, two reverse primers were designed to cover the variation site: S-R (5'-CTATGTAGAACGCCTCGTGAG-3', located in Exon 9) for the wild allele, and r1-R (5'-CTTCACACATGATGTTCCTCG-3', located in Exon 25) for the mutant r_1 allele. A third primer, r1-F (5'-AGACAGGGACACTCTTGAGAAG-3', located in Exon 8), was designed as the common forward primer. The amplification reactive mixture (25 μl) contained 100 ng genomic DNA, 1 μM of each primer, 150 μM of dNTP, 2 mM of MgCl$_2$, 1 U of rTaq DNA polymerase and 2.5 μl of 10× PCR buffer. PCR was performed for 30 cycles of 30 s at 94°C, 1 min at 55°C, and 2 min at 72°C. PCR products were analyzed by 1.5% agarose gel electrophoresis and ethidium bromide staining. When the primer pair r1-F/r1-R was used for PCR amplification, the resistant homozygote (r_1r_1) and heterozygote (r_1S) had a 410 bp fragment, while the susceptible homozygote (SS) did not have this fragment. However, when the primer pair r1-F/S-R was used, the susceptible homozygote (SS) and heterozygote (r_1s) had a 650 bp fragment, while the resistant homozygote (r_1r_1) did not have this fragment.

Introgression of the r_1 allele from the GYBT strain into the SCD strain

The program for backcrossing and family selection was illustrated in fig. 1. Males from the GYBT strain (r_1r_1) were crossed (mass mating, 50 pairs) with females from the SCD strain (SS) to produce F_1 progeny (r_1S). The F_1 males were crossed (mass mating, 50 pairs) with the SCD females to produce BC1 progeny ($r_1S + SS$, 1:1). Then, the BC1 males were backcrossed individually with the SCD females (single-pair mating). After AS-PCR detection of genotypes of the

Ha_BtR gene of all BC1 males, only single-pair families derived from the r_1S father were kept to produce BC2 ($r_1S + SS$, 1:1). In the same way, single-pair families between the BC2 males with the r_1S allele and the SCD females were kept to produce BC3 ($r_1S + SS$, 1:1), and single-pair families between the BC3 males with the r_1S allele and the SCD females were kept to produce BC4 ($r_1S + SS$, 1:1). The males and females of BC4 were crossed to produce BC4F$_1$ progeny ($r_1r_1 + r_1S + SS$, 1:2:1). The BC4F$_1$ progeny were selected with $5\,\mu g\,cm^{-2}$ Cry1Ac, which is expected to kill all r_1S and SS individuals; and only r_1r_1 homozygotes were kept to generate a homozygous strain of r_1r_1 (SCD-r1). In cases where there was any ambiguity, single-pair families between SCD-r1 males and females were established, and all parents of these single-pair families were subjected to an AS-PCR examination to confirm their homogeneity for the r_1 allele of Ha_BtR gene.

Bioassay of Bt toxins and chemical insecticides

The toxicity of the various *B. thuringiensis* proteins to *H. armigera* was determined by a surface contamination bioassay. The toxin suspensions of the proteins were diluted with 0.01 M pH7.4 phosphate buffer solution (PBS) to generate five to seven serial dilutions. PBS was used as a control. A disc of artificial diet (16 mm dia.) was put into a 24-well plate and made to fit into the inner wall and bottom of the plate by gentle pressure. 100 μl of the toxin solution was applied to the diet surface and allowed to air dry. One second-instar larva, starved for four hours, was placed in each well of the plate and covered with two layers of nylon net to prevent escape. Forty-eight larvae were tested for each concentration. The mortality and body mass of the survivors were measured after five days being kept at $26 \pm 1°C$ with a 16:8 (L:D) photoperiod and 60% RH. Both dead larvae and those with a body mass of less than 5 mg were recorded as dead.

Technical grade fenvalerate (97%, Sumitomo, Japan) or monocrotophos (94%, Nantong Pesticide Factory, China) was dissolved in acetone and a series of concentrations was prepared. For each concentration, at least 40 third instar larvae (9–12 mg) were treated topically on the thorax with 0.25 μl insecticide by Hamilton syringe. After 48 h, the mortality was assessed. Larvae were classified as dead if they were unable to move after being prodded with a needle.

Inheritance analysis

Virgin female moths from the SCD-r1 strain were mass-crossed with male moths of the SCD strain and *vice visa*. The responses of F$_1$ hybrids from the two reciprocal crosses to activated Cry1Ac were determined. The dominance of resistance was calculated using the method of Stone (1968). The degree of dominance (D) values range from -1 (completely recessive) to $+1$ (completely dominant).

Survival and development in the laboratory

For each strain, 300 newly-hatched neonates were placed individually in glass tubes (20 ml volume) with artificial diet under $26 \pm 1°C$. Larval development status was individually recorded. Within 48–72 h of pupation, pupae were weighed. Emergence of adults was recorded daily and adults were paired in the oviposition cages. The eggs were collected at an interval of 24 h. The net replacement rate (R_0), the number of

Table 1. Molecular detection of the r_1 allele of Ha_BtR gene of *H. armigera*.

Cross/strain	N	Genotype		
		r_1r_1	r_1S	SS
BC$_1$ (♂ parents)	25	0	25	0
BC$_2$ (♂ parents)	35	0	19	16
BC$_3$ (♂ parents)	27	0	13	14
BC$_4$ (♂ parents)	26	0	13	13
BC$_4$F$_1$ (♂ parents)	69	0	33	36
BC$_4$F$_1$ (♀ parents)	69	0	31	38
SCD-r1 (♂ parents)	38	38	0	0
SCD-r1 (♀ parents)	38	38	0	0

N, Number of moths checked.

daughters that replace an average female over a course of a generation (Birch, 1948), was calculated for each strain by the formula $R_0 = (n \cdot I_e \cdot I_a)/2$, where n is the mean number of eggs per female, I_e is the fraction of eggs which are fertile, I_a is the fraction of adults which are reproductive and 2 is the sex ratio coefficient. The relative fitness was calculated as the R_0 of the resistant SCD-r1 strain divided by the R_0 of the susceptible SCD strain.

Data analysis

Bioassay data were analysed using Poloplus® software (LeOra Software, California, 2003). The mean larval development time and the pupal duration of the different strains were compared using analysis of variance (ANOVA).

Results

Introgression of the r_1 allele

The r_1 allele of Ha_BtR gene was introgressed into a susceptible SCD strain by crossing between GYBT and SCD strains, followed by four iterations of backcrossing to the SCD strain. Single-pair families resulting from backcrossing were subjected to family selection with allele specific PCR, and only progeny derived from male moths heterozygous for the r_1 allele were selected for further backcrossing. Molecular detection data is detailed in table 1. Progeny (BC$_4$F$_2$) from single-pair families from heterozygous male and heterozygous female moths of BC$_4$F$_1$ were subjected to selection with $5\,\mu g\,cm^{-2}$ of Cry1Ac, which is expected to kill all r_1S and SS according to previous experience from the GYBT strain (Yang *et al.*, 2007). Thirty-eight single-pair families produced from survivors of BC$_4$F$_2$ under Cry1Ac selection were established, and all parents of these single-pair families were confirmed to be homozygous for the r_1 allele of Ha_BtR gene. The strain derived from the 38 single-pair families was designated as SCD-r1. The SCD and SCD-r1 strains are a pair of near isogenic lines (NILs) for the Ha_BtR locus. It was estimated, using the principle of Mendelian segregation, that the SCD-r1 strain shared 96.7% (31/32) of its genome with that of the recipient SCD strain.

Cross resistance spectrum

After the mutated r_1 allele of Ha_BtR was introgressed into the recurrent parent SCD strain, the toxicity of Cry1Ac, Cry1Aa, Cry1Ab and Cry2Aa against the SCD-r1 and SCD strains was compared (table 2). Compared with the SCD

Table 2. Toxicity of activated Cry1Ab, Cry1Ac and Cry2Aa against the SCD and SCD-r1 strains of *H. armigera*.

Strain	Bt Cry toxin	LC$_{50}$ (95%CL) (µg cm^{-2})*	Slope$\pm$SE	*N*	Resistance ratio
SCD	Cry1Ac	0.13 (0.11–0.17)	2.95 (0.34)	240	–
	Cry1Aa	1.20 (0.89–1.58)	2.85 (0.31)	240	–
	Cry1Ab	1.42 (0.63–2.69)	2.53(0.29)	240	–
	Cry2Aa	2.82 (2.24–3.49)	4.72 (1.08)	120	–
SCD-r1	Cry1Ac	56.9 (46.5–70.4)	2.12(0.24)	240	438
	Cry1Aa	12% @ 50 µg cm^{-2}	–	48	>41
	Cry1Ab	43.9 (27.1–56.5)	2.80 (0.58)	240	31
	Cry2Aa	3.46 (2.73–4.28)	4.35 (0.94)	120	1.2

* LC$_{50}$s for which the 95% confidence limits (95%CL) did not overlap were considered to be significantly different.
N, Number of second-instar larvae tested.

Table 3. Toxicity of fenvalerate and monocrotophos against the SCD and SCD-r1 strains of *H. armigera*.

Strain	Insecticide	LD$_{50}$ (95%CL) (µg Larva^{-1})*	Slope$\pm$SE	*N*	Toxicity ratio**
SCD	Fenvalerate	0.009 (0.007–0.01)	3.15$\pm$0.34	240	–
	Monocrotophos	0.82 (0.50–1.53)	2.14$\pm$0.24	320	–
SCD-r1	Fenvalerate	0.006 (0.004–0.008)	2.51$\pm$0.27	320	0.7
	Monocrotophos	0.52 (0.28–0.80)	2.01$\pm$0.26	305	0.6

* LD$_{50}$s for which the 95% confidence limits (95%CL) did not overlap were considered to be significantly different.
N, Number of third-instar larvae tested.
** Toxicity ratio = LD$_{50}$ (SCD-r1)/LD$_{50}$(SCD).

Table 4. Toxicity of Cry1Ac against the susceptible SCD strain, resistant SCD-r1 strain and their F$_1$ progeny of *H. armigera*.

Strain	LC$_{50}$ (95%CL) (µg cm^{-2})	Slope$\pm$SE	*N*	Resistance ratio	Dominance level
SCD	0.13 (0.11–0.17)	2.95 (0.34)	240	–	
SCD-r1	56.9 (46.5–70.4)	2.12 (0.24)	240	438	
SCD-r1♂ × SCD♀	0.14(0.09–0.19)	1.84 (0.19)	240	1.0	−0.98
SCD-r1♀ × SCD♂	0.13(0.10–0.16)	1.74 (0.19)	240	1.0	−1.0

N, Number of second-instar larvae tested.

strain, the SCD-r1 strain exhibited high levels of resistance to Cry1Ac (438-fold), medium levels of resistance to Cry1Aa (>41-fold) and Cry1Ab (31-fold), but there was no resistance to Cry2Aa (1.2-fold). This indicates that the loss of function mutation of *Ha_BtR* alone is enough to confer high levels of resistance to Cry1Ac and significant cross resistance to the other two Cry1A toxins tested.

Toxicity of two chemical insecticides fenvalerate and monocrotophos against the SCD-r1 strain was tested by topical application bioassay, and the LD$_{50}$ was not significantly different between the two strains (table 3), suggesting that loss of function mutation of *Ha_BtR* does not confer any pleiotropic effects on susceptibility of *H. armigera* to the two nerve-toxic insecticides.

Inheritance mode

Toxicological responses of the resistant SCD-r1, susceptible SCD and their F$_1$ progeny to Cry1Ac toxin are summarized in table 4. The responses to Cry1Ac of F$_1$ progeny from reciprocal crosses between the SCD-r1 and SCD strains were not statistically different. This indicates that the Cry1Ac resistance is autosomal. Using the method of Stone (1968), the degree of dominance (D) in resistance was calculated to be −0.98 and −1 at LC$_{50}$, suggesting that Cry1Ac resistance in the SCD-r1 strain was completely recessive.

Relative fitness of the SCD and SCD-r1 strains

Life tables of the SCD and SCD-r1 strains fed on artificial diet were constructed to determine net replacement rate (R$_0$), and no difference was observed between R$_0$ of the two strains (table 5).

The larval development time of the resistant SCD-r1 strain was significant longer than that of the SCD strain, but pupal development time of the two strains was not significantly different (table 6).

Table 5. Life table and relative fitness of the SCD and SCD-r1strains of *H. armigera* reared on artificial diet.

Life history parameter	Strain	
	SCD	SCD-r1
Number of neonates	300	300
Number of pupae	199	182
Number of adults	178	171
Estimated no. of female moths (at 1:1 sex ratio)	89	85.5
Mean eggs laid per female	750.3	734.1
Percent egg hatch	50.1	54.4
Net replacement rate (females/female)	111.5	113.8
Relative fitness*	1	1.02

* Relative fitness = R_0 of SCD-r1 strain)/R_0 of SCD strain.

Table 6. Developmental time (day) of larvae and pupae of the SCD and SCD-r1 strains of *H. armigera*.

Development stage	N	Strain*	
		SCD	SCD-r1
Larva	200	16.80 ± 2.02 a	18.98 ± 4.65 b
Pupa (♀)	100	12.52 ± 0.83 a	12.61 ± 0.85 a
Pupa (♂)	100	13.60 ± 0.87 a	13.58 ± 0.96 a

N, Number of evaluated individuals for each strain.
* Means between strains followed by the different letters are significantly different ($P = 0.05$).

Discussion

Repeated backcrossing has been frequently applied to the genetic analysis of insecticide resistance. Not only can this method be useful in the genetic analysis of resistance, it can be used to move a major resistance gene into a susceptible genetic background and, thereby, isolate it from other genes that affect the resistance phenotype (Roush & Daly, 1990). Marker-assisted backcrossing is routinely applied for gene introgression in plant and animal breeding (Frisch & Melchinger, 2005), but it is infrequently used in the study of insecticide resistance. In the current study, a marker-assisted backcrossing regime worked successfully to introduce a deletion mutation allele of *Ha_BtR* into a susceptible SCD strain. The results further confirmed that the loss of function mutation of the *Ha_BtR* gene alone can confer resistance to Cry1Ac and cross resistance to Cry1Aa and Cry1Ab. Cry1Ac resistance in the GYBT strain of *H. armigera* is incompletely recessive, but it is completely recessive in the SCD-r1 strain. Levels of cross resistance to Cry1Ab and Cry1Aa of the SCD-r1 strain differed a little to those of the GYBT strain. These indicated some minor factor or factors affecting resistance phenotype were removed through the repeated backcross regime employed in the present study.

Since the first Cry toxin receptors were cloned in 1995, much progress has been made in our understanding of toxin specificity and mode of action (Pigott & Ellar, 2007). The best-characterized receptors of Cry1A toxins are aminopeptidase N (APN) proteins and cadherin proteins from lepidopterans. Bravo *et al.* (2007) proposed that both cadherin and APN receptors are required for full Cry1A toxicity against *Manduca sexta*. The toxin and receptor binding is suggested to be sequential; an activated toxin monomer binds initially to a cadherin receptor (BT-R_1) and then to a second receptor (class 1 APN). The cadherin binding results in a conformational change of activated Cry1Ac that facilitates cleavage of helix $\alpha-1$ from Cry1Ac domain 1 by a membrane-bound protease. Further activated toxin oligomerizes to form a tetrameric prepore and subsequently binds to APN, which finally results in pore formation and intoxication. This model is consistent with the finding of Chen *et al.* (2007) that peptide fragments from membrane-proximal regions of the cadherin protein (Bt-R_1) unexpectedly increase the toxicity of Cry1A. These fragments could accelerate the hypothesized additional toxin processing step. A recent study reported that modified Bt

Cry1A toxins (Cry1AbMod and Cry1AcMod) lacking one α-helix did not require cadherin to form oligomers, and the modified Cry1A toxins killed cadherin-silenced *M. sexta* and Bt-resistant *P. gossypiella* that had cadherin deletion mutations (Soberón *et al.*, 2007). It would be interesting to test the concept of the mode of action of modified Cry1A in the SCD-r1 and SCD strains of *H. armigera* developed in the present study to determine how universal the effects of mutations in cadherins are.

Pleiotropy is the ability of a single allele to have more than one distinguishable effect. Selection for insect resistance to insecticides generally results in a reduction in the fitness of the resistant insects, which is caused by the antagonistic pleiotropy of the resistant alleles (Roush & Daly, 1990; Chevillon *et al.*, 1997; Gould, 1998). In the absence of the pesticide selection, resistant strains of insects often show fitness costs, such as longer developmental time (Oppert *et al.*, 2000; Carrière *et al.*, 2006), lower fertility and fecundity (Alyokhin & Ferro, 1999), lower emergence from diapause (Carrière *et al.*, 2001) and decreased ability to response to alarm pheromones (Foster *et al.*, 2005) or to avoid predation (Agnew *et al.*, 2004; Berticat *et al.*, 2004).

However, it is often difficult to associate fitness disadvantages specifically with resistance, for resistant and susceptible strains may differ in fitness attributes independently of resistance (Roush & Daly, 1990). The extent of fitness costs could have been overestimated or underestimated in studies based on comparisons between strains, and overestimation would have been more common if selection for resistance reduces population size and accelerates accumulation of deleterious mutations in resistant strains (Carrière *et al.*, 2006). So, it is critical to compare all three genotypes (rr, rs, ss) in a similar genetic background, preferably in a strain that has been repeatedly backcrossed to a strain without previous pesticide exposure. Bird & Akhurst (2004, 2005) minimized divergence between strains by using four generations of serial backcrossing to increase isogenicity of their Bt-susceptible and Bt-resistant strains of *H. armigera*. Carrière *et al.* (2006) used hybrid strains of *P. gossypiella* to generate a similar genetic background for Bt resistance genotypes. In the current study, a pair of near-isogenic strains of the SCD-r1 and SCD strains of *H. armigera* was developed to homogenize the genetic background other than the resistance locus. The fitness cost of slower larval development in the resistant strain appears, therefore, to be associated with a specific resistance mechanism (loss of function mutation of *Ha_BtR*).

Fitness costs imposed by resistance genes can be magnified under stressful conditions, such as poor quality food resources or harsh weather conditions. The fitness costs

associated with Bt resistance in *Trichoplusia ni*, *H. armigera* and *P. xylostella* increased with declining host plant suitability (Janmaat & Myers, 2005; Bird & Akhurst, 2007; Raymond *et al*., 2007). Fitness costs of resistance to spinosad in *P. xylostella* increased in scale at unfavourably low and high temperatures (Liu *et al*., 2007). The current study investigated the fitness costs of Bt-resistant *H. armigera* under optimized conditions (reared on artificial diet in the laboratory); further work on relative fitness of the SCD and SCD-r1 strains of *H. armigera* under field conditions is ongoing.

Acknowledgements

References

Agnew, P., Berticat, C., Bedhomme, S., Sidobre, C. & Michalakis, Y. (2004) Parasitism increases and decreases the costs of insecticide resistance in mosquitoes. *Evolution* **58**, 579–586.

Anilkumar, K.J., Rodrigo-Simón, A., Ferré, J., Pusztai-Carey, M., Sivasupramaniam, S. & Moar, W.J. (2008) Production and characterization of *Bacillus thuringiensis* Cry1Ac-resistant cotton bollworm, *Helicoverpa zea* (Boddie). *Applied and Environmental Microbiology* **74**, 462–469.

Alyokhin, A.V. & Ferro, D.N. (1999) Relative fitness of Colorado potato beetle (Coleoptera: Chrysomelidae) resistant and susceptible to the *Bacillus thuringiensis* Cry3A toxin. *Journal of Economic Entomology* **92**, 510–515.

Berticat, C., Duron, O., Heyse, D. & Raymond, M. (2004) Insecticide resistance genes confer a predation cost on mosquitoes, *Culex pipiens*. *Genetical Research* **83**, 189–196.

Birch, L.C. (1948) The intrinsic rate of natural increase of an insect population. *Journal of Animal Ecology* **17**, 15–26.

Bird, L.J. & Akhurst, R.J. (2004) Relative fitness of Cry1A-resistant and -susceptible *Helicoverpa armigera* (Lepidoptera: Noctuidae) on conventional and transgenic cotton. *Journal of Economic Entomology* **97**, 1699–1709.

Bird, L.J. & Akhurst, R.J. (2005) Fitness of Cry1A-resistant and -susceptible *Helicoverpa armigera* (Lepidoptera: Noctuidae) on transgenic cotton with reduced levels of Cry1Ac. *Journal of Economic Entomology* **98**, 1311–1319.

Bird, L.J. & Akhurst, R.J. (2007) Effects of host plant species on fitness costs of Bt resistance in *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Biological Control* **40**, 196–203.

Bravo, A., Gill, S.S. & Soberón, M. (2007) Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. *Toxicon* **49**, 423–435.

Carrière, Y., Ellers-Kirk, C., Patin, A.L., Sims, M.A., Meyer, S., Liu, Y-B., Dennehy, T.J. & Tabashnik, B.E. (2001) Overwintering cost associated with resistance to transgenic cotton in the pink bollworm (Lepidoptera: Gelechiidae). *Journal of Economic Entomology* **94**, 935–941.

Carrière, Y., Ellers-Kirk, C., Biggs, R.W., Nyboer, M.E., Unnithan, G.C., Dennehy, T.J. & Tabashnik, B.E. (2006) Cadherin-based resistance to *Bacillus thuringiensis* cotton in

hybrid strains of pink bollworm: fitness costs and incomplete resistance. *Journal of Economic Entomology* **99**, 1925–1935.

Chen, J., Hua, G., Jurat-Fuentes, J.L., Abdullah, M.A. & Adang, M.J. (2007) Synergism of *Bacillus thuringiensis* toxins by a fragment of a toxin-binding cadherin. *Proceedings of the National Academy of Sciences USA* **104**, 13901–13906.

Chevillon, C., Denis Bourguet, D., Rousset, F.O., Pasteur, N. & Raymond, M. (1997) Pleiotropy of adaptive changes in populations: comparisons among insecticide resistance genes in *Culex pipiens*. *Genetical Research* **70**, 195–204.

Foster, S.P., Denholm, I., Thompson, R., Poppy, G.M. & Powell, W. (2005) Reduced response of insecticide-resistant aphids and attraction of parasitoids to aphid alarm pheromone: a potential fitness trade-off. *Bulletin of Entomological Research* **95**, 37–46.

Frisch, M. & Melchinger, A.E. (2005) Selection theory for marker-assisted backcrossing. *Genetics* **170**, 909–917.

Gahan, L.J., Gould, F. & Heckel, D.G. (2001) Identification of a gene associated with Bt resistance in *Heliothis virescens*. *Science* **293**, 857–860.

Gould, F. (1998) Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. *Annual Review of Entomology* **43**, 701–726.

Gould, F., Anderson, A.R., Bumgarner, L. & Moar, W. (1995) Selection and genetic analysis of a *Heliothis virescens* (Lepidoptera: Noctuidae) strain with high levels of resistance to *Bacillus thuringiensis* toxins. *Journal of Economic Entomology* **88**, 1545–1559.

James, C. (2006) Global status of commercialized Biotech/GM crops, 2006. ISAAA Briefs No. 35, Ithaca, New York, ISAAA.

Janmaat, A.F. & Myers, J. (2005) The cost of resistance to *Bacillus thuringiensis* varies with the host plant of *Trichoplusia ni*. *Proceedings of the Royal Society B: Biological Sciences* **272**, 1031–1038.

Liu, Y-B., Tabashnik, B.E., Meyer, S.K., Carrière, Y. & Bartlett, L.C. (2001) Genetics of pink bollworm resistance to *Bacillus thuringiensis* toxin Cry1Ac. *Journal of Economic Entomololgy* **94**, 248–252.

Liu, Z.M., Liu, S.S., Liu, Y.Q. & Ye, G.Y. (2007) Temperature-related fitness costs of resistance to spinosad in the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *Bulletin of Entomological Research* **97**, 627–635.

Morin, S., Biggs, R.W., Sisterson, M.S., Shriver, L., Ellers-Kirk, C., Higginson, D., Holley, D., Gahan, L.J., Heckel, D.G., Carrière, Y., Dennehy, T.J., Brown, J.K. & Tabashnik, B.E. (2003) Three cadherin alleles associated with resistance to *Bacillus thuringiensis* in pink bollworm. *Proceedings of the National Academy of Sciences USA* **100**, 5004–5009.

Oppert, B., Hammel, R., Thorne, J.E. & Kramer, K.J. (2000) Fitness costs of resistance to *Bacillus thuringiensis* in the indianmeal moth, *Plodia interpunctella*. *Entomologia Experimentalis et Applicata* **96**, 281–287.

Pigott, C. & Ellar, D.J. (2007) Role of receptors in *Bacillus thuringiensis* crystal toxin activity. *Microbiology and Molecular Biology Reviews* **71**, 255–281.

Raymond, B., Sayyed, A.H. & Wright, D.J. (2007) Host plant and population determine the fitness costs of resistance to *Bacillus thuringiensis*. *Biology Letters* **3**, 82–85.

Roush, R.T. & Daly, J.C. (1990) The role of population genetics in resistance research and management. pp. 97–152 *in* Roush, R.T. & Tabashnik, B.E. (*Eds*) *Pesticide Resistance in Arthropods*. London, Chapman and Hall.

Soberón, M., Pardo-López, L., López, I., Gómez, I., Tabashnik, B.E. & Bravo, A. (2007) Engineering modified Bt toxins to counter insect resistance. *Science* **318**, 1460–1462.

Stone, B.F. (1968) A formula for determining degree of dominance in case of monofactorial inheritance of resistance to chemicals. *Bulletin of the World Health Organization* **38**, 325–326.

Xu, X., Yu, L. & Wu, Y. (2005) Disruption of a cadherin gene associated with resistance to Cry1Ac δ-endotoxin of *Bacillus thuringiensis* in *Helicoverpa armigera*. *Applied and Environmental Microbiology* **71**, 948–954.

Yang, Y., Chen, H., Wu, S., Yang, Y., Xu, X. & Wu, Y. (2006) Identification and molecular detection of a deletion mutation responsible for a truncated cadherin of *Helicoverpa armigera*. *Insect Biochemistry and Molecular Biology* **36**, 735–740.

Yang, Y., Chen, H., Wu, Y., Yang, Y. & Wu, S. (2007) Mutated cadherin alleles from field population of *Helicoverpa armigera* confer resistance to *Bacillus thuringiensis* toxin Cry1Ac. *Applied and Environmental Microbiology* **73**, 6939–6944.