High-Level Resistance to *Bacillus thuringiensis* Toxin Cry1Ac and Cadherin Genotype in Pink Bollworm

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ABSTRACT Resistance to transgenic cotton, *Gossypium hirsutum* L., producing *Bacillus thuringiensis* (Bt) toxin Cry1Ac is linked with three recessive alleles of a cadherin gene in laboratory-selected strains of pink bollworm, *Pectinophora gossypiella* (Saunders), a major cotton pest. Here, we analyzed a strain (MOV97-R) with a high frequency of cadherin resistance alleles, a high frequency of resistance to 10 μg of Cry1Ac per milliliter of diet, and an intermediate frequency of resistance to 1,000 μg of Cry1Ac per ml of diet. We selected two strains for increased resistance by exposing larvae from MOV97-R to diet with 1,000 μg of Cry1Ac per ml of diet. In both selected strains, two to three rounds of selection increased survival at 1,000 μg of Cry1Ac per ml of diet to at least 76%, indicating genetic variation in survival at this high concentration and yielding >4,300-fold resistance relative to a susceptible strain. Variation in cadherin genotype did not explain variation in survival at 1,000 μg of Cry1Ac per ml of diet, implying that one or more other loci affected survival at this concentration. This conclusion was confirmed with results showing that when exposure to Cry1Ac stopped, survival at 1,000 μg of Cry1Ac per ml of diet dropped substantially, but survival at 10 μg Cry1Ac per ml of diet remained close to 100% and all survivors had two cadherin resistance alleles. Although survival at 1,000 μg of Cry1Ac per ml of diet is not required for resistance to Bt cotton, understanding how genes other than cadherin confer increased survival at this high concentration may reveal novel mechanisms of resistance.

KEY WORDS genetics, resistance, genetically modified crops, *Bacillus thuringiensis*, *Pectinophora gossypiella*

Transgenic corn, *Zea mays* L., and cotton, *Gossypium hirsutum* L., producing *Bacillus thuringiensis* (Bt) toxins for control of some key insect pests grew on 26 million ha worldwide in 2005 (James 2005). Such Bt crops can help to reduce reliance on insecticides, but their efficacy will be cut short if pest populations evolve resistance (Gould 1998, Shelton et al. 2002, Tabashnik et al. 2004a, Tabashnik and Carrière 2007). Field-evolved resistance to Bt crops has not been reported yet (Carrière et al. 2003; Tabashnik et al. 2003, 2005b, 2006; Stodola et al. 2006), but many pests have been selected for resistance to Bt toxins in the laboratory (Tabashnik 1994, Ferré and Van Rie 2002). Furthermore, resistance to Bt sprays has evolved in greenhouse populations of cabbage looper, *Trichoplusia ni* (Hübner) (Janmaat and Myers 2003), and in field populations of diamondback moth, *Plutella xylostella* (L.) (Tabashnik 1994, Ferré and Van Rie 2002).

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Understanding the genetic basis and mechanism of resistance to Bt toxins is useful for developing and implementing strategies to delay and monitor pest resistance (Tabashnik 1994, Gould 1998, Tabashnik et al. 2006). In principle, resistance to Bt toxins could evolve by a variety of mutations disrupting any step in the mode of action, including binding of toxin to midgut membrane receptors (Heckel 1994, Bravo et al. 2004). The best known type of resistance to Bt toxins is "mode 1" resistance of Lepidoptera, which entails reduced toxin binding, recessive inheritance, limited cross-resistance, and >500-fold resistance to one or more Cry1A toxins (Tabashnik et al. 1998).

Mutations in genes encoding cadherin proteins that bind Bt toxin Cry1Ac are associated with laboratory-selected mode 1 resistance to Cry1Ac in at least three major cotton pests [tobacco budworm, *Heliothis virescens* (F.); pink bollworm *Pectinophora gossypiella* (Saunders); and cotton bollworm, *Helicoverpa armigera* (Hübner); Gahan et al. 2001, Morin et al. 2003, Xu et al. 2005]. However, mode 1 resistance is not linked with cadherin in some field- and laboratory-selected strains of diamondback moth, a cole crop pest (Baxter et al. 2005).

In pink bollworm, three recessive alleles (*r1*, *r2*, and *r3*) of a cadherin gene (*BtR*) are tightly linked with resistance to Cry1Ac and to Bt cotton that produces Cry1Ac (Morin et al. 2003, 2004; Tabashnik et al. 2004b, 2005a, Carrière et al. 2006a). At a diagnostic concentration of Cry1Ac (10 μg of Cry1Ac per ml of diet), survival is close to 0% for larvae with one or none of these *r* alleles and close to 100% for larvae with two copies of these *r* alleles in any combination. For example, the MOV97-R strain from the Mohave Valley of western Arizona had a high frequency of two cadherin resistance alleles (*r1* and *r3*), high survival on Bt cotton, and high survival at 10 μg of Cry1Ac per ml of diet (Tabashnik et al. 2005a). However, MOV97-R had only intermediate survival at 1,000 μg Cry1Ac per ml of diet (Tabashnik et al. 2005a).

Here, we tested hypotheses about the genetic basis of variation in survival at 1,000 μg of Cry1Ac per ml of diet. First, to determine whether the variation in survival at this concentration was heritable, we selected two strains derived from MOV97-R at 1,000 μg of Cry1Ac per ml of diet. Their increased survival at this concentration after selection indicated genetically based variation. Second, to determine whether this observed genetic variation was associated with variation in cadherin genotype among *rr* individuals, we compared survival at 1,000 μg of Cry1Ac per ml of diet among *r1r1*, *r1r3*, and *r3r3* in MOV97-R. The results show that variation in cadherin genotype among *rr* individuals did not account for variation in survival at 1,000 μg of Cry1Ac per ml of diet, implying that one or more additional loci affect survival at this concentration. This conclusion was confirmed with results showing that when exposure to Cry1Ac stopped, survival at 1,000 μg of Cry1Ac per ml of diet fell substantially, but survival at 10 μg of Cry1Ac per ml of diet remained close to 100% and all survivors were *rr*.

Materials and Methods

Larval Rearing, Selection, and Bioassays. Pink bollworm larvae were reared in the laboratory on wheat germ diet (Liu et al. 2001b). For selection and bioassays, we used one neonate and 4–6 g of diet per 33-ml cup (Liu et al. 2001b, Tabashnik et al. 2002). The source of Cry1Ac was MVPII (Dow Agrosciences, San Diego, CA), a liquid formulation containing protoxin encapsulated in *Pseudomonas fluorescens* (Tabashnik et al. 2002). In each round of selection, 200 larvae from each strain were exposed to diet containing 1,000 μg of Cry1Ac per ml of diet, and the survivors were reared to continue the strain. In bioassays, we used 0 (control), 10, and 1,000 μg of Cry1Ac per ml of diet. Forty to 200 larvae were exposed to each concentration. After 21 d at 29 ± 2°C, live fourth instars and pupae were scored as survivors. Unless noted otherwise, survival was not adjusted for control mortality. Adjusted survival was calculated by dividing survival on treated diet by survival on untreated diet.

Insect Strains. We used four laboratory-selected resistant strains of pink bollworm: MOV97-R, MOV97-R1000A, MOV97-R1000B, and MOV97-R1000P. MOV97-R was derived from MOV97, which had been started in 1997 from individuals collected from cotton fields in the Mohave Valley of western Arizona (Tabashnik et al. 2005a). MOV97-R (referred to as MOV97-R_{10} by Carrière et al. 2001a,b) was started by selecting a subset of the F10 generation of MOV97 with 10 μg of Cry1Ac per ml of diet. MOV97-R was selected at 10 μg of Cry1Ac per ml of diet every even generation through the F50 generation and in the F69 and F75 generations.

MOV97-R1000A and MOV97R-1000B were derived from MOV97-R and selected for increased resistance as follows: MOV97-R1000A was started in February 2004 with the 77 survivors from 200 larvae of the F68 generation of MOV97-R exposed to 1,000 μg of Cry1Ac per ml of diet. MOV97-R1000B was started in March 2004 with the 82 survivors from 200 larvae of the F1 generation of MOV97-R1000A exposed to 1,000 μg of Cry1Ac per ml of diet. Two additional rounds of selection were done with MOV97-R1000A (F5 and F7) and MOV97-R1000B (F3 and F5). After the final round of selection, MOV97-R1000A and MOV97-R1000B were reared without exposure to Cry1Ac for five generations and pooled to make MOV97-R1000P, which was reared without exposure to Cry1Ac for another nine generations.

Response to Selection with Cry1Ac. To determine the response to selection, larvae from MOV97-R, MOV97-R1000A, and MOV97R-1000B were tested simultaneously in February 2005. At the time of this bioassay, the rounds of selection at 1,000 μg of Cry1Ac per ml of diet were none for MOV97-R (F78), two for MOV97-R1000A (F7), and three for MOV97-R1000B (F5). The numbers of larvae tested per strain at each concentration were 40 for 0 and 10 μg of Cry1Ac per ml of diet and 200 for 1,000 μg of Cry1Ac per ml of diet (total = 840 larvae). Survival and pupal weight were recorded after 21 and 22 d, respectively.

Stability of Resistance. To determine the stability of resistance to Cry1Ac, larvae from MOV97-R1000P were tested after 14 generations without exposure to Cry1Ac, including five generations when MOV97-R1000A and MOV97-R1000B were reared separately and nine generations when they were pooled as MOV97-R1000P. The numbers of larvae from MOV97-R1000P tested at each concentration were 40 for 0 and 10 μg of Cry1Ac per ml of diet and 200 for 1,000 μg of Cry1Ac per ml of diet (total = 280 larvae).

DNA Analyses to Determine Cadherin Genotype. We used DNAzol (Molecular Research Center, Cincinnati, OH) as described previously (Tabashnik et al. 2005a) to purify DNA from larvae. We used previously described allele-specific polymerase chain reaction (PCR) methods to determine cadherin genotype (Morin et al. 2004).

Association between Resistance to Cry1Ac and Cadherin Genotype. To test the hypothesis that cadherin genotype is associated with variation in survival at 1,000 μg of Cry1Ac per ml of diet, we compared cadherin genotype and allele frequencies between survivors at 10 and 1,000 μg of Cry1Ac per ml of diet in the F65 and F78 generations of MOV97-R. If a

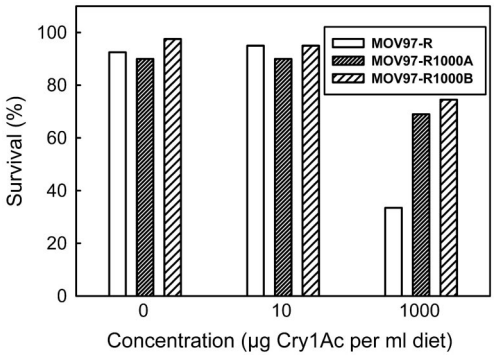

Fig. 1. Responses to Cry1Ac in two strains selected at 1,000 μg of Cry1Ac per ml of diet (MOV97-R1000A and MOV97-R1000B) and their parental resistant strain (MOV97-R). The numbers of larvae tested per concentration per strain were 40 for 0 and 10 μg of Cry1Ac per ml of diet and 200 for 1,000 μg of Cry1Ac per ml of diet. G-tests of independence with Williams' correction (df = 1 in each test) showed a significant difference in survival between MOV97-R versus MOV97-R1000A and MOV97-R1000B at 1,000 μg of Cry1Ac per ml of diet ($G = 80.7$, $P < 0.0001$), but not at 0 ($G = 0.061$, $P = 0.80$) or 10 μg of Cry1Ac per ml of diet ($G = 0.26$, $P = 0.61$).

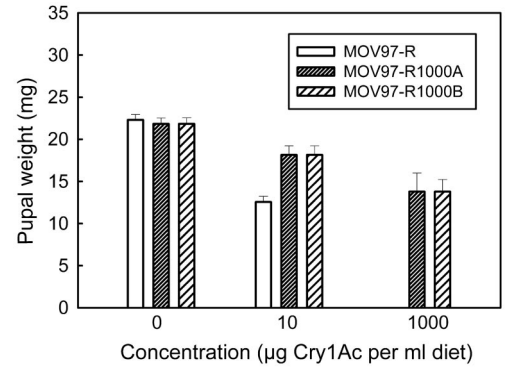

Fig. 2. Pupal weight of individuals from resistant strains MOV97-R, MOV97-R1000A, and MOV97-R1000B reared on larval diet for 22 d with 0, 10, or 1,000 μg of Cry1Ac per ml of diet. No pupae were obtained from MOV97-R at the highest concentration. In total, 169 pupae were weighed, including 10 from MOV97-R1000A and MOV97-R1000B at 1,000 μg of Cry1Ac per ml of diet. At 0 and 10 μg of Cry1Ac per ml of diet, sample size for each strain × concentration combination ranged from 13 to 36. Two-way ANOVA of results for 0 and 10 μg of Cry1Ac per ml of diet revealed significant effects of concentration ($F = 70.9$; df = 1, 153; $P < 0.0001$) and a strain × concentration interaction ($F = 10.7$; df = 2, 153; $P < 0.001$). Specific contrasts of the parental strain (MOV97-R) versus the two derived strains (MOV97R-1000A and MOV97-R1000B) showed a significant difference at 10 μg of Cry1Ac per ml of diet ($F = 44.4$; df = 1, 57; $P < 0.001$), but not on untreated diet ($F = 0.16$; df = 1, 96; $P = 0.69$).

particular cadherin genotype (e.g., *r3r3*) had greater survival than other genotypes at 1,000 μg of Cry1Ac per ml of diet, we would expect a significantly higher frequency of that genotype in survivors at 1,000 versus 10 μg of Cry1Ac per ml of diet.

Data Analysis. We used G-tests of independence with Williams' correction (Sokal and Rohlf 1995) to compare frequencies of survivors from MOV97-R versus MOV97-R1000A and MOV97R-1000B (pooled) at 0, 10, and 1,000 μg of Cry1Ac per ml of diet. We used chi-square tests to compare frequencies between survivors at 10 and 1,000 μg of Cry1Ac per ml of diet of 1) *rr* genotypes (*r1r1*, *r1r3*, and *r3r3*) and 2) *r1* and *r3* alleles. We used two-way analysis of variance (ANOVA) to test for effects on pupal weight of pink bollworm strain, Cry1Ac concentration (0 and 10 μg of Cry1Ac per ml of diet), and strain × concentration interactions. We did not include results from 1,000 μg of Cry1Ac per ml of diet in this ANOVA because no pupae from MOV97-R were obtained at this concentration. We applied specific contrasts to compare pupal weight of MOV97-R versus MOV97-R1000A and MOV97R-1000B for individuals reared on diet with 0 or 10 μg of Cry1Ac per ml of diet. ANOVA and specific contrasts were done with a general linear model and type III sum of squares (PROC GLM in SAS 8.0e, SAS Institute 1999).

Results

Response to Selection with Cry1Ac: Survival and Pupal Weight. Selection of MOV97-R1000A and MOV97-R1000B with 1,000 μg of Cry1Ac per ml of diet increased their survival at this concentration relative to their parent strain MOV97-R (Fig. 1). This response to selection reveals genetically based variation in survival of MOV97-R at 1,000 μg of Cry1Ac per ml of diet. However, mortality at 0 or 10 μg of Cry1Ac per ml of diet did not differ between the parent strain MOV97-R and the derived strains MOV97-R1000A and MOV97-R1000B (Fig. 1). These results show that the gene or genes conferring increased survival at 1,000 μg of Cry1Ac per ml of diet did not affect survival at 0 or 10 μg of Cry1Ac per ml of diet.

After 22 d on diet with 1,000 μg of Cry1Ac per ml of diet, pupae were obtained from MOV97-R1000A and MOV97-R1000B but not from MOV97-R (Fig. 2). On diet with 10 μg of Cry1Ac per ml of diet, pupal weight was greater for MOV97-R1000A and MOV97-R1000B than for MOV97-R (Fig. 2). On untreated diet, pupal weight did not differ among strains (Fig. 2). These results show that a gene or genes linked with increased survival at 1,000 μg of Cry1Ac per ml of diet increased pupal weight at 10 μg of Cry1Ac per ml of diet without affecting pupal weight on untreated diet.

Association between Cadherin Genotype and Resistance to Cry1Ac. Confirming expectations based on previous results (Morin et al. 2003; Tabashnik et al. 2004b, 2005a,b), the cadherin *r* allele frequency and survival at 10 μg of Cry1Ac per ml of diet were high in the MOV97-R strain. In the F65 generation of MOV97-R, the *r* allele frequency was 0.85 (0.10 for *r1* and 0.75 for *r3*; $n = 20$ alleles from 10 larvae) and adjusted survival at 10 μg of Cry1Ac per ml of diet was 86% ($n = 40$ larvae). Also consistent with previous results, all larvae from MOV97-R surviving 10 or 1,000

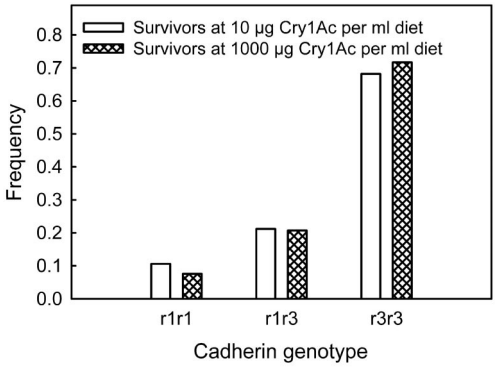

Fig. 3. Cadherin genotype frequencies in larvae from MOV97-R. Genotype frequency did not differ between larvae surviving 10 versus 1,000 μg of Cry1Ac per ml of diet ($\chi^2 = 0.46$, df = 2, $P = 0.79$). Sample sizes were 66 and 92 larvae at 10 and 1,000 μg of Cry1Ac per ml of diet, respectively.

μg of Cry1Ac per ml of diet were *rr* (*r1r1*, *r1r3*, or *r3r3;* F65 and F78, $n = 262$ larvae genotyped).

Bioassays of the F65 and F78 generations of MOV97-R yielded mean adjusted survival of 93.2% at 10 μg of Cry1Ac per ml of diet ($n = 80$ larvae) and 48.1% at 1,000 μg of Cry1Ac per ml of diet ($n = 240$ larvae). PCR analyses of the survivors from these bioassays show that cadherin genotype and allele frequency of *rr* individuals did not differ significantly between survivors of 10 and 1,000 μg of Cry1Ac per ml of diet (Figs. 3 and 4). Thus, among *rr* individuals in MOV97-R, cadherin genotype did not affect variation in survival at 1000 μg of Cry1Ac per ml of diet. In conjunction with results from our selection experiment showing heritable variation in survival at 1,000 μg Cry1Ac per ml of diet, the data on cadherin genotype suggest that survival at 1,000 μg of Cry1Ac per ml of diet was affected by one or more genes other than the cadherin gene.

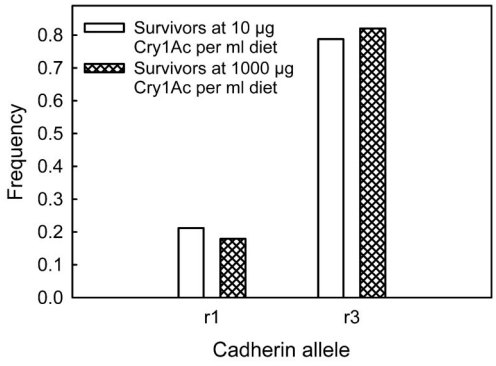

Fig. 4. Cadherin allele frequencies in larvae from MOV97-R. Allele frequency did not differ between larvae surviving 10 versus 1,000 μg of Cry1Ac per ml of diet ($\chi^2 = 0.53$, df = 1, $P = 0.47$). Sample sizes were 132 and 184 alleles (two per larva) at 10 and 1,000 μg of Cry1Ac per ml of diet, respectively.

Stability of Resistance. After 14 generations of rearing without exposure to Cry1Ac, adjusted survival at 1,000 μg of Cry1Ac per ml of diet dropped to 20.3% in MOV97-R1000P from 76.7% in MOV97-R1000A and 76.4% in MOV97-R1000B ($n = 200$ larvae per strain; $t = 1020$, df = 1, $P < 0.001$). The decline in survival at 1,000 μg of Cry1Ac per ml of diet implies that a fitness cost is associated with the gene or genes conferring increased survival at this concentration. However, 14 generations of rearing without exposure to Cry1Ac did not decrease survival at 10 μg of Cry1Ac per ml of diet (MOV97-R1000P = 96.6% versus MOV97-R1000A = 100% and MOV97-R1000B = 97.4%, $n = 40$ larvae per strain; $t = 0.51$, df = 1, $P = 0.70$). The decline in survival at 1,000 but not at 10 μg of Cry1Ac per ml of diet confirms that the gene or genes conferring increased survival at 1,000 μg of Cry1Ac are not linked with the cadherin locus. In addition, after 14 generations of rearing without exposure to Cry1Ac, PCR analyses of cadherin genotype showed that all 28 survivors at 10 μg of Cry1Ac per ml of diet were *rr* (14% *r1r1*, 43% *r1r3*, and 43% *r3r3*). These results are consistent with previous results showing that two cadherin *r* alleles are required for survival at 10 μg of Cry1Ac per ml of diet (Morin et al. 2003, Tabashnik et al. 2005a).

Discussion

The results show that the laboratory-selected MOV97-R strain had genetic variation in survival at 1,000 μg of Cry1Ac per ml of diet. Several lines of evidence support the conclusion that survival at this high concentration was affected by a gene or genes not linked with the cadherin gene. First, two or three generations of selection at 1,000 μg of Cry1Ac per ml of diet increased survival at this concentration, demonstrating genetic variation in this trait. Second, survival of larvae from MOV97-R at 1,000 μg of Cry1Ac per ml of diet did not differ among the cadherin genotypes (*r1r1*, *r1r3*, and *r3r3*) or alleles (*r1* and *r3*). Third, rearing without exposure to Cry1Ac for 14 generations decreased survival at 1,000 μg of Cry1Ac per ml of diet without changing survival at 10 μg of Cry1Ac per ml of diet or the prevalence of cadherin resistance alleles.

The stable resistance to 10 μg of Cry1Ac per ml of diet probably occurred because the frequency of cadherin resistance alleles was at or close to 100% when exposure to Cry1Ac stopped. In contrast, the decrease in survival at 1,000 μg of Cry1Ac per ml of diet when exposure to Cry1Ac stopped indicates genetic variation in this trait. Although this decline is indicative of a fitness cost, no such cost was detected on untreated diet for survival or pupal weight in MOV97-R1000A and MOV97-R1000B relative to MOV97-R. This suggests that fitness costs associated with increased survival at 1,000 μg of Cry1Ac per ml of diet affected other life history traits such as development time, mating success, or fecundity.

The results with MOV97-R showing that selection with a high concentration of Cry1Ac boosted resis-

tance above an initial plateau are similar to previously reported results with three other strains that showed mode 1 resistance to Bt toxins: the NO-Q strain of diamondback moth (Tabashnik et al. 1995), the YHD2 strain of tobacco budworm (Gould et al. 1995), and the AZP-R strain of pink bollworm (Tabashnik et al. 2002). Initial selection of MOV97-R at 10 μg of Cry1Ac per ml of diet yielded an LC_{50} value of 400 μg of Cry1Ac per ml of diet (Tabashnik et al. 2005a). As described here, subsequent selection at 1,000 μg of Cry1Ac per ml of diet yielded adjusted survival at this high concentration of 76.7% in MOV97-R1000A and 76.4% MOV97-R1000B. These results translate to an LC_{50} value of >1,000 μg of Cry1Ac per ml of diet and >4,300-fold resistance relative to the susceptible pink bollworm strain APHIS-S (Tabashnik et al. 2002). In AZP-R, which was derived by pooling pink bollworm from many Arizona cotton fields, including the source fields for MOV97-R, initial laboratory selection with 1, 3.2, and 10 μg of Cry1Ac per ml of diet produced 300-fold resistance to Cry1Ac relative to APHIS-S (Tabashnik et al. 2000). Subsequent selection of AZP-R at 100 μg of Cry1Ac per ml of diet yielded an LC_{50} value of 730 μg of Cry1Ac per ml of diet and 3,100-fold resistance relative to APHIS-S (Tabashnik et al. 2002). Consequently, it seems that pink bollworm harbors genetic variation for at least two levels of resistance to Cry1Ac.

For AZP-R, analysis of bioassays testing progeny from various crosses suggested that Cry1Ac resistance was controlled primarily by one or a few major loci (Tabashnik et al. 2002). However, a single locus, two-allele model was not sufficient to explain the results at 10 and 320 μg of Cry1Ac per ml of diet, implying effects of more than one locus or more than two alleles (Tabashnik et al. 2002). Subsequent molecular work revealed at least four alleles at the cadherin locus (*r1*, *r2*, *r3*, and *s*) in AZP-R. Survival of AZP-R at 10 μg of Cry1Ac per ml of diet can be explained by variation at this cadherin locus (Morin et al. 2003). We do not know whether survival at higher concentrations of Cry1Ac conferred by *r1* and *r3* is less than that conferred by *r2*, which was absent in MOV97-R and thus was not examined here. However, survival on Bt cotton plants did not vary among cadherin *rr* genotypes in AZP-R (Morin et al. 2003) or in SAF97-R, which has *r1* and *r2* (Tabashnik et al. 2005a). An alternative hypothesis is that, as in MOV97-R, resistance to high concentrations of Cry1Ac in AZP-R is affected by at least two independently segregating loci.

A critical practical question is whether performance on Bt cotton plants is affected by the increased level of resistance to Cry1Ac conferred by the gene or genes other than cadherin in MOV97-R. Evidence from greenhouse experiments shows that survival at 1,000 μg of Cry1Ac per ml of diet is not required for survival on Bt cotton. For example, the SAF97-R strain of pink bollworm had only 5% survival at 1,000 μg of Cry1Ac per ml of diet, but it was resistant to Bt cotton (Tabashnik et al. 2005a). Indeed, survival of SAF97-R was not lower on Bt cotton (10.5%) than on non-Bt cotton (6.9%) (Tabashnik et al. 2005a). In contrast, survival on Bt cotton plants is consistently correlated with cadherin *r* allele frequency across strains and within hybrid strains (Morin et al. 2003, Tabashnik et al. 2005a, Carrière et al. 2006a), supporting the idea that DNA screening for cadherin *r* alleles in pink bollworm is useful for resistance monitoring (Morin et al. 2004, Carrière et al. 2006b, Tabashnik et al. 2006).

We do not know whether the gene or genes other than cadherin conferring increased survival at 1,000 μg of Cry1Ac per ml of diet affect resistance to Cry1Ac when cadherin *r* alleles are absent. In the presence of a high frequency of cadherin *r* alleles in MOV97-R, however, selection for increased survival at 1,000 μg of Cry1Ac per ml of diet also increased pupal weight at 10 μg of Cry1Ac per ml of diet. In greenhouse experiments, resistant pink bollworm had lower pupal weight on Bt cotton than on non-Bt cotton, which is a component of incomplete resistance (Liu et al. 2001a, Carrière et al. 2006a). If the gene or genes conferring increased pupal weight on diet with 10 μg of Cry1Ac per ml of diet also increase pupal weight or otherwise improve fitness on Bt cotton, they could speed resistance evolution in the field. Possibilities for proteins encoded by such genes include putative Bt toxin receptors other than cadherin such as aminopeptidase N or alkaline phosphatase (Bravo et al. 2004, Jurat-Fuentes and Adang 2004); proteinases that activate Bt toxins (Li et al. 2005) as well as any other proteins that influence the responses of insects to Cry1Ac or MVPII (the formulated version of Cry1Ac that we used). In any case, understanding the role of such genes may help to identify novel resistance mechanisms and elucidate the mode of action of Bt toxins.

Acknowledgments

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