Asymmetrical cross-resistance between *Bacillus thuringiensis* toxins Cry1Ac and Cry2Ab in pink bollworm

Bruce E. Tabashnik1,2, Gopalan C. Unnithan3, Luke Masson4, David W. Crowder5, Xianchun Li6, and Yves Carrier7

1Department of Entomology, University of Arizona, Tucson, AZ 85721; and 2Biotechnology Research Institute, National Research Council of Canada, Montréal, QC, Canada H4P 2R2

Edited by May R. Berenbaum, University of Illinois, Urbana, IL, and approved May 21, 2009 (received for review February 6, 2009)

Transgenic crops producing *Bacillus thuringiensis* (Bt) toxins kill some key insect pests and can reduce reliance on insecticide sprays. Sustainable use of such crops requires methods for delaying evolution of resistance by pests. To thwart pest resistance, some transgenic crops produce 2 different Bt toxins targeting the same pest. This “pyramid” strategy is expected to work best when selection for resistance to 1 toxin does not cause cross-resistance to the other toxin. The most widely used pyramid is transgenic cotton producing Bt toxins Cry1Ac and Cry2Ab. Cross-resistance between these toxins was presumed unlikely because they bind to different larval midgut target sites. Previous results showed that laboratory selection with Cry1Ac caused little or no cross-resistance to Cry2A toxins in pink bollworm (*Pectinophora gossypiella*), a major cotton pest. We show here, however, that laboratory selection of pink bollworm with Cry2Ab caused up to 420-fold cross-resistance to Cry1Ac as well as 240-fold resistance to Cry2Ab. Inheritance of resistance to high concentrations of Cry2Ab was recessive. Larvae from a laboratory strain resistant to Cry1Ac and Cry2Ab in diet bioassays survived on cotton bolls producing only Cry1Ac, but not on cotton bolls producing both toxins. Thus, the asymmetrical cross-resistance seen here does not threaten the efficacy of pyramided Bt cotton against pink bollworm. Nonetheless, the results here and previous evidence indicate that cross-resistance occurs between Cry1Ac and Cry2Ab in some key cotton pests. Incorporating the potential effects of such cross-resistance in resistance management plans may help to sustain the efficacy of pyramided Bt crops.

To delay pest resistance, some second-generation Bt crops produce 2 distinct Bt toxins that are active against the same pest. This approach, which is called a “pyramid,” is expected to delay pest resistance most effectively when selection for resistance to 1 of the toxins does not cause cross-resistance to the other toxin (14). Other factors favoring success of pyramided Bt crops are abundant refuges of non-Bt host plants near Bt crops and the following conditions for each toxin in the pyramid: functionally recessive inheritance of resistance, low initial resistance allele frequency, and fitness costs associated with resistance (3, 14–16).

The most widely used pyramided Bt crop is cotton producing Cry1Ac and Cry2Ab, which was registered in December 2002 (http://www.epa.gov/pesticides/biopesticides/pips/pip_list.htm) and planted on more than 1 million ha in the United States in 2006, 2007, and 2008 (www.monsanto.com/pdf/investors/2008/2008.biotech.acres.pdf). Cross-resistance between Cry1Ac and Cry2Ab has been presumed unlikely, because these toxins differ substantially in amino acid sequence and bind to different target sites in the larval midgut (17–20). Both Cry1Ac and Cry2Ab are active against some key lepidopteran pests, including pink bollworm (*Pectinophora gossypiella*), a major cotton pest in the southwestern United States and in many other countries (21). Bt cotton producing only Cry1Ac has been exceptionally effective against pink bollworm in Arizona; susceptibility to Cry1Ac has not decreased in field populations of this pest despite more than a decade of exposure (22–25). Previous work showed that laboratory selection of pink bollworm with Cry1Ac yielded high levels of resistance to Cry1Ac, but little or no cross-resistance to Cry2A toxins (26–28; http://www.epa.gov/scipoly/sap/meetings/2006/october/unnithan.et_al_04_cry2ab.baselines.pdf). Here we show, however, that laboratory selection of pink bollworm with Cry2Ab yielded resistance to Cry2Ab and cross-resistance to Cry1Ac. We term this “asymmetrical cross-resistance” because selection with Cry2Ab caused cross-resistance to Cry1Ac, but selection with Cry1Ac did not cause cross-resistance to Cry2Ab. Asymmetrical cross-resistance could be important for resistance management but has received little attention previously. In particular, most previous tests for cross-resistance include selection with only 1 of the 2 toxins being evaluated and thus cannot detect asymmetrical cross-resistance (2, 18).


Conflict of interest statement: Although preparation of this article was not supported by organizations that may gain or lose financially through its publication, the authors have received support for other research from Monsanto, Cotton Inc., the Cotton Foundation, and the Arizona Cotton Growers Association. B.E.T. is a coauthor of a patent application filed with the World Intellectual Property Organization on engineering modified Bt toxins to counter pest resistance, which is related to published research [2007 Science 318:1640–1642].

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

1To whom correspondence should be addressed. E-mail: brucet@ag.arizona.edu.
Results

Effects of Selection with Cry2Ab on Susceptibility to Cry2Ab, Cry1Ac, and Cry2Aa. Laboratory selection of pink bollworm strains BX-R1 and BX-R2 with Cry2Ab in diet yielded resistance of both strains to Cry2Ab, as well as cross-resistance to Cry1Ac and Cry2Aa (Tables 1 and 2, and Fig. 1). Confirming previous results (26, 27), the AZP-R strain selected with Cry1Ac had little or no cross-resistance to Cry2Ab or Cry2Aa (Tables 1 and 2). Relative to the susceptible strain APHIS-S, the maximum resistance ratios for BX-R1 were 240 for Cry2Ab and 420 for Cry1Ac. Maximum resistance ratios for BX-R2 were 74 for Cry2Ab and 21 for Cry1Ac. Relative to their hybrid parent strain BX-H, maximum increases in LC50 values were 110-fold for Cry2Ab and 190-fold for Cry1Ac for BX-R1, and 44-fold for Cry2Ab and 9.2-fold for Cry1Ac for BX-R2. Relative to BX-H, selection with Cry2Ab increased survival of BX-R1 and BX-R2 exposed to a diagnostic concentration (10 μg toxin/ml diet) of Cry2Ab, Cry1Ac, or Cry2Aa (Tables 1 and 2). For BX-R1, survival at the diagnostic concentration of Cry2Ab was slightly higher in the F22–24 generations (92%) than in the F16–17 generations (88%), even though the LC50 of Cry2Ab was lower in the F22–24 generations (27 μg toxin/mL diet) than in the F16–17 generations (99 μg toxin/mL diet) (Table 1). The consistently high survival at the diagnostic concentration indicates that resistance did not decrease substantially.

Selection with Cry2Ab also increased survival in response to simultaneous exposure to Cry2Ab and Cry1Ac in diet tests. On diet treated with a combination of 10 μg Cry2Ab plus 100 μg Cry1Ac/mL diet, survival was 75% for BX-R1 (F17, n = 65) versus 0% for BX-H (F41, n = 30, Fisher’s exact test, P < 0.0015).

Table 1. Responses of pink bollworm to Bt toxins Cry1Ac and Cry2Ab in diet bioassays

<table>
<thead>
<tr>
<th>Strain</th>
<th>Dates</th>
<th>Gens.*</th>
<th>n</th>
<th>LC50 (95% FL)†</th>
<th>RR‡</th>
<th>Survival, %§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cry2Ab</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BX-R1</td>
<td>Feb. 2005</td>
<td>F16–17</td>
<td>786</td>
<td>99 (52–530)</td>
<td>240</td>
<td>88</td>
</tr>
<tr>
<td>BX-R1</td>
<td>Sept.–Nov. 2005</td>
<td>F22–24</td>
<td>700</td>
<td>27 (23–32)</td>
<td>64</td>
<td>92</td>
</tr>
<tr>
<td>BX-R2</td>
<td>Feb. 2005</td>
<td>F5</td>
<td>334</td>
<td>4.2 (3.2–5.3)</td>
<td>10</td>
<td>23</td>
</tr>
<tr>
<td>BX-H</td>
<td>Aug. 2005</td>
<td>F47</td>
<td>400</td>
<td>0.7 (0.58–0.83)</td>
<td>1.7</td>
<td>0</td>
</tr>
<tr>
<td>AZP-R</td>
<td>Feb. 2005</td>
<td>F72</td>
<td>408</td>
<td>0.76 (0.65–0.88)</td>
<td>1.8</td>
<td>0</td>
</tr>
<tr>
<td>AZP-R</td>
<td>Aug. 2005</td>
<td>F78</td>
<td>449</td>
<td>0.80 (0.70–0.89)</td>
<td>1.9</td>
<td>0</td>
</tr>
<tr>
<td>APHIS-S</td>
<td>Feb. 2005</td>
<td>NA</td>
<td>424</td>
<td>0.42 (0.28–0.55)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>APHIS-S</td>
<td>Aug. 2005</td>
<td>NA</td>
<td>250</td>
<td>0.42 (0.31–0.52)</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2. Responses of pink bollworm to Bt toxin Cry2Aa in diet bioassays

<table>
<thead>
<tr>
<th>Strain</th>
<th>Gens.*</th>
<th>n</th>
<th>LC50 (95% FL)†</th>
<th>RR‡</th>
<th>Survival, %§</th>
</tr>
</thead>
<tbody>
<tr>
<td>BX-R1</td>
<td>F24–25</td>
<td>513</td>
<td>8.4 (6.3–11)</td>
<td>25</td>
<td>43</td>
</tr>
<tr>
<td>BX-R2</td>
<td>F14</td>
<td>509</td>
<td>7.9 (6.0–10)</td>
<td>24</td>
<td>41</td>
</tr>
<tr>
<td>BX-H</td>
<td>F50–51</td>
<td>420</td>
<td>0.61 (0.47–0.77)</td>
<td>1.8</td>
<td>0</td>
</tr>
<tr>
<td>APHIS-S</td>
<td>F76–78</td>
<td>410</td>
<td>0.95 (0.70–1.2)</td>
<td>2.9</td>
<td>1.9</td>
</tr>
</tbody>
</table>

All bioassays with Cry2Aa were started during November to December 2005.

*Generations tested in bioassays. NA, not available.
†Concentration killing 50% with 95% fiducial limits in parentheses, in μg of toxin per ml of diet.
‡Resistance ratio, the LC50 of a strain divided by the LC50 of APHIS-S from the same time period.
§Survival at 10 μg of toxin per ml of diet, adjusted for control mortality; sample size for each survival estimate ranged from 60 to 380 (mean = 120).

Fig. 1. Effect of selection with Cry2Ab on resistance to Cry1Ac. Solid symbols correspond to LC50 values from the first set of bioassay dates (December 2004 to March 2005), and open symbols correspond to LC50 values from the second set of bioassay dates (July to November 2005; Table 1). A significant portion of the variation in the logarithm of LC50 of Cry1Ac is explained by variation in the logarithm of LC50 of Cry2Ab (y = 1.02x + 1.52, R² = 0.83, df = 6, P = 0.0015).
Fig. 2. Responses to Cry2Ab of pink bollworm larvae from a resistant strain (BX-R), a susceptible strain (APHIS-S), and their F1 progeny (BX-R X APHIS-S). Each point represents mean mortality (with standard error bars) of 40–160 larvae fed diet treated with Cry2Ab, adjusted for control mortality (see Materials and Methods). Inheritance was completely recessive at 3, 10, 30, and 100 μg Cry2Ab/mL diet (h = 0) and somewhat less recessive at lower concentrations (h = 0.02, 0.089–0.097, and 0.19–0.23, at 1, 0.3, and 0.1 μg Cry2Ab/mL diet, respectively, see Materials and Methods).

Inheritance of Resistance to Cry2Ab. Results of a cross between the resistant BX-R strain and the susceptible APHIS-S strain showed that resistance to Cry2Ab was inherited as a completely recessive trait at concentrations of 3, 10, 30, and 100 μg Cry2Ab/mL diet (h = 0) (Fig. 2). As the concentration of Cry2Ab decreased, resistance was slightly less recessive (Fig. 2). Results did not differ between the F1 hybrid progeny from the 2 reciprocal crosses (BX-R females × APHIS-S males and APHIS-S females × BX-R males), indicating that the resistance was autosomally inherited.

Survival on Bolls of Bt Cotton and Non-Bt Cotton. Despite their increased survival on diet treated with Cry2Ab and Cry1Ac, BX-R1 larvae did not survive on cotton bolls producing both of these toxins (Fig. 3). In greenhouse boll bioassays, survival of BX-R1 larvae did not differ significantly between non-Bt cotton (2.0 larvae per boll, n = 15 bolls) and cotton producing only Cry1Ac (2.6 larvae per boll, n = 20 bolls; Mann-Whitney U-test, P = 0.68). However, no larvae survived on 110 bolls of cotton producing Cry1Ac and Cry2Ab, which was significantly lower than survival on the 2 other types of cotton (Mann-Whitney U-test, P < 0.0001). These results were confirmed in laboratory boll bioassays where survival was 25% on non-Bt cotton, 14% on cotton producing Cry1Ac, and 0% on cotton producing Cry2Ab and Cry1Ac (n = 50 or 60 neonates infesting 5 or 6 bolls, respectively, for each type of cotton). As with the greenhouse boll bioassays, survival in laboratory boll bioassays did not differ significantly between non-Bt cotton and cotton producing only Cry1Ac (Fisher’s exact test, P = 0.12), but survival on cotton producing both toxins was significantly lower than survival on the other 2 types of cotton (Fisher’s exact test, P < 0.0001).

Discussion

Together with previous results, the data reported here imply that cross-resistance between Bt toxins Cry1Ac and Cry2Ab was asymmetrical in the pink bollworm strains examined. Whereas previous laboratory selection of pink bollworm with Cry1Ac produced little or no cross-resistance to Cry2A toxins (26, 27), selection with Cry2Ab here yielded up to 420-fold cross-resistance to Cry1Ac (Table 1). As far as we know, asymmetrical cross-resistance between Cry1 and Cry2 toxins has not been reported before. For example, in the cotton pest Helicoverpa armigera, selection with Cry1Ac did not cause cross-resistance to Cry2Ab and selection with Cry2Ab did not cause cross-resistance to Cry1Ac (29–32). However, most previous studies could not detect asymmetrical cross-resistance, because insects selected with Cry1 toxins were tested for cross-resistance to Cry2 toxins, but the reciprocal experiment was not done (2, 18).

Although the mechanism of pink bollworm’s asymmetrical cross-resistance between Cry1Ac and Cry2Ab remains to be determined, the available evidence excludes some explanations. In pink bollworm strains selected with Cry1Ac including AZP-R, resistance to Cry1Ac is tightly linked with mutations in a gene encoding a cadherin protein that binds Cry1Ac in the larval midgut (33–35). Because the strains selected with Cry1Ac had little or no cross-resistance to Cry2A toxins (Table 1, and refs. 26, 27), we infer that the cadherin mutations alone do not confer high levels of cross-resistance to Cry2A toxins. Furthermore, given that Cry1Ac and Cry2Ab bind to different sites in the larval midgut of pink bollworm (19), it is unlikely that mutations affecting any single toxin-binding protein confer resistance to Cry2Ab and cross-resistance to Cry1Ac. We hypothesize that the observed resistance to Cry2Ab requires resistance alleles at 2 or more loci, including the cadherin locus (or a locus linked with the cadherin locus) and at least 1 locus not linked with the cadherin locus. If so, selection with Cry2Ab could increase the frequency of cadherin mutants as well as resistance alleles at 1 or more independent loci affecting susceptibility to Cry2Ab. Conversely, selection with Cry1Ac alone could increase the frequency of cadherin mutants without affecting the frequency of alleles at other loci conferring resistance to Cry2Ab. Additional experiments are needed to test this and other hypotheses.

For several reasons, the type of asymmetrical cross-resistance between Cry1Ac and Cry2Ab seen in our laboratory selection experiments does not threaten control of pink bollworm by pyramided Bt cotton in the field. First, the conditions of our experiments differed markedly from conditions in the field. In particular, the laboratory selection experiments with Cry2Ab in diet started with a hybrid strain of pink bollworm (BX-H) that had close to 10% survival at a diagnostic concentration of Cry1Ac (10 μg toxin/mL diet) (Table 1). In contrast, after a decade of exposure to cotton producing only Cry1Ac, survival at the diagnostic concentration of Cry1Ac was essentially 0% in field-derived strains of pink bollworm (24, 25). Second, laboratory-selected strains of pink bollworm showed recessive inheritance of resistance to high concentrations of Cry2Ab (Fig. 2) and Cry1Ac (22, 28), which favors sustained efficacy of pyramided Bt cotton. Third, pink bollworm larvae from the BX-R1 strain...
selected with Cry2Ab did not survive on bolls of Bt cotton producing Cry1Ac and Cry2Ab, despite their high levels of resistance to both toxins in diet bioassays and survival on Bt cotton bolls producing only Cry1Ac. This pattern probably reflects the 160-fold higher toxin concentration in bolls for Cry2Ab (792 µg toxin/g) versus Cry1Ac (4.95 µg toxin/g) in bolls producing Cry1Ac only and bolls producing both toxins (36). The concentration of Cry2Ab in bolls was 8 to 29 times higher than the LC50 values of Cry2Ab for BX-R1 (Table 1). Conversely, the LC50 values of Cry1Ac for BX-R1 (Table 1) were more than 100 times greater than the concentration of Cry1Ac in bolls (Table 1). Thus, it seems likely that the Cry2Ab in bolls killed BX-R1 larvae, but the Cry1Ac did not.

Whereas selection with Cry1A toxins caused little or no cross-resistance to Cry2A toxins in several cases (18, 29–31, 37), notable exceptions include the major U.S. cotton pests Helicoverpa zea and Helicoverpa armigera. Laboratory selection of H. virescens strain CP73–3 with Cry1Ac caused 50-fold resistance to Cry1Ac and 53-fold cross-resistance to Cry2Aa (38). Selection with Cry1Ac also caused some cross-resistance to Cry2Aa in the KCB and YHD2 strains of H. virescens (39, 40). Genetic linkage analysis identified loci in H. virescens that contributed to resistance to both Cry1Ac and Cry2Aa (41). However, as in pink bollworm and H. armigera (30), H. virescens resistance to Cry2Aa was not caused by the cadherin gene linked with Cry1Ac resistance (41). Similar to pink bollworm, field populations of H. virescens have remained susceptible to Cry1Ac despite many years of exposure to Bt cotton producing only Cry1Ac (9). Slower processing of protoxin to activated toxin was identified in 2 of 3 strains of H. virescens with resistance to Cry1Ac and Cry2Aa (42). Although resistance was similar to the protoxin and activated toxin forms of Cry1Ab in pink bollworm strain AZP-R (26), more work is needed to determine if altered processing of protoxin contributes to the asymmetrical cross-resistance seen here.

As in some strains of H. virescens, resistance to Cry1Ac was genetically correlated with resistance to Cry2Aa in field populations of H. zea sampled during 2000 (43). In addition, responses to Cry1Ac and Cry2Ab were genetically correlated in field populations of H. zea sampled during 2001 and 2002 (44). An independent study showed that for 61 populations of H. zea tested from 2002 to 2004, the LC50 values of Cry2Ab were positively correlated with those of Cry1Ac (45). Although H. zea has been exposed to cotton producing only Cry1Ac since 1996 and some field populations have evolved resistance to Cry1Ac (4, 8–9), cotton producing Cry1Ac and Cry2Ab was registered in December 2002 and first exceeded 1 million ha planted in the United States in 2006. Thus, cross-resistance to Cry2Ab caused by resistance to Cry1Ac is a plausible explanation for the observed positive correlation between LC50 values for Cry1Ac and Cry2Ab (45). An alternative scenario is that the positive correlation was caused by selection first for resistance to Cry1Ac followed by selection for resistance to Cry2Ab. Survival on cotton with both toxins would be more likely for individuals with Cry2Ab resistance alleles if they were already resistant to Cry1Ac (14). However, because cotton producing both Cry1Ac and Cry2Ab was relatively rare from 2002 to 2004, the positive correlation between LC50 values for Cry1Ac and Cry2Ab detected during this period probably resulted from cross-resistance, rather than sequential resistance to the 2 toxins. Cross-resistance is more problematic in H. zea than in pink bollworm or H. virescens, because H. zea showed nonrecessive inheritance of resistance to Cry1Ac and Cry2Aa (43, 46), relatively low inherent susceptibility to Cry1Ac and Cry2Ab (36), and survival in the field on cotton plants producing Cry1Ac and Cry2Ab (47).

As the second and future generations of insecticidal transgenic crops are deployed, crops with pyramids of toxins such as Cry1Ac and Cry2Ab will become increasingly widespread. Indeed, pyramided Bt corn with Cry1Ac and Cry2Ab was registered in the United States in 2008 (www.epa.gov/ EPA-IMPACT/2008/July/Day-24/116947.htm). To maximize the benefits of pyramided crops, it will be important to apply insights gained from experiments and field experience. The results reported here suggest that it may be useful to perform separate selection experiments with each of the toxins in a pyramid to fully delineate patterns of cross-resistance. Evidence to date suggests that the ideal conditions for pyramid performance may not always be attained. In particular, despite the dissimilarity in structure and toxin-binding sites for Cry1Ac and Cry2Ab, cross-resistance occurs between these toxins in some key target pests. Previous results suggest that refuges of host plants that do not produce Bt toxins can delay pest resistance (4, 7). To determine the optimal abundance of such refuges for pyramided Bt crops, it may be useful to account for the potential effects of cross-resistance between the toxins in pyramids.

Materials and Methods

Insect Strains. We used 6 strains of pink bollworm from APHIS-S, AZP-R, BX-H, BX-R, BX-R2, and BX-R. APHIS-S is a susceptible strain that had been reared for many years without exposure to Bt toxins (45). BX-R1, BX-R2, and BX-R are resistant strains that were started by pooling survivors of exposure to Cry1Ac in diet from 10 strains derived in 1997 from Arizona cotton fields (22). AZP-R had been selected repeatedly with 10 or 100 µg Cry1Ac/mL diet, yielding >1,000-fold resistance to Cry1Ac, but little cross-resistance to Cry2Aa or Cry2Ab (27, 28). BX-H is a hybrid strain that included a mixture of Cry1Ac-resistant and susceptible individuals (34). BX-R1 and BX-R were started with individuals from BX-H and selected for resistance to Cry2Ab as described below. After BX-R1 and BX-R2 achieved substantial resistance to Cry2Ab, 875 pupae from these 2 strains were pooled to create BX-R in December 2006. Larvae of all strains were reared on wheat germ diet (48).

BT Toxins. We used the protoxin form of Bt toxins in all experiments. The source of Cry1Ac was MVP II obtained from Dow Agrosciences (28). The Cry2Ab in the inheritance experiment was produced by a recombinant acrystalliferous strain of Bt subsp. kurstaki (HD73 cry-1) that was transformed with the cry2Ab gene from strain HD1 of Bt subsp. kurstaki (49). For all other experiments, Cry2Ab2 was obtained from Monsanto Inc. in powder from transgenic corn. The powder was made by grinding freeze-dried leaves of corn (Mon84006) that produced Cry2Ab2. Cry2Aa2 provided by William Moar (Auburn University, Auburn, AL) was purified from transformed Eschericia coli that produced Cry2Aa2 protoxin inclusion bodies (27). We refer to Cry2Ab2 as Cry2Aab and Cry2Aa2 as Cry2Aab2 because the amino acid sequence is the same for Cry2Ab and Cry2Ab2. In the present study, Cry2Ab and Cry2Ab2, as well as for Cry2Aa2 and Cry2Aa (http://www.lifesci.sussex.ac.uk/home/Neil.Crickmore/Bt/).
Inheritance of Resistance to Cry2Ab. To evaluate dominance of resistance, maternal effects, and sex linkage, we used diet bioassays with Cry2Ab protein toxin as described above to test APHIS-S, BX-R, and the F1 hybrid progeny resulting from crosses between BX-R females and APHIS-S males and the reciprocal cross (APHIS-S females × BX-R males) (28). The entire experiment was replicated in 2 different time periods starting in April and May 2007.

Statistical Analysis. We estimated the concentration of toxin killing 50% of larvae (LC50) and its 95% fiducial limits from diet bioassay data by using the program DBC6O (50). LC50 values with nonoverlapping 95% fiducial limits are significantly different. Resistance ratios were calculated as the LC50 of a strain divided by the LC50 of the susceptible APHIS-S strain tested during the same time period. We also calculated adjusted mortality at individual toxin concentrations, including a diagnostic concentration of 10 μg Cry2Ab/mL diet, as: 100% − adjusted survival, where adjusted survival equals [survival (%) on treated diet divided by (%) on diet without toxin] × 100%. In some cases, larvae were tested with bioassays in 2 to 3 consecutive generations, and data were pooled across generations to yield robust estimates of susceptibility parameters. To test the hypothesis that selection with Cry2Ab caused cross-resistance to Cry1Ac, we used linear regression with the logarithm of LC50 of Cry2Ab as the explanatory variable and the logarithm of LC50 of Cry1Ac as the response variable (51). To test the hypothesis that selection with Cry2Ab yielded resistance to a combination of Cry1Ac and Cry2Ab, we used Fisher’s exact test to make the following pairwise comparisons of survival between strains: BX-R versus BX-H, BX-R2 versus BX-H, BX-R1 versus AZP-R, and BX-R2 versus AZP-R.

We estimated dominance (h2) (52) based on adjusted survival for APHIS-S, BX-R, and their F1 hybrid progeny at each of 7 concentrations: 0.1, 0.3, 1, 3, 10, 30, and 100 μg Cry2Ab/mL diet. Values of h range from zero (completely recessive) to 1 (completely dominant). The highest concentrations tested against APHIS-S were 1, 3, and 10 μg Cry2Ab/mL diet, which all caused adjusted mortality to 100%. To calculate h for 30 and 100 μg Cry2Ab/mL diet, we assumed that these higher concentrations also killed 100% of APHIS-S. The lowest concentration tested against BX-R was 1 μg Cry2Ab/mL diet, which caused 4.2% adjusted mortality. To calculate h for 0.1 and 0.3 μg Cry2Ab/mL diet, we assumed that these lower concentrations killed zero to 4.2% of BX-R, which generated a range of values for h at 0.1 and 0.3 μg Cry2Ab/mL diet.

ACKNOWLEDGMENTS. We thank Timothy Dennehy for initiating this research when he was at the University of Arizona. We thank Robert Biggs, Ali Mazza, and the staff of the Extension Arthropod Resistance Management Laboratory for technical assistance, and Mark Sisterson and David Onstad for comments on the manuscript. We thank Monsanto for Cry2Ab, William Moar (Auburn University, AL) for Cry2Aa, and Dow for Cry1Ac. The research was supported by U.S. Department of Agriculture-National Research Initiative Grants 2006-35352-17365 and 2008-35352-0390.


