SUSCEPTIBILITY OF SOUTHWESTERN PINK BOLLWORM TO CRY2Ab:

BASELINE RESPONSES IN 2001 AND 2002

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Summary

Susceptibility of pink bollworm (PBW), Pectinophora gossypiella, to the Bt toxin Cry2Ab was evaluated on collections made in the Southwestern U.S. in 2001 and 2002 and contrasted with laboratory strains. PBW were collected from cotton fields, cultured in the laboratory, and tested using diet-incorporation bioassays. A total of 6 collections in 2001 and 14 collections in 2002 were successfully reared and bioassayed. Significant differences between strains in susceptibility to Cry2Ab were found each year, though the range of susceptibility was substantially greater in 2001 than in 2002. LC_{50} estimates of strains ranged from 0.220 to 4.56 µg Cry2Ab/ml and 0.0840 to 0.723 µg Cry2Ab/ml in 2001 and 2002, respectively. Only one field strain, a collection from Tornillo, Texas, was substantially less susceptible to Cry2Ab than was the laboratory strain, AZP-R, which was highly resistant to Cry1Ac. Based on these baseline responses, concentrations of 1.0 and 10 µg Cry2Ab/ml diet have been identified for routine monitoring of pink bollworm. Selection of 2001 and 2002 strains with Cry2Ab is underway in the laboratory but has not yielded intense resistance to Cry2Ab. Thus, at this time we have no indication of significant resistance of Southwestern pink bollworm to the second Bt toxin being deployed in transgenic insecticidal cotton.

Introduction

Pink bollworm (*Pectinophora gossypiella*) (PBW), is one of the most economically damaging pests of Southwestern cotton. High susceptibility of this pest to the Bt toxin expressed in Bollgard® cotton, Cry1Ac, promoted rapid and intensive adoption of this technology in the Southwest (Frisvold et al. 2000). Loss of target pest susceptibility as a result of resistance has been anticipated to be perhaps the greatest biological limitation of transgenic insecticidal crops (Mellon and Rissler 1998). Resistance problems seemed especially likely in Arizona cotton following selection in the laboratory of high levels of resistance of PBW to Cry1Ac (Bartlett 1995, Simmons et al. 1998, Patin et al. 1999, Liu et al. 1999, Tabashnik et al. 2000, Sims et al. 2001). Despite the isolation and characterization of highly resistant pink bollworm from Arizona fields, statewide monitoring of resistance and parallel evaluations of the field performance of Bt cotton have shown that resistance has not increased measurably after 8 years of intensive use of Bollgard cotton (Dennehy et al. 2004).

Addition of the Cry2Ab toxin to Cry1Ac in the second generation of Bt cotton, Bollgard II, was done to increase toxicity to key pests like *Helicoverpa zea* and *Spodoptera exigua,* to broaden the range of pests controlled, and to combat resistance development in key pests through redundant killing. Redundant killing insures that rare individuals homozygous for resistance to one toxin will be killed by the second toxin. Herein, we report baseline susceptibility to Cry2Ab of pink bollworm collections made in Arizona, California, New Mexico, and Texas in 2001 and 2002.

Materials and Methods

Collections. Cotton bolls infested with pink bollworm were collected from August through November during both 2001 and 2002. Collections were made in Arizona and Texas in 2001 and from Arizona, California, New Mexico and Texas in 2002. The goal was to establish a laboratory culture with ≥ 200 PBW from each site.

At each location 300-2000 cotton bolls were collected from non-Bt cotton fields in areas adjacent to Bt cotton fields. Bolls were taken to the University of Arizona Extension Arthropod Resistance Management Laboratory (EARML) in Tucson and put in boll boxes (17.6 cm x 50.4 cm x 35.2 cm). Bolls were spread on wire racks approximately 3 cm above sheets of white paper towels on the floor of the boll boxes. Fourth instar PBW larvae cut out of infested bolls and dropped onto the paper towels below. These larvae were transferred to pupation boxes consisting of tightly sealed, 1.7 liter rectangular Rubbermaid® containers with layers of moist paper towels on the bottom. When fewer than 200 larvae were obtained from boll boxes, bolls were cracked manually to obtain the desired number of larvae. To prevent or disrupt larval diapause the paper towels were kept moist and the larvae were disturbed regularly until pupation.

Rearing. We reared PBW in environmental chambers using modified versions of the methods of Bartlett and Wolf (1985) and the wheat germ diet described by Adkinson et al. (1960). Neonate offspring of field-collected PBW were transferred individually with brushes into 1 oz plastic cups. These cups had tight fitting lids and contained about 5 g of diet each. One or two neonate larvae were placed into each cup and the tops were affixed. Neonate to pupal development was conducted in the dark; all other stages were maintained on a 16 h photophase. Pupae from each culture were collected from the rearing cups and bulked in groups of ≤ 50 individuals in 16 oz paper cups, to permit collection of eggs. These cups had wire-meshed lids on top of which pieces of shop towel were placed to serve as an oviposition substrate. Adults were provided a 10% sugar solution. Eggs were harvested at regular intervals and held in sealed containers until they hatched. Neonates were then used to infest diet for the next generation. All life stages were held at 29±1 °C.

Bioassaying Susceptibility to Cry2Ab. Six field strains in 2001 and 14 field strains in 2002 were evaluated for susceptibility to Cry2Ab. Tests of each culture with Cry2Ab commenced after they were first bioassayed for susceptibility to Cry1Ac. Thus, the evaluations of susceptibility to Cry2Ab reported herein were made on strains that had been in culture for 14 to 21 generations (12 to 18 months) for 2001 collections and 4 to 10 generations (4 to 9 months) for 2002 collections. Additionally, susceptibility of two laboratory strains of pink bollworm was evaluated at the same time that the 2002 collections were being tested. APHIS was a susceptible strain that has been maintained in the laboratory for over 30 years without exposure to pesticides. Prior to 1996, field collected pink bollworm were periodically added to this strain. The AZP-R strain was highly resistant to Cry1Ac. It was collected in Arizona in 1997 and repeatedly selected with Cry1Ac in the laboratory (Patin et al. 1999, Tabashnik et al. 2000). This strain has been shown to possess three mutations in the cadherin protein gene that are very tightly linked with resistance to Cry1Ac (Morin et al 2003). Greenhouse trials on Bollgard cotton have shown that this strain had 46% survival on Bt cotton, relative to survival on non-Bt cotton (Liu et al. 2001).

A 21 day diet-incorporation bioassay (Patin et al. 1999) was used. The source of Cry2Ab toxin was freeze-dried Cry2Ab corn leaf powder obtained from Monsanto. The material we used was originally estimated by Monsanto, in 2001, to contain 2.7 mg Cry2Ab toxin/g of leaf powder. Monsanto analyses of this same batch of leaf powder in 2003 yielded a modified estimate of 6.014 mg Cry2Ab/ ml leaf powder. All mortality and lethal concentration values in this report have been adjusted to reflect this latter estimate. A modified version of the wheat germ diet described by Adkinson et al. (1960) was made in 3-4 liter batches, in a KitchenAid® food processor. Liquid diet was subdivided by weight into 1 liter glass beakers, and held in a water bath at 50-60 °C. Cry2Ab corn powder and food color (0.1µl/ml) was added to the liquid diet and blended thoroughly, using an electric hand mixer. The food color was added to ensure thorough mixing of the corn leaf powder in the diet. Cry2Ab concentrations routinely used for bioassays were 0 (control), 0.01, 0.1 and 1.0 µg/ml diet. Populations that had multiple survivors of 1.0 µg/ml assays were tested at higher concentrations. After mixing the toxin, the diet was cooled and cubed using a commercial cheese slicer. Five to six g of diet per cup was dispensed into 1 oz plastic cups. Cups were then closed and held in the refrigerator until used. Neonate larvae were placed, one per cup, on the diet and the lids of infested cups were affixed. Replicates in multiples of 10 cups each were set up for each concentration tested. Tables 2 and 3 detail the total numbers of neonates tested with each concentration of Cry2Ab evaluated.

After 21 days neonates that developed to ≥ 4th instar stage (Watson and Johnson 1974) were scored as alive. Cups from which 4th instar larvae exited by chewing through the plastic were scored as alive if: 1) they contained frass of the size/quantity produced by a 4th instar; 2) the exit hole was of the size produced by a 4th instar; and 3) the diet in the cup showed evidence of feeding consistent with development to the 4th instar. Corrected mortality was computed using Abbott's correction (Abbott 1925). Estimates of LC_{50} and LC_{90}, and corresponding 95% confidence limits, were calculated using probit analysis (LeOra software 1987, Robertson and Preisler 1992).

Bioassaying Susceptibility to Cry1Ac. Susceptibility of the APHIS and AZP-R laboratory reference strains of pink bollworm to Cry1Ac was estimated using the same 21-day diet-incorporation bioassay methodology described above for Cry2Ab. MVP-II® Bioinsecticide obtained from Ecogen was diluted with sterilized, distilled water to produce a stock solution of Cry1Ac toxin. The stock was then added to liquid wheat germ diet (Adkinson et al. 1960) in amounts appropriate to create the desired final concentrations.

Results and Discussion

Laboratory Reference Strains

Cry1Ac. The AZP-R strain was over 1000-fold less susceptible to Cry1Ac than was the APHIS strain (Figure 1). The LC_{50} of APHIS to Cry1Ac was 0.574 (95% F.L. 0.39-0.74) µg Cry1Ac/ml (Table 1). The LC_{50} of AZP-R was > 1000 µg Cry1Ac/ml. A poor fit of data to the probit model resulted in our inability to derive 95% statistical limits for this estimate. However, corrected mortality observed in bioassays of AZP-R with 1000 µg Cry1Ac/ml was 29.8% (95% C.L. 18-42%) (Table 1). Thus, we know with 95% statistical certainty that the concentration of Cry1Ac resulting in 50% mortality of AZP-R was well over 1000 µg Cry1Ac/ml.

Cry2ab. The AZP-R and APHIS strains were significantly different in susceptibility to Cry2Ab but the differences were very small. Whereas these strains differed by over 1000-fold in susceptibility to Cry1Ac, AZP-R was only ca. two-fold less susceptible to Cry2Ab (Figure 1) than was APHIS. The LC_{50} and LC 90 of AZP-R were 1.84 and 3.91 µg Cry2Ab/ml, respectively (Table 1). APHIS had LC_{50} and LC_{90} estimates of 0.876 and 2.22 µg Cry2Ab/ml, respectively (Table 1).

2001 Field Strains

Responses to Cry2Ab of five Arizona collections and one Texas collection made in 2001 are illustrated in Figure 2. Strains differed significantly at both the LC_{50} and LC_{90} levels (Table 2). The most susceptible strains were collected in Paloma and Mohave, Arizona, and had LC_{50} estimates of 0.220 and 0.230 µg Cry2Ab/ml, respectively. The least susceptible strain was collected in Tornillo, Texas, and had an LC_{50} of 4.56 µg Cry2Ab/ml. Thus, the least and most susceptible strains differed ca. twenty-fold in susceptibility to Cry2Ab at the LC_{50}. Differences in the range of LC_{90}s were even greater (Table 2). Strains from Marana, Safford and Coolidge, Arizona, were intermediate to these extremes (Figure 2). For comparison purposes, strains were grouped into those with LC_{50}s above and below 1.0 µg Cry2Ab/ml. Mohave, Paloma, and Marana all had LC_{50} values below 1.0 µg Cry2Ab/ml (Table 2). Safford, Coolidge and Tornillo had LC_{50}s above 1.0 µg Cry2Ab/ml.

The response of the Tornillo strain was atypical of Arizona collections. Moreover, the Tornillo strain was significantly less susceptible to Cry2Ab than was the AZP-R strain, based on non-overlapping 95% limits of LC_{50} and LC_{90} estimates (Tables 1 and 2). We are currently completing experiments in which the 2001 Tornillo strain has been repeatedly exposed to Cry2Ab in the laboratory. Preliminary conclusions are that we have not observed a striking response to selection of this strain. These selection studies should be concluded by May of 2004.

These data provide as basis for detecting future differences or changes in susceptibility of pink bollworm to Cry2Ab. Based on the 2001 results, we conclude that 1.0 and 10 µg Cry2Ab/ml would be useful concentrations to use for routine monitoring of pink bollworm.

2002 Field Strains

Overall susceptibility to Cry2Ab of collections made in 2002 (Figure 3) was greater than in 2001 (Figure 2), and 2002 collections had substantially smaller differences between the least and most susceptible strains. Whereas 3 out of 6 of the 2001 strains evaluated had LC_{50}s exceeding 1.0 µg Cry2Ab/ml (Table 2), none of the 14 strains evaluated from 2002 had LC_{50}s exceeding this level, and all but the Las Cruces populations had LC_{50}s below 0.5 µg/ml (Table 3). The strains most susceptible to Cry2Ab were collected in Arizona; the strains least susceptible to Cry2Ab were collected from the Blythe/Palo Verde Valley area of California, and near Las Cruces, New Mexico (Figure 3). However, the Las Cruces collection was not significantly less susceptible to Cry2Ab than was the AZP-R laboratory strain, and the most and least susceptible 2003 collections differed in LC_{50} for Cry2Ab by less than 8-fold (Table 3).

Selection of the 2002 collections with Cry2Ab is underway. Preliminary indications are that we have not been able to select for a high intensity of resistance to Cry2Ab. Baseline responses obtained in 2002 confirmed the utility of 1.0 and 10 µg/ml for routine monitoring of pink bollworm susceptibility to Cry2Ab.

Conclusion Regarding Resistance to Cry2Ab

We found significant differences between strains in pink bollworm susceptibility to Cry2Ab in 2001 and 2002. However, only the collection from Tornillo, Texas, gave us reason for concern in that it was substantially less susceptible to Cry2Ab

than was AZP-R. Selection with Cry2Ab of Tornillo and other 2001 and 2002 strains is underway in the laboratory but has not yielded intense resistance to Cry2Ab. Thus, at this time we have no indication of significant resistance of Southwestern pink bollworm to the second Bt toxin being deployed in transgenic insecticidal cotton. Based on baseline responses to Cry2Ab reported herein, we have identified concentrations of 1.0 and 10 µg Cry2Ab/ml for future monitoring of pink bollworm susceptibility to this toxin.

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Table 1. Corrected mean mortality of laboratory strains of pink bollworm tested in 2003 for susceptibility to Cry2Ab in diet-incorporation bioassays. The APHIS strain was susceptible to Cry1Ac; the AZP-R strain was highly resistant to Cry1Ac. LC_{50} and LC_{90} estimates (95% F.L.) were generated using the POLO program (Robertson and Preisler 1992).

Cry1Ac (concentration)	***0.01 µg/ml***	***0.1 µg/ml***	***1.0 µg/ml***	***3.2 µg/ml***	**LC_{50}** (95% F.L.)	**LC_{90}** (95% F.L.)
APHIS (Cry1Ac-Susceptible)	2.45	4.55	62.4	87.7	0.574 (0.39-0.74)	2.02 (1.6-2.8)
		10 µg/ml	***100 µg/ml***	***1000 µg***		
AZP-R(Cry1Ac-Resistant)		0 (*SEM 0)	2.16 (*SEM 1.44)	29.8 (*SEM 5.66)	>1000*	>3000*
Cry2Ab (concentration)			***0.22 µg***	***0.22 µg***		
APHIS (Cry1Ac-Susceptible)			1.34	82.6	0.876 (0.69-1.1)	2.22 (1.7-3.4)
AZP-R (Cry1Ac-Resistant)			0	60.08	1.84 (1.6-2.1)	3.91 (3.5-4.6)

* Due to poor fit to the probit model, 95% limits of LC_{50} and LC_{95} could not be derived for AZP-R response to Cry1Ac. Instead, mean and standard errors of corrected mortality are provided for the concentrations tested.

Table 2. Susceptibility to Cry2Ab of pink bollworm collections made in 2001 in Arizona (5 strains) and Texas (1 strain). LC_{50} and LC_{90} estimates (95% F.L.) were generated using the POLO program (Robertson and Preisler 1992).

Coolidge, Arizona

Concentration µg/ml		No. of	Mean percent	Mean percent	Corrected	LD50***	LD90***
Modified	Original*	Neonates	survival**	mortality	mortality	µg/ml	µg/ml
0	0	170	96.4	3.53			
0.022	0.022 (0.01)	100	93.6	6.36	2.34	2.15	4.79
.0.22	0.22 (0.1)	180	94.4	5.56	1.50	(1.8-2.4)	(3.9-6.6)
2.2	1.0 (2.228)	170	45.2	54.7	52.7		
7.0	3.2 (7.039)	110	2.73	97.2	97.1		

Marana, Arizona

Concentration µg/ml		No. of	Mean percent	Mean percent	Corrected	LD50	LD90
Modified	Original	Neonates	survival	mortality	mortality	µg/ml	µg/ml
0	0	140	95.0	5.00			
0.022	0.010	140	90.0	10.0	5.26	0.524	1.29
.0.22	0.10	180	82.2	17.7	12.4	(.42-.66)	(.99-1.8)
2.2	1.0	160	1.87	98.1	98.0		

Mohave, Arizona

Concentration µg/ml		No. of	Mean percent	Mean percent	Corrected	LD50	LD90
Modified	Original	Neonates	survival	mortality	mortality	µg/ml	µg/ml
0	0	150	88.7	11.3			
0.022	0.010	120	71.3	28.7	19.6	0.230	0.332
.0.22	0.10	170	49.4	50.6	44.3		
2.2	1.0	180	0.00	100	100		

Paloma Ranch, Arizona

Concentration µg/ml		No. of	Mean percent	Mean percent	Corrected	LD50	LD90
Modified	Original	Neonates	survival	mortality	mortality	µg/ml	µg/ml
0	0	140	82.9	17.1			
0.022	0.010	150	70.0	30.0	15.5	0.222	0.327
.0.22	0.10	160	38.8	61.3	46.8		
2.2	1.0	150	0.00	100	100		

Safford, Arizona

Concentration µg/ml		No. of	Mean percent	Mean percent	Corrected	LD50	LD90
Modified	Original	Neonates	survival	mortality	mortality	µg/ml	µg/ml
0	0	110	95.5	4.55			
0.022	0.010	110	97.3	2.73	0.00	1.37	5.77
.0.22	0.10	100	95.0	5.00	0.480		
2.2	1.0	150	7.33	92.7	92.3		

Tornillo, Texas

Concentration µg/ml		No. of	Mean percent	Mean percent	Corrected	LD50	LD90
Modified	Original	Neonates	survival	mortality	mortality	µg/ml	µg/ml
0	0	130	96.2	3.85			
0.022	0.010	150	96.0	4.00	0.160		
.0.22	0.10	160	93.8	6.25	2.50	4.57	19.6
2.2	1.0	140	65.7	34.3	31.7	(3.6-5.5)	(14-33)
7.0	3.2	150	42.0	58.0	56.3		
22	10	80	1.25	98.8	98.7		

*Original estimate of concentration of Cry2Ab in the corn leaf powder was 2.7 mg Cry2Ab/g dry weight. A second analysis by Monsanto yielded the modified estimate of 6.014 mg/g.

**Survivors were recorded as larvae that developed to ≥ 4th instar

***95% Confidence intervals for LC50 and LC90 are given in parenthesis for those instances in which they could be derived from probit analysis. No confidence intervals are provided for data sets that had high degrees of departure from the probit model (Robertson and Preisler 1992).

Table 3. Susceptibility to Cry2Ab of pink bollworm collections made in 2002 in Arizona (9 strains), California (3 strains), New Mexico (1 strain), and Texas (1 strain). LC_{50} and LC_{90} estimates (95% F.L.) were generated using the POLO program (Robertson and Preisler 1992).

2002 Arizona populations

02-26 Tacna

Concentration (µg/ml)	Total No. of Neonates	Mean percent Survival*	Mean percent mortality	Corrected mortality	LC_{50}** µg/ml	LC_{90}** µg/ml
0	110	82.7	17.2		0.0920	0.549
0.022	100	76.0	24.0	8.13		
0.221	110	24.5	74.5	70.3		
2.228	130	0.00	100	100		

02-27 Somerton

Concentration (µg/ml)	Total No. of Neonates	Mean percent survival	Mean percent mortality	Corrected mortality	LC_{50} µg/ml	LC_{90} µg/ml
0	100	81.0	19.0		0.183	0.257
0.022	110	86.3	13.6	0.00		
0.221	100	20.0	80.0	75.3		
2.228	140	0.00	100	100		

02-29 Coolidge

Concentration (µg/ml)	Total No. of Neonates	Mean percent survival	Mean percent mortality	Corrected mortality	LC_{50} µg/ml	LC_{90} µg/ml
0	100	90.0	10.0		0.224	0.322
0.022	100	93.0	7.00	0.00		
0.221	110	47.2	52.7	47.4		
2.228	130	0.00	100	100		

02-30 Magma

Concentration (µg/ml)	Total No. of Neonates	Mean percent survival	Mean percent mortality	Corrected mortality	LC_{50} µg/ml	LC_{90} µg/ml
0	100	94.0	6.00		0.238	0.342
0.022	100	92.0	8.00	2.13		
0.221	100	56.0	44.0	40.4		
2.228	130	0.00	100	100		

02-31 Maricopa

Concentration (µg/ml)	Total No. of Neonates	Mean percent survival	Mean percent mortality	Corrected mortality	LC_{50} µg/ml	LC_{90} µg/ml
0	100	89.0	11.0		0.201	0.297
0.022	100	89.0	11.0	0.00		
0.221	130	33.8	66.1	61.9		
2.228	180	0.00	100	100		

02-32 Goodyear

Concentration (µg/ml)	Total No. of Neonates	Mean percent survival	Mean percent mortality	Corrected mortality	LC_{50} µg/ml	LC_{90} µg/ml
0	100	92.0	8.00		0.102	0.268
0.022	100	90.0	10.0	2.17		
0.221	100	17.0	83.0	81.5		
2.228	130	0.00	100	100		

02-36 Marana Ag Center

Concentration (µg/ml)	Total No. of Neonates	Mean percent survival	Mean percent mortality	Corrected mortality	LC_{50} µg/ml	LC_{90} µg/ml
0	70	91.4	8.57		0.218	0.319
0.022	70	85.7	14.2	6.25		
0.221	70	42.8	57.1	53.1		
2.228	100	0.00	100	100		

02-44 Mohave Valley

Concentration (µg/ml)	Total No. of Neonates	Mean percent survival	Mean percent mortality	Corrected mortality	LC_{50} µg/ml	LC_{90} µg/ml
0	40	87.5	12.5		0.094	0.315
0.022	50	82.0	18.0	6.29		
0.221	50	16.0	84.0	81.7		
2.228	20	0.00	100	100		

02-52 Safford

Concentration (µg/ml)	Total No. of Neonates	Mean percent survival	Mean percent mortality	Corrected mortality	LC_{50} µg/ml	LC_{90} µg/ml
0	50	88.0	12.0		0.084	0.298
0.022	50	80.0	20.0	9.09		
0.221	110	14.5	85.4	83.4		
2.228	100	0.00	100	100		

2002 California population
Blythe/Palo Verde, Site 1

Concentration (µg/ml)	Total No. of Neonates	Mean percent survival	Mean percent mortality	Corrected mortality	LC_{50} µg/ml	LC_{90} µg/ml
0	140	89.2	10.7		0.201	0.353
0.022	140	87.1	12.8	2.40		
0.221	140	36.4	63.5	59.2		
2.228	150	0.00	100	100		

02 Blythe/Palo Verde, Site 3

Concentration (µg/ml)	Total No. of Neonates	Mean percent survival	Mean percent mortality	Corrected mortality	LC_{50} µg/ml	LC_{90} µg/ml
0	60	85.0	15.0		0.31	1.114
0.022	60	86.6	13.3	0.00		
0.221	60	65.0	35.0	23.5		
2.228	70	1.43	98.5	98.1		

02 Imperial Valley, Site 2

Concentration (µg/ml)	Total No. of Neonates	Mean percent survival	Mean percent mortality	Corrected mortality	LC_{50} µg/ml	LC_{90} µg/ml
0	80	92.5	7.50		0.187	0.756
0.022	90	92.2	7.78	0.300		
0.221	100	40.0	60.0	56.7		
2.228	160	1.25	98.7	98.4		

2002 New Mexico population
02-34 Las Cruces

Concentration (µg/ml)	Total No. of Neonates	Mean percent survival	Mean percent mortality	Corrected mortality	LC_{50} µg/ml	LC_{90} µg/ml
0	90	96.6	3.33		0.723	4.033
0.022	100	94.0	6.00	2.76		
0.221	100	78.0	22.0	19.3		
2.228	100	19.0	81.0	80.3		

2002 Texas population
02-53 Esperanza

Concentration (µg/ml)	Total No. of Neonates	Mean percent survival	Mean percent mortality	Corrected mortality	LC_{50} µg/ml	LC_{90} µg/ml
0	100	92.0	8.00		0.227	0.331
0.022	130	90.0	10.0	2.04		
0.221	100	49.0	51.0	43.8		
2.228	120	100	100	100		

*Survivors were recorded as larvae that developed to ≥ 4th instar

**95% Confidence intervals for LC50 and LC90 are given in parenthesis for those instances in which they could be derived from probit analysis. No confidence intervals are provided for data sets that had high degrees of departure from the probit model (Robertson and Preisler 1992).

Figure 1. Susceptibility to Cry1Ac and Cry2Ab of two laboratory strains of pink bollworm from Arizona. The APHIS strain was maintained in the laboratory for over 3 decades without exposure to insecticides. The AZP-R strain was collected in the field in 1997 and repeatedly exposed to Cry1Ac in the laboratory. Probit lines with LC_{50} and LC_{90} estimates (± 95% F.L.) are provided for all culture/toxin combinations except AZP-R/Cry1Ac. Response of AZP-R to Cry1Ac departed significantly from the probit model and thus is provided as mean corrected mortality observed at the concentrations tested. Mean corrected mortality of AZP-R was 2.16% (SEM 1.44) in bioassays of 100 µg Cry1Ac/ml diet and 29.8% (SEM 5.96) in bioassays of 1000 µg Cr1Ac/ml diet. Concentration response data for all evaluations are provided in Table 1.

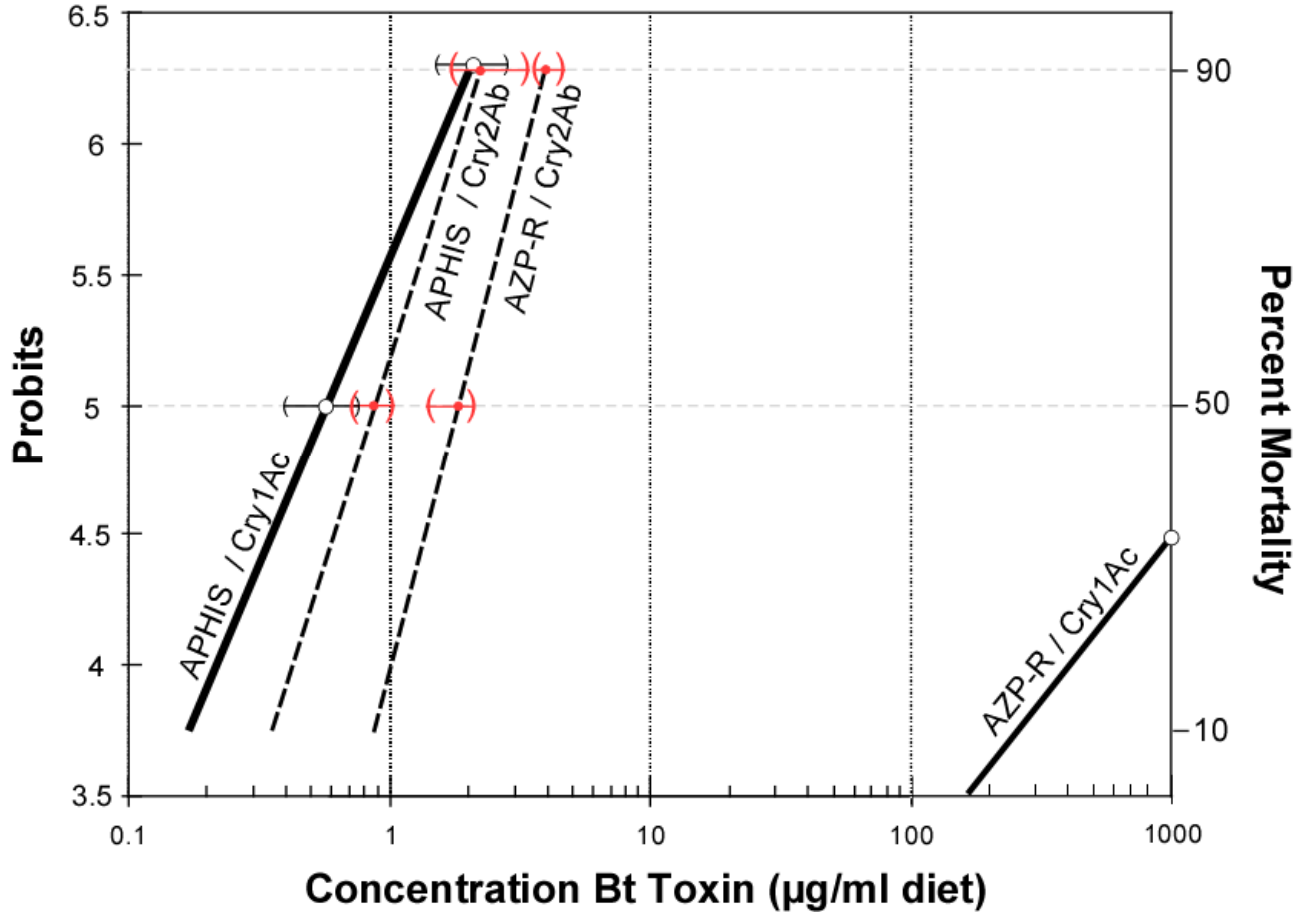

Figure 2. Susceptibility to Cry2Ab of pink bollworm collections made in 2001 in Arizona (5 strains) and Texas (1 strain). Probit lines with LC_{50} and LC_{90} estimates (95% F.L.) were generated using the POLO program (Robertson and Preisler 1992). Responses indicate that concentrations of 1.0 and 10 µg/ml would be suitable for routine monitoring of susceptibility to Cry2Ab. Concentration response results and probit analysis estimates for all evaluations are provided in Table 2.

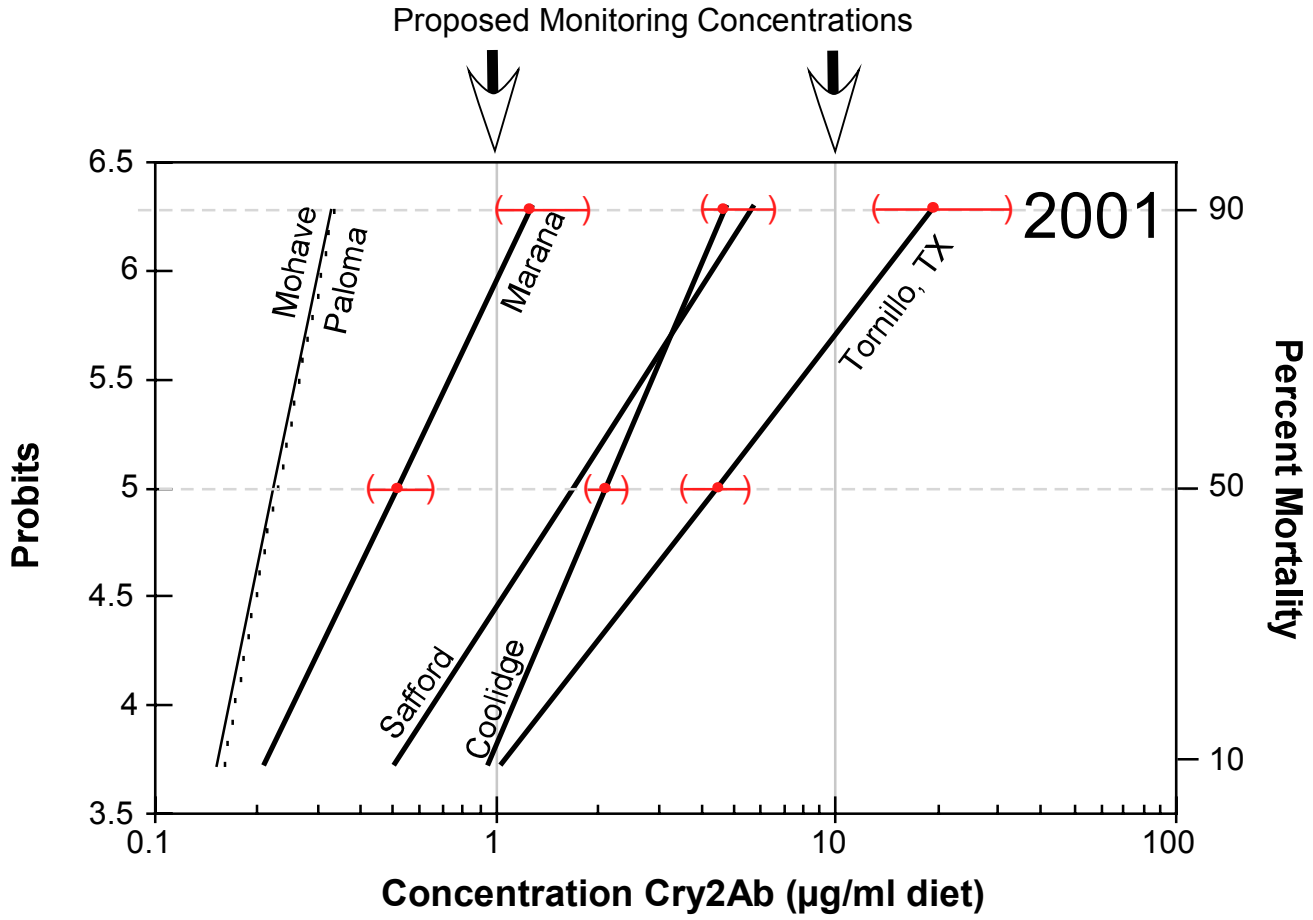

Figure 3. Susceptibility to Cry2Ab of pink bollworm collections made in 2002 in Arizona (9 strains), California (3 strains), New Mexico (1 strain), and Texas (1 strain). Probit lines with LC_{50} and LC_{90} estimates (95% F.L.) were generated using the POLO program (Robertson and Preisler 1992). 2002 results confirmed the conclusion from 2001 monitoring that concentrations of 1.0 and 10 µg Cry2Ab/ml are suitable for routine monitoring of susceptibility to Cry2Ab. Concentration response results and probit analysis estimates for all evaluations are provided in Table 3.