The University of Arizona • Extension Arthropod Resistance Management Laboratory

SUSCEPTIBILITY OF SOUTHWESTERN PINK BOLLWORM TO *Bt* TOXINS CRY1AC AND CRY2Ab2:

FINAL RESULTS OF 2003 SEASON STUDIES

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Summary

Monitoring of Arizona pink bollworm (PBW), Pectinophora gossypiella, susceptibility to Bt toxin Cry1Ac was conducted annually from 1997-2003. Similar studies with Cry2Ab2 were conducted with collections from 2001-2003. Larvae were collected from cotton fields located throughout the Southwest, cultured in the laboratory, and tested for susceptibility to Cry toxins using diet-incorporation bioassays. The total collections made in 2003 and successfully reared and bioassayed for susceptibility to Cry1Ac were: 16 from Arizona, five from California, two from Texas and one from New Mexico. Susceptibility to Cry2Ab2 was estimated in 12 of these same strains from Arizona, four from California, two from Texas and one from New Mexico.

In 1997, laboratory selection of pink bollworm collected in Arizona and exposed to Cry1Ac in diet produced a strain capable of survival on Bollgard cotton. Subsequent studies showed that 10 μg Cry1Ac/ml of insect diet was a reliable diagnostic concentration for detection of pink bollworm homozygous for resistance to Cry1Ac. Survivors of 10 μg Cry1Ac/ml were detected in 2003 in five Arizona strains and one California strain at frequencies ranging from 0.32 to 3.1% of subjects tested. The grand mean frequency of PBW survival of 10 μg Cry1Ac/ml in 2003 collections was: Arizona 0.24% (range 0.0-1.5%) and California 0.73% (range 0.0-3.1%). However, bioassays of a susceptible culture, used each year as an internal control, showed that the potency of the Cry1Ac stock used to test 2003 collections had declined slightly and was resulting in 97.7%, rather than 100% mortality of this strain. No survivors of 10 μg Cry1Ac/ml were detected in the New Mexico and Texas collections.

Survivors of 10 μg Cry1Ac/ml bioassays were significantly more abundant in Arizona from 2001 to 2003 than they were in 1998 and 1999. However, the frequency of resistant survivors in bioassays was low in 2001-2003, and markedly lower than in 1997. Moreover, molecular tests to detect mutations in a cadherin-encoding gene previously shown to confer strong resistance to Cry1Ac, confirmed the presence of resistance alleles only in one strain in 2003, the Imperial Valley Site 2 strain.

Based on contrasts with baseline data collected in 2001 and 2002, all 19 pink bollworm strains evaluated were highly susceptible to Cry2Ab2. Mean mortality ranged from 84.7 to 100% and from 98.1 to 100% in bioassays of 1.0 μg and 10 μg/ml Cry2Ab2/ml, respectively.

Field evaluations of efficacy of Bt cotton were conducted by the Arizona Cotton Research and Protection Council in adjacent pairs of Bt and non-Bt fields at 43 Arizona locations. Statewide, large pink bollworm larvae were found in an average of 29.0% (range 0 to 100%) of non-Bt bolls sampled from borders of refuge fields. Bolls from adjacent Bt cotton fields yielded an average of 0.208% (range 0 to 1.40%) bolls infested with large larvae. Of 17 infested bolls collected from Bt cotton fields, all but two tested negative for Cry1Ac.

We conclude from these findings that there was no indication that pink bollworm resistance to Cry1Ac was a problem at the locations sampled in 2003 and Bt cotton continued to exhibit exceptional field performance in Arizona. Furthermore, field populations yielded no indications of imminent problems with resistance to Cry2Ab2.

Introduction

Registration of Bt cotton in the US in 1996 marked the beginning of a major change in pest management in Arizona cotton. Pink bollworm (*Pectinophora gossypiella*), one of the most economically damaging pests of Arizona cotton, is highly susceptible to the toxin produced by Bt cotton, Cry1Ac. Producer gains from use of Bt cotton in Arizona averaging $15,000 per farm (Frisvold et al. 2000) have promoted rapid adoption of this technology. Additionally, the environment and integrated pest management have benefited from decreased use of conventional insecticides associated with adoption of Bt cotton. In 1995, the year preceding registration of Bt cotton, an average of over six insecticide applications were made per acre of cotton in Arizona (Sims et al. 2001). Insecticide use in Arizona cotton declined strikingly since 1995, reaching a low of less than two treatments per acre in 2000. These dramatic reductions are attributable in large part to the combined effects of Bt cotton used to control pink bollworm and to improved management of whiteflies with insect growth regulators (Dennehy et al. 2002, Naranjo et al. 2003).

Loss of target pest susceptibility as a result of resistance is anticipated to be the greatest biological limitation of transgenic insecticidal crops (Mellon and Rissler 1998). This is due foremost to the many months each year that pests are exposed to toxins in plants. Resistance seemed all the more probable in Arizona cotton following the successful selection in the laboratory of high levels of resistance of pink bollworm to the Bt toxin in Bollgard® cotton, Cry1Ac (Bartlett 1995, Simmons et al. 1998, Patin et al. 1999, Liu et al. 1999, Tabashnik et al. 2000, Sims et al. 2001).

The > 3000-fold resistance to Cry1Ac selected in the AZP-R strain of pink bollworm was shown to be conferred by one or few major autosomal genes (Tabashnik et al. 2002). Homozygous susceptible and F_1 heterozygote individuals were killed by bioassays of 10 µg Cry1Ac/ml diet. Thus, 10 µg/ml provided a reliable diagnostic concentration for monitoring resistant pink bollworm. The frequency of pink bollworm resistance to Cry1Ac was unexpectedly high in 1997 collections but declined to undetectable levels by 1999 and 2000 (Patin et al. 1999, Tabashnik et al. 2000).

Herein, we report the 2003 season results of Arizona's multi-agency collaboration to monitor resistance of pink bollworm to Bt toxins using laboratory-based bioassays, and complementary evaluations of the field performance of Bt cotton.

Materials and Methods

Susceptibility of Arizona PBW to the Bt Endotoxins, Cry1Ac and Cry2Ab2

Collections. Collections from Arizona cotton fields commenced in August and continued through December, 2003. They were made from 16 sites in Arizona, five in California, and two in Texas, and one in New Mexico (Table 1). The goal was to establish cultures with ≥ 200 PBW from each site. At each location 300 to 2,000 bolls were collected, mainly from non-Bt cotton fields in areas adjacent to Bt 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).

Boll boxes suspended infested bolls on wire racks approximately three cm above sheets of paper toweling on the bottom of the boxes. Fourth instar larvae cut out of infested bolls and dropped onto the paper toweling. Larvae were transferred to pupation boxes consisting of tightly sealed, 1.7 liter rectangular Rubbermaid® containers enclosing sheets of paper toweling. For cultures from which fewer than 200 larvae were obtained from boll boxes, bolls were manually opened to collect additional PBW. To prevent or disrupt diapause, larvae that had cut out of bolls and webbed up were disturbed, one to five times per week, by pulling the paper toweling apart and spraying it lightly with water.

Rearing. We reared PBW using a modified version of the method of Bartlett and Wolf (1985). Offspring of field-collected PBW were reared singly or in pairs in one ounce cups containing approximately five g diet each. Bioassays of susceptibility to Cry1Ac were conducted from November to April, 2004, on the $F_{2\text{-}8}$ generations. Bioassays with Cry2Ab2 were then done on the $F_{5\text{-}7}$ generations during the months of April to August, 2004.

Bioassaying PBW Susceptibility to Cry1Ac. Susceptibility of each collection of pink bollworm (Table 1) to Cry1Ac was determined using 21-day diet-incorporation bioassays (Patin et al. 1999). 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 final concentrations of 0 (control), 1.0, and 10 µg

Cry1Ac/ml diet solution. Diet was made in two four-liter batches, subdivided by weight into beakers, and held in water baths at 50-60°C, after which toxin and food coloring were blended thoroughly into the liquid diet. Food coloring was added to ensure thorough mixing of toxin in the diet. Diet was allowed to cool to room temperature and was then refrigerated at 6-8 °C for 48-72 h, after which it was cubed using a commercial cheese slicer. The cubed diet was sealed in plastic bags, and returned to the refrigerator. Approximately five g of diet per cup was dispensed into one ounce medicine cups with tight fitting lids. Diet was used in bioassays within four weeks.

Neonate larvae were placed individually in the one ounce cups and the tops were affixed. Subjects were assigned to replicates consisting of 10-25 bioassay cups for each concentration. Bioassay cups were placed in plastic trays and incubated in darkness at 29±1 °C for 21 days, after which mortality and developmental stage of survivors (Watson and Johnson 1974) were recorded. Subjects developing to $\geq 4^{th}$ instar were scored as alive. Cups in which 4^{th} instar larvae had exited by chewing out of the plastic were scored as alive if: 1) they contained frass of the size produced by a 4^{th} instar; 2) the exit hole was the size produced by a 4^{th} instar; and 3) the cup contained evidence of feeding consistent with development to 4^{th} instar. Corrected mortality was computed using Abbott's formula (Abbott 1925).

For each population, our goal was to complete a minimum of four replications of 10 larvae each tested in control bioassays containing no toxin, an equivalent number of bioassays containing 1.0 µg Cry1Ac, and nine replicates of 10 larvae each tested in bioassays containing 10 µg Cry1Ac. Bioassays normally commenced in the F_2 generation and, if necessary to complete the desired number of replicates, continued through the F_7 generation, contingent on the numbers of eggs produced per generation. Results obtained from each population were pooled to obtain a single estimate of mortality for each concentration. The totals of subjects bioassayed were 4619 from Arizona, 1905 from California, and 325 and 460 from New Mexico and Texas, respectively. This comprised a range of 70 to 220 larvae tested per concentration for each collection.

Bioassaying Susceptibility to Cry2Ab2. Nineteen field strains were evaluated for susceptibility to Cry2Ab2. Bioassays were conducted using the 21-day diet-incorporation method described above. The source of toxin was freeze-dried corn leaf powder containing Cry2Ab2, produced by Monsanto in St. Louis, MO. It was estimated by Monsanto to contain 6.014 mg Cry2Ab2 toxin/g of leaf powder.

For each population, our goal was to complete a minimum of 5 replications of 10 larvae each for control groups, and 10 replicates of 10 larvae each for the concentrations of 1.0 and 10 µg/ml Cry2Ab2. Bioassays normally commenced by the F_5 generation and, if necessary to complete the desired number of replicates, were continued through the F_8 generation, contingent on the numbers of eggs produced per generation. Results obtained from each population were pooled to obtain a single estimate of mortality for each concentration. The total subjects bioassayed was 3102 from Arizona, 1100 from California, and 270 and 530 from New Mexico and Texas, respectively. This comprised a range of 12 to 210 larvae tested per concentration for each collection.

APHIS Laboratory Reference Strain. The Cry1Ac-susceptible laboratory strain, APHIS, has been test each year since 1998 to serve as an internal standard for our bioassays (Table 3). This has facilitated contrasts of susceptibility estimates between years. APHIS has been maintained in the laboratory for at least 30 years without exposure to pesticides. Prior to 1996, field collected pink bollworm were periodically added to the strain. Surprisingly, APHIS has had consistently lower susceptibility to Cry toxins than most field populations. It is, however, devoid of major mutations conferring resistance to the Cry toxins we have tested.

Field Efficacy of Bollgard Cotton in Arizona

These studies were conducted by the Arizona Cotton Research and Protection Council, based in Phoenix, Arizona. Thirty-nine pairs of adjacent fields of Bt and non-Bt cotton fields were sampled throughout Arizona. Fields were grower-managed. From August to November, 2003, depending on pink bollworm population buildup and harvest date, each pair of Bt and non-Bt fields was sampled twice, as close as practical to the date of harvest. On each sampling date, 150 bolls were collected from the non-Bt (refuge) field and 500 bolls were sampled from the adjacent Bt field of each pair, yielding total boll numbers of 300 and 1000 for the non-Bt and Bt fields, respectively. Boll collections were made within 50 meters of the common edges of each pair of fields. No more than one boll was taken from any plant.

Boll samples were labeled, transported to ACRPC field offices, and placed in boll boxes (17.6 cm x 50.4 cm x 35.2 cm) in groups of 50 per box. Boll boxes suspended infested bolls on wire racks approximately three cm above sheets of paper toweling on the bottom of the boxes. Two to three weeks after making collection, bolls were opened to record numbers of larvae $\geq 3^{rd}$ instar and pupae within. Additionally, counts were made of 4^{th} instar larvae, pupae and adults that had exited bolls in the boxes. Because non-Bt bolls often had very high infestation rates, a variable sample size was used. When a

single box of 50 bolls yielded eight or more individuals of ≥ 3rd instar, the other two boxes from that sample of 150 bolls was not evaluated. ANOVA was used to detect differences in mean survival of PBW between sites and years.

When possible, bolls from Bt fields in which PBW were found to have survived to ≥ 4th instar were tested for the presence of Cry1Ac toxin. Two or three seeds of such bolls were tested individually using the ImmunoStrip test system (Agdia, Elkhart, IN). Bolls were then designated as a) positive for Cry1Ac, b) negative for Cry1Ac, or c) mosaic (containing seeds testing positive and negative) for Cry1Ac. Heavily damaged bolls often could not be tested because of insufficient seed material. Archived samples of Bt and non-Bt cotton seeds served as internal controls for these evaluations.

Results and Discussion

Interpreting PBW Bioassay Data

Pink bollworm that survive 10 µg/ml discriminating concentration bioassays of Cry1Ac are homozygous for the major Mendelian factor that confers resistant to Cry1Ac. This conclusion is based on over seven years of investigations in Arizona. Susceptible field strains (Patin et al. 1999, Tabashnik et al. 2000) as well as susceptible laboratory strains (Table 3) had no survivors of 10 µg/ml Cry1Ac bioassays.

Laboratory selection of pink bollworm collected in Arizona in 1997, and that survived 10 µg/ml bioassays, yielded a strain (AZP-R) with strong resistance to Cry1Ac (Simmons et al. 1998). Tabashnik et al. (2000) computed the frequency of resistance in Arizona field populations based of survival of 10 µg/ml bioassays. Greenhouse evaluations showed that the resistant AZP-R strain had 46% survival on Bt cotton, relative to survival on non-Bt cotton (Liu et al. 2001). Morin et al. (2003) showed that resistance to Cry1Ac in bioassays, and survival on transgenic Bt cotton in greenhouse experiments of laboratory-selected pink bollworm from Arizona and Texas were linked with the presence of three mutant alleles of a cadherin-encoding gene. Larvae with two of these resistance alleles in any combination were resistant, whereas those with one or none were susceptible to Cry1Ac.

APHIS Laboratory Reference Strain. This strain has served as an internal control for our bioassay method since we began the monitoring program in 1997. Prior to the testing of 2003 strains (conducted in 2004), bioassays of 10 µg Cry1Ac/ml resulted in 100% mortality of this susceptible strain (Table 3). In 2004, during the time when 2003 strains were being tested, bioassays of APHIS with 10 µg/ml Cry1Ac yielded 97.7% mean mortality. These results provided a plausible explanation for our finding in 2003 collections of a small number of survivors of 10 µg/ml Cry1Ac (Figure 2) that did not possess major resistance alleles (see below). We believe this to be indication that the stock solution of MVPII used in 2004 had slightly reduced potency. It has been replaced with fresh material provided by DowAgrosciences.

Susceptibility of Arizona PBW to Bt Endotoxin, Cry1Ac

Arizona Collections--2003. Grand mean mortality of 16 Arizona strains of pink bollworm was 12.5%, 72.3% and 99.8% at concentrations of 0, 1.0 and 10 µg/ml, respectively (Table 2). The lowest mortality observed in discriminating concentration bioassays of 10 µg/ml was 98.6% (corrected = 98.5%); this was observed with the collection from Safford/Thatcher, Arizona. Corrected grand mean mortality for 2003 collections was 68.3% at 1.0 µg/ml and 99.8% at 10 µg/ml. Thus, when corrected for control mortality, an average of 31.6% of individuals bioassayed in 2002 collections survived exposure to 1.0 µg/ml and 0.236% survived 10 µg/ml bioassays.

Change in AZ Collections 1997-03. Figure 1 shows change in corrected survivorship in bioassays of 1.0 and 10 µg/ml Cry1Ac from 1997 to 2003. We detected survivors of 10 µg/ml bioassays of PBW strains collected in Arizona in 2001, 2002, and 2003, but at much lower frequencies than observed in 1997. We previously reported that Arizona pink bollworm were significantly less susceptible to Cry1Ac in 1997 than 1998 (P=0.031, F=5.36, df=1), 1999 (P=0.015, F=6.95, df=1) or 2000 (P=0.007, F=8.52, df=1) in bioassays of 10 µg Cry1Ac/ml (Dennehy et al. 2003). Mean mortality (corrected) in bioassays of 10 µg/ml increased from 94.1% in 1997 to 99.9%, 100%, and 100% in 1998, 1999, and 2000, respectively. In the 2001 season, this value dropped to 98.9% corrected mortality. Our 2003 results showed slightly less survivorship in 10 µg/ml bioassays than 2001. However, this change is small and not statistically significant. In conclusion, mean levels of survivorship in discriminating concentration bioassays of Cry1Ac in Arizona pink bollworm remained low in 2003 and were lower than in 1997.

Individuals that survived bioassays of 10 µg Cry1Ac/ml (Figure 2), and developed into viable adults, were crossed with the APHIS strain by the Carrière laboratory. Offspring of successful single-pair lines were bioassayed for resistance in the F_2 generation to establish resistant strains. F_2 offspring were also tested by the Tabashnik laboratory for resistance alleles after amplification with the polymerase chain reaction method and sequencing of known major mutations in a cadherin-encoding

gene. At the time of writing this report, only the survivors of Imperial Valley Site 2 collection have yielded a resistant strain and tested positive for resistance alleles.

CA, NM and TX Collections--2003. Seven of the eight strains evaluated from California, New Mexico, and Texas had no survivors of 10 µg/ml Cry1Ac bioassays (Table 2). The Imperial Valley Site 2 sample from California had 3.14% corrected survival. As indicated above, this strain was subsequently shown to possess the same mutations in a cadherin-encoding gene described from our first isolation of resistance in 1997 collections. We now have this strain in culture and have successfully selected it for higher levels of resistance.

Statewide averages of survivorship in bioassays of 1.0 µg/ml Cry1Ac varied greatly between years from 1997 to 2003. Most notably, it increased from 13.9% in 2002 to 31.6% in 2003 (Figure 1). Tabashnik et al. (2002) showed that pink bollworm resistance to Cry1Ac was recessive at a high concentration of Cry1Ac but the dominance of resistance increased as the concentration of Cry1Ac decreased. Thus, the possibility exists that higher survival of 1.0 µg/ml bioassays reflected increases in pink bollworm that were heterozygous for resistance. However, we doubt that this is the explanation for the increased survival in bioassays of 1.0 µg/ml Cry1Ac in 2003 collections, relative to 2002. The aforementioned reduced potency of our Cry1Ac stock could easily account for this change. Mortality of our susceptible laboratory strain, APHIS, in 1.0 µg/ml bioassays declined by more than 30% from 2003 (when 2002 collections were tested) to 2004 (Table 3).

Susceptibility of Arizona PBW to the Bt Endotoxin, Cry2Ab2

Baseline data from 2001 2002. EARML estimates of baseline susceptibility of pink bollworm to Cry2Ab2 were previously reported to Monsanto, based on six collections in 2001 (Figure 3) and 14 collections in 2002 (Figure 4) made in the Southwestern U.S. Significant differences in susceptibility between strains were found both years, though the range of susceptibility was substantially greater in 2001 than in 2002. The monitoring concentrations of 1.0 and 10 µg/ml Cry2Ab2 reported herein were based on these earlier findings.

2003 Collections. Relative to 2001 and 2002 baselines, all 19 pink bollworm strains evaluated from 2003 were highly susceptible to Cry2Ab2. Mean mortality ranged from 84.7 to 100%, and from 98.3 to 100% in bioassays of 1.0 µg/ml and 10 µg/ml Cry2Ab2/ml, respectively (Table 4). Only four individuals out of a total of 2040 subjects tested survived bioassays of 10 µg/ml Cry2Ab2/ml. These comprised single survivors from collections made in southwestern Arizona (Texas Hill) and the Palo Verde Valley of California (Blythe/Palo Verde, Site 1), and two survivors from Mesquite, New Mexico. Selection of these two strains is underway to evaluate their significance.

Field Efficacy of Bollgard Cotton in Arizona

A total of 8825 non-Bt bolls and 38400 Bt bolls were inspected from a total of 39 pairs of fields in 2003. Non-Bt bolls yielded 2418 pink bollworm. Bolls from Bt fields yielded 81 PBW. Thus, on a statewide basis, non-Bt fields averaged 29.0% infested bolls, while Bt fields averaged 0.208% infested bolls (Figure 5). Infestation rates in individual Bt cotton fields ranged from 0 to 1.40%.

Tests of infested bolls collected from Bt fields, conducted by Arizona Cotton Research and Protection Council personnel, revealed that a large proportion of infested bolls did not express Bt in the seeds. Tests of 17 infested bolls collected from Bt fields in 2003 yielded only two that were positive for toxin. This means that the true frequency of pink bollworm surviving in Bt cotton is substantially lower than the average we report of 0.208%. Non-Bt bolls in Bt fields could result from contamination of non-Bt seed in the seed bag, contamination in the hopper of the planter at the time of planting, or volunteer non-Bt plants originating from seed that remained in the ground from the previous season.

Infestation levels of Bt fields have averaged ≤ 0.300% over the past nine years (Figure 5). This amounts to ≤ three PBW per 1000 bolls. Irrespective of the low level of contamination of Bt fields with non-Bt cotton, the efficacy of Bt cotton against pink bollworm in Arizona is exceptional and has changed little since Bollgard was first commercialized in 1996 (Figure 4).

Conclusions

Extensive monitoring of pink bollworm from throughout the Southwestern US in 2003 confirmed that resistance to Cry1Ac has remained at low or undetectable frequencies in field populations. Bollgard continued to perform remarkably well against pink bollworm at 39 Arizona locations evaluated in 2003. At this time we have no indications whatsoever of imminent problems with pink bollworm resistance to Cry1Ac in Arizona or elsewhere in the Southwest. All 19 populations evaluated for susceptibility to Cry2Ab2 were highly susceptible. Thus, we found no indications of imminent resistance problems in the Southwest as they pertain to Bollgard II.

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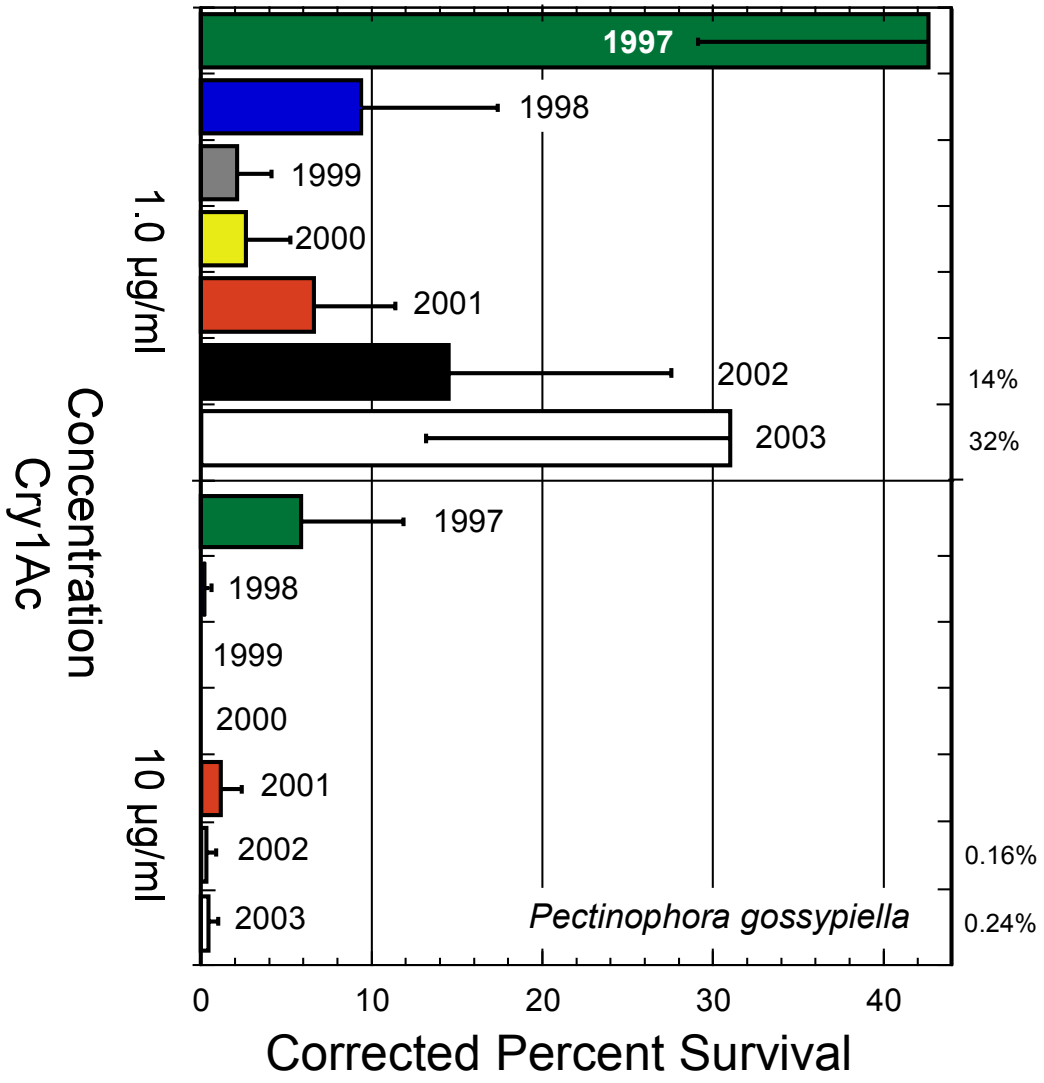

Figure 1. Changes in pink bollworm susceptibility to Cry1Ac in Arizona from 1997 to 2003. Shown are mean values (± standard deviation) of corrected survival observed in replicated bioassays of 1.0 and 10 µg CrylAc/ml diet of field collections made throughout Arizona in 1997 (n=9), 1998 (n=12), 1999 (n=14), 2000 (n=17), 2001 (n=17), 2002 (n=13) and 2003 (n=16). See Table 2 for summary statistics of collections made in Califoria, New Mexico, and Texas.

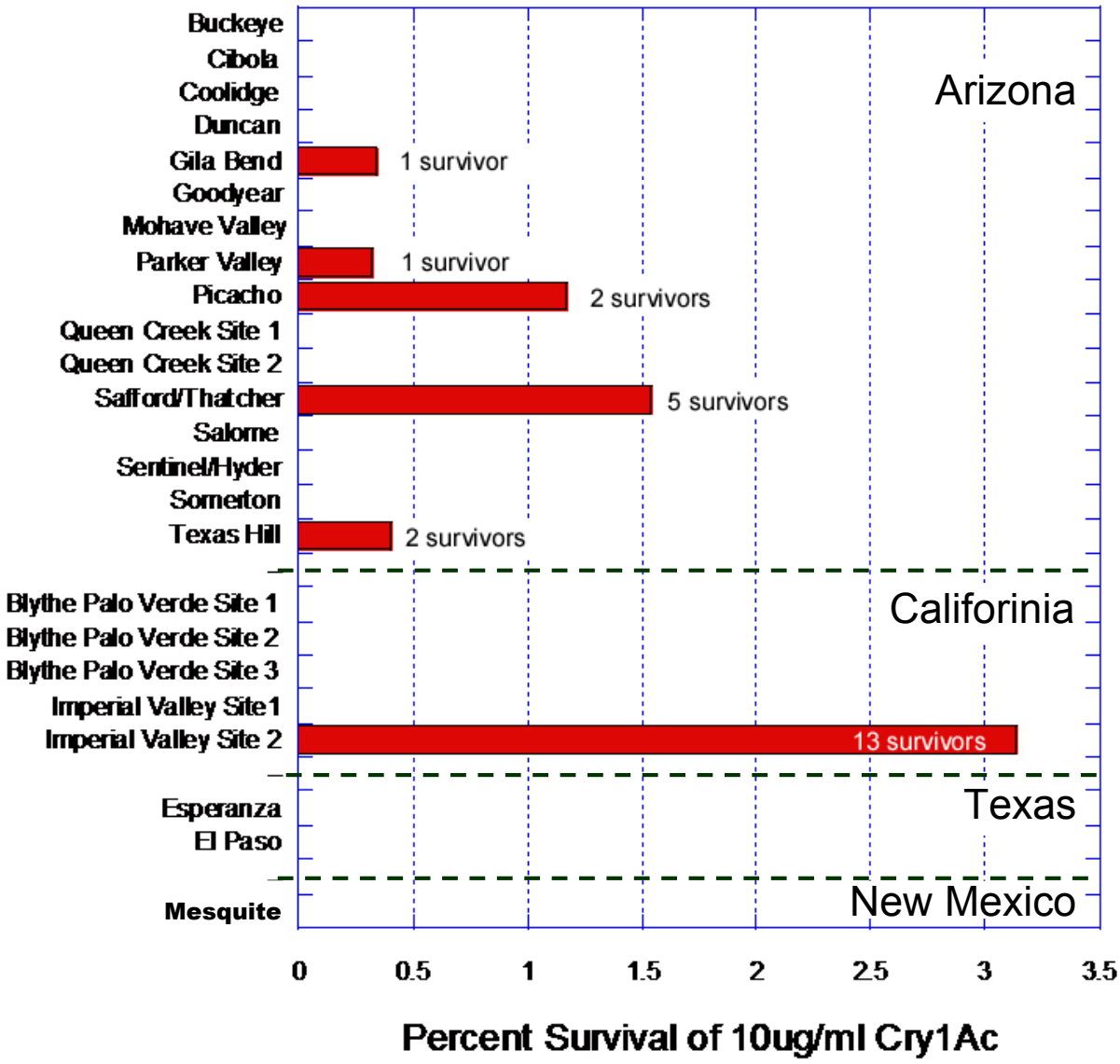

Figure 2. Survival in 2003 of discriminating concentration bioassays of 10 µg Cry1Ac/ml diet. Individuals that developed into viable adults were crossed with the APHIS strain by the Carrière laboratory and offspring of single-pair lines were bioassayed for resistance in the F_2 generation. F_2 offspring were also tested by the Tabashnik laboratory for resistance alleles after amplification with the polymerase chain reaction method and sequencing of known major mutations in a cadherin-encoding gene. At the time of writing this report, only the survivors of Imperial Valley Site 2 collection have yielded a resistant strain and tested positive for resistance alleles.

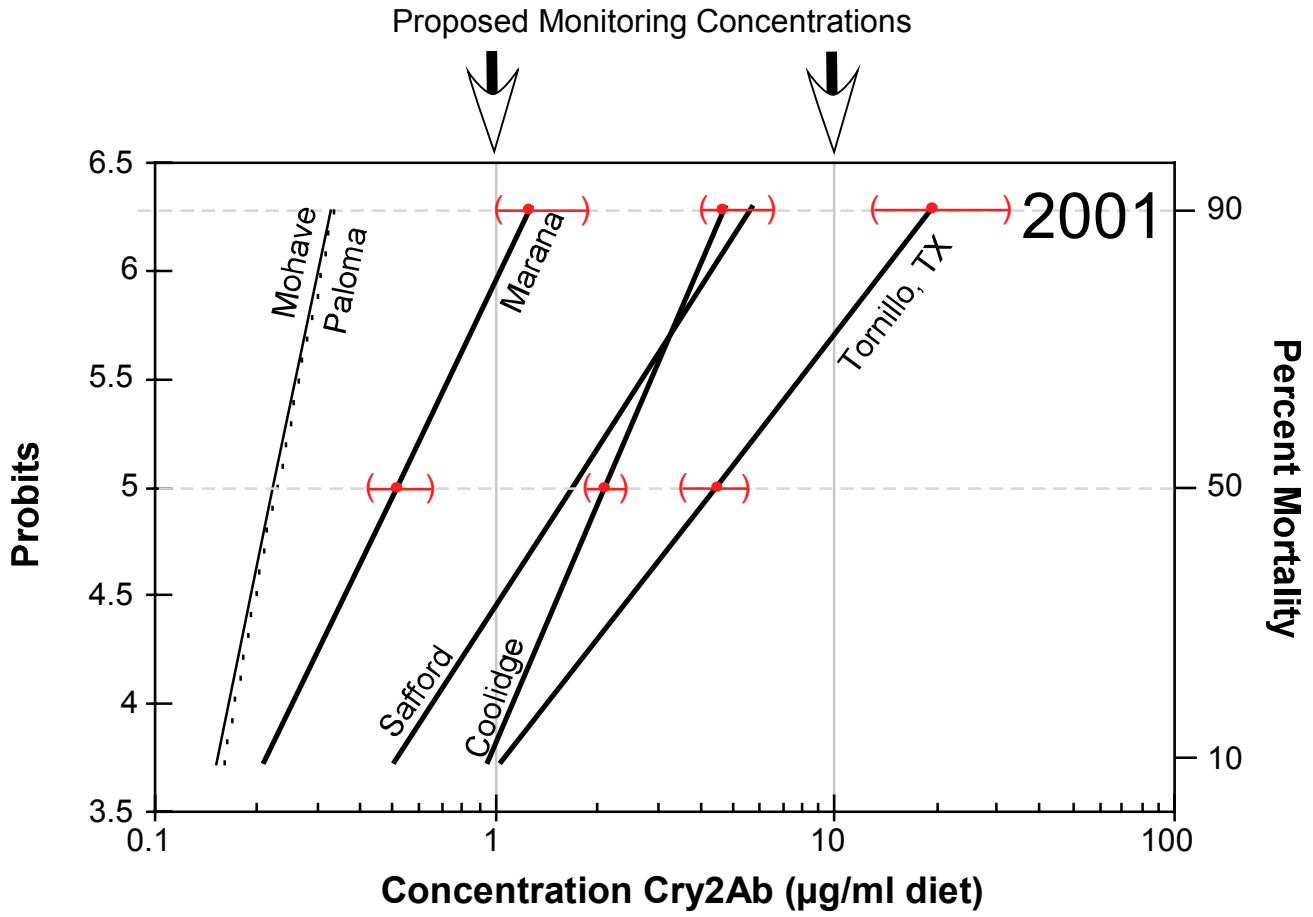

Figure 3. Susceptibility to Cry2Ab2 of pink bollworm collections made in 2001 in Arizona (five strains) and Texas (one strain). Probit lines with LC_{50} and LC_{90} estimates (95% F.L.) were generated using the POLO program (Robertson and Preisler 1992). Responses indicate that concentrations of 1.0 and 10 µg/ml would be suitable for routine monitoring of susceptibility to Cry2Ab2.

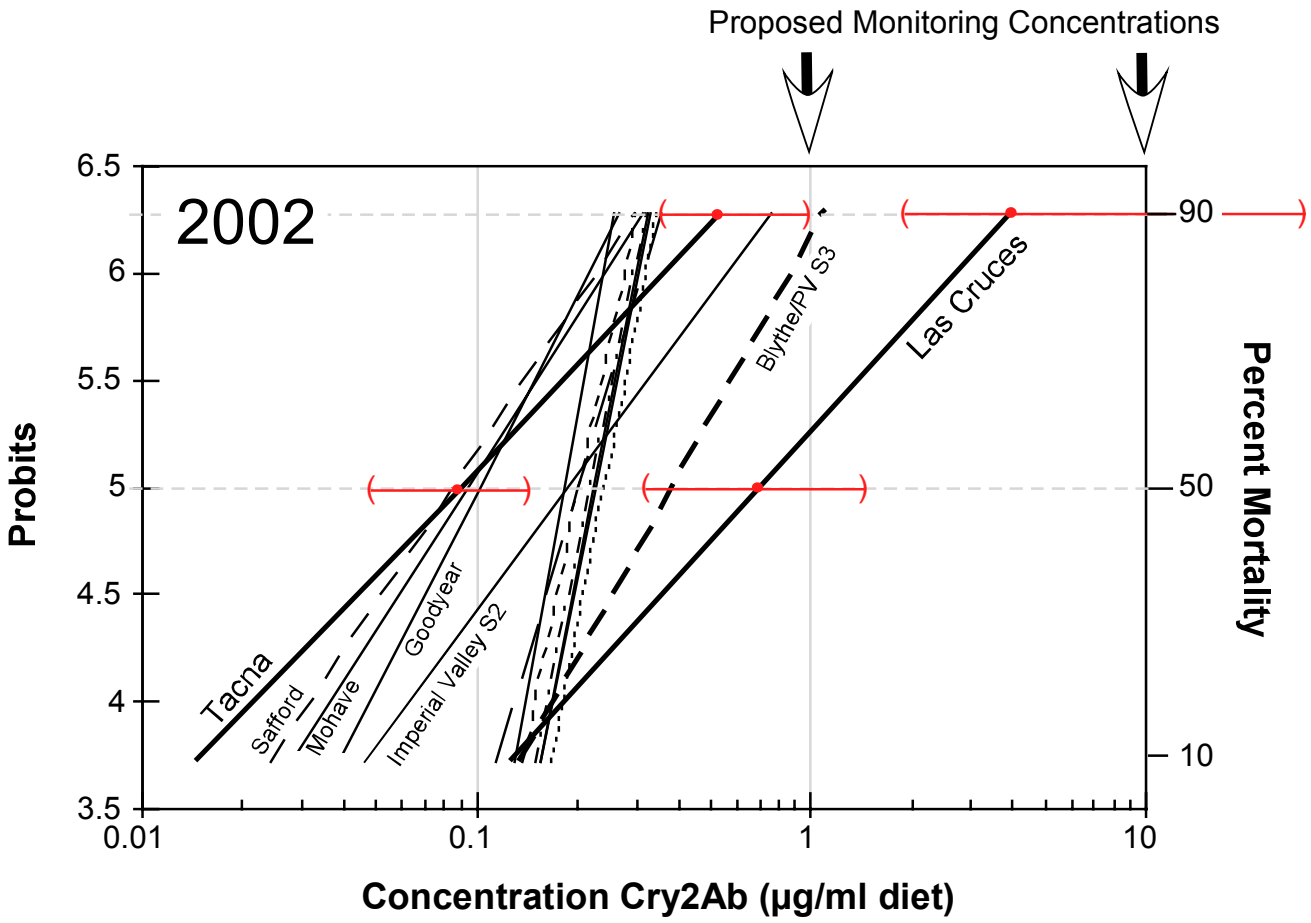

Figure 4. Susceptibility to Cry2Ab2 of pink bollworm collections made in 2002 in Arizona (9 strains), California (3 strains), New Mexico (1 strain), and Texas (1 strain). Probit lines with LC_{50} and LC_{90} estimates (95% F.L.) were generated using the POLO program (Robertson and Preisler 1992).

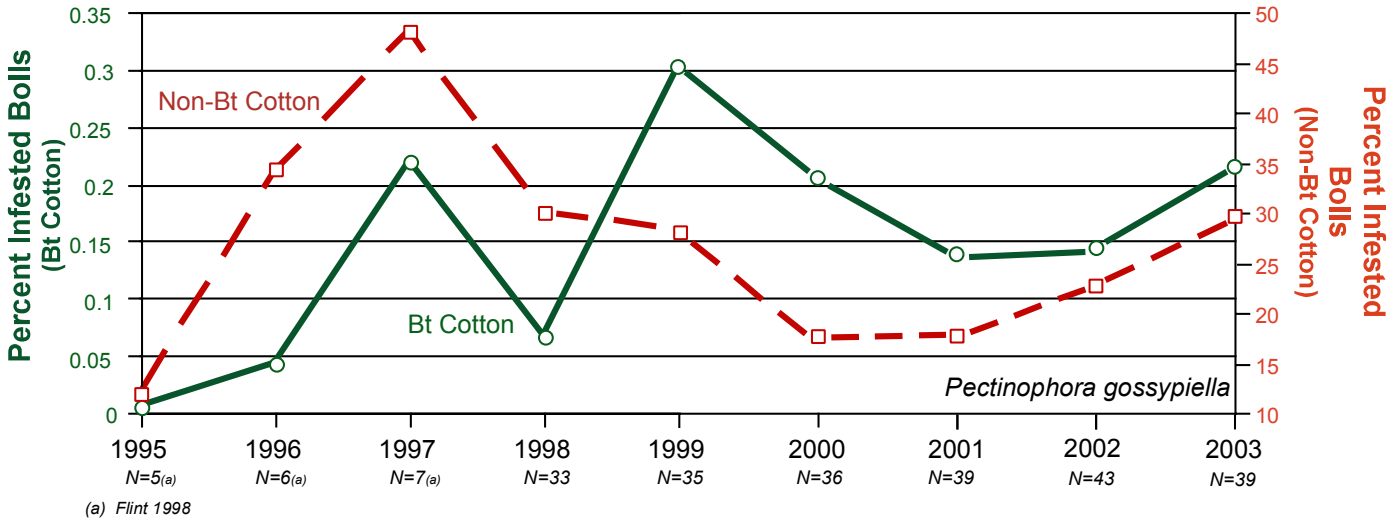

Figure 5. Sustained efficacy of Bt cotton in Arizona: 1995 to 2003. Data from 1995 to 1997 were reported by Flint et al. (1995) and Flint and Park (1996). All other data were collected by the Arizona Cotton Research and Protection Council. Shown are means of percent boll infestation (bolls with ≥ 3rd instar PBW) for pairs of Bt cotton (left axis) and non-Bt cotton fields (right axis) sampled each year from 1995 to 2003. The numbers of pairs of Bt and non-Bt fields (*N*) is indicated for each year.

Table 1. Pink bollworm strains evaluated in 2003.

Location	State	Waypoint	Cry1Ac	Cry2Ab2
Buckeye	AZ	03-20	✓	✓
Cibola	AZ	03-40	✓	
Coolidge	AZ	03-44	✓	✓
Duncan	AZ	03-46	✓	✓
Gila Bend	AZ	03-55	✓	✓
Goodyear	AZ	03-48	✓	✓
Mohave Valley	AZ	03-26	✓	✓
Parker Valley	AZ	03-27	✓	✓
Picacho	AZ	03-41	✓	
Queen Creek, Site 1	AZ	03-22	✓	
Queen Creek, Site 2	AZ	03-43	✓	✓
Safford/Thatcher	AZ	03-01	✓	✓
Salome	AZ	03-42	✓	✓
Sentinel/Hyder	AZ	03-54	✓	
Somerton	AZ	03-28	✓	✓
Texas Hill	AZ	03-29	✓	✓
Blythe Palo Verde, Site 1	CA	03-34	✓	✓
Blythe Palo Verde, Site 2	CA	03-35	✓	
Blythe Palo Verde, Site 3	CA	03-36	✓	✓
Imperial Valley, Site 1	CA	03-37	✓	✓
Imperial Valley, Site 2	CA	03-39	✓	✓
Esperanza	TX	03-51	✓	✓
El Paso	TX	03-52	✓	✓
Mesquite	NM	03-32	✓	✓
APHIS, laboratory strain	lab		✓	✓

Table 2. Susceptibility to Cry1Ac of pink bollworm collections made in 2003.

ARIZONA

All 2003 Arizona Collections

Cry1Ac

	----------Sums of All Assays----------								--------Grand Means--------			
Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	69	7	210	230	4	90	610	534	87.54%	12.46%		
1	262	179	137	7	0	25	610	169	27.70%	72.30%	68.35%	31.65%
10	5042	275	9	0	0	2	5328	11	0.21%	99.79%	99.76%	0.236%

Total Individuals Tested 6548

1 03-01-I Safford/Thatcher

Cry1Ac

Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	4	0	17	11	0	8	40	36	90.00%	10.00%		
1	11	19	8	0	0	2	40	10	25.00%	75.00%	72.22%	27.78%
10	334	21	5	0	0	0	360	5	1.39%	98.61%	98.46%	1.54%

2 03-20 Buckeye

Cry1Ac

Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	2	0	8	20	0	10	40	38	95.00%	5.00%		
1	7	11	20	0	0	2	40	22	55.00%	45.00%	42.11%	57.89%
10	331	29	0	0	0	0	360	0	0.00%	100.00%	100.00%	0.00%

3 03-22-I Queen Creek Site 1

Cry1Ac

Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	6	0	8	17	2	7	40	34	85.00%	15.00%		
1	23	7	9	0	0	1	40	10	25.00%	75.00%	70.59%	29.41%
10	336	24	0	0	0	0	360	0	0.00%	100.00%	100.00%	0.00%

4 03-26 Mohave Valley

Cry1Ac

Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	4	1	14	15	0	6	40	35	87.50%	12.50%		
1	14	18	7	0	0	1	40	8	20.00%	80.00%	77.14%	22.86%
10	353	7	0	0	0	0	360	0	0.00%	100.00%	100.00%	0.00%

5 03-27 Parker Valley

Cry1Ac

Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	2	0	16	18	0	4	40	38	95.00%	5.00%		
1	16	8	13	0	0	3	40	16	40.00%	60.00%	57.89%	42.11%
10	304	25	1	0	0	0	330	1	0.30%	99.70%	99.68%	0.32%

6 03-28 Somerton
Cry1Ac

Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	5	0	13	20	0	12	50	45	90.00%	10.00%		
1	11	21	8	3	0	7	50	18	36.00%	64.00%	60.00%	40.00%
10	412	38	0	0	0	0	450	0	0.00%	100.00%	100.00%	0.00%

7 03-29 Texas Hill
Cry1Ac

Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	4	0	18	25	1	12	60	56	93.33%	6.67%		
1	13	19	24	2	0	2	60	28	46.67%	53.33%	50.00%	50.00%
10	517	21	1	0	0	1	540	2	0.37%	99.63%	99.60%	0.40%

8 03-40 Cibola
Cry1Ac

Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	3	1	18	6	0	2	30	26	86.67%	13.33%		
1	11	17	2	0	0	0	30	2	6.67%	93.33%	92.31%	7.69%
10	263	7	0	0	0	0	270	0	0.00%	100.00%	100.00%	0.00%

9 03-41-I Picacho
Cry1Ac

Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	11	0	0	16	0	3	30	23	76.67%	23.33%		
1	16	1	8	0	0	5	30	13	43.33%	56.67%	43.48%	56.52%
10	186	47	2	0	0	0	235	2	0.85%	99.15%	98.89%	1.11%

10 03-42 Salome
Cry1Ac

Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	3	1	8	5	0	3	20	16	80.00%	20.00%		
1	12	8	0	0	0	0	20	0	0.00%	100.00%	100.00%	0.00%
10	180	0	0	0	0	0	180	0	0.00%	100.00%	100.00%	0.00%

11 03-43 Queen Creek Site 2
Cry1Ac

Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	3	0	9	22	0	6	40	37	92.50%	7.50%		
1	16	11	12	0	0	1	40	13	32.50%	67.50%	64.86%	35.14%
10	340	20	0	0	0	0	360	0	0.00%	100.00%	100.00%	0.00%

12 03-44 Coolidge
Cry1Ac

Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	7	1	19	13	0	0	40	32	80.00%	20.00%		
1	22	10	6	1	0	1	40	8	20.00%	80.00%	75.00%	25.00%
10	351	9	0	0	0	0	360	0	0.00%	100.00%	100.00%	0.00%

13 03-46 Duncan
Cry1Ac

Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	4	1	21	8	0	6	40	35	87.50%	12.50%		
1	34	4	2	0	0	0	40	2	5.00%	95.00%	94.29%	5.71%
10	359	1	0	0	0	0	360	0	0.00%	100.00%	100.00%	0.00%

14 03-48 Goodyear
Cry1Ac

Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	5	1	7	10	0	7	30	24	80.00%	20.00%		
1	21	5	4	0	0	0	30	4	13.33%	86.67%	83.33%	16.67%
10	260	10	0	0	0	0	270	0	0.00%	100.00%	100.00%	0.00%

15 03-54 Sentinel / Hyder
Cry1Ac

Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	3	1	16	9	1	0	30	26	86.67%	13.33%		
1	14	10	6	0	0	0	30	6	20.00%	80.00%	76.92%	23.08%
10	214	1	0	0	0	0	215	0	0.00%	100.00%	100.00%	0.00%

16 03-55-I Gila Bend
Cry1Ac

Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	3	0	18	15	0	4	40	37	92.50%	7.50%		
1	21	10	8	1	0	0	40	9	22.50%	77.50%	75.68%	24.32%
10	302	15	0	0	0	1	318	1	0.31%	99.69%	99.66%	0.34%

CALIFORNIA

All 2003 California Collections

Cry1Ac

	----------Sums of All Assays----------								--------Grand Means--------			
Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	11	1	40	114	3	41	210	198	94.29%	5.71%		
1	54	66	59	3	0	28	210	90	42.86%	57.14%	54.55%	45.45%
10	1688	189	5	7	0	1	1890	13	0.69%	99.31%	99.27%	0.73%

Total Individuals Tested 2310

1 03-34 Blythe Palo Verde Site 1

Cry1Ac

Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	3	0	10	15	0	12	40	37	92.50%	7.50%		
1	11	14	14	0	0	1	40	15	37.50%	62.50%	59.46%	40.54%
10	347	13	0	0	0	0	360	0	0.00%	100.00%	100.00%	0.00%

2 03-35 Blythe Palo Verde Site 2

Cry1Ac

Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	2	0	11	19	0	8	40	38	95.00%	5.00%		
1	11	11	12	0	0	6	40	18	45.00%	55.00%	52.63%	47.37%
10	351	9	0	0	0	0	360	0	0.00%	100.00%	100.00%	0.00%

3 03-36 Blythe Palo Verde Site 3

Cry1Ac

Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	1	0	11	24	0	4	40	39	97.50%	2.50%		
1	12	23	5	0	0	0	40	5	12.50%	87.50%	87.18%	12.82%
10	348	12	0	0	0	0	360	0	0.00%	100.00%	100.00%	0.00%

4 03-37 Imperial Valley Site1

Cry1Ac

Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	2	0	4	22	3	9	40	38	95.00%	5.00%		
1	11	10	11	0	0	8	40	19	47.50%	52.50%	50.00%	50.00%
10	344	16	0	0	0	0	360	0	0.00%	100.00%	100.00%	0.00%

5 03-39 Imperial Valley Site 2

Cry1Ac

Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	3	1	4	34	0	8	50	46	92.00%	8.00%		
1	9	8	17	3	0	13	50	33	66.00%	34.00%	28.26%	71.74%
10	298	139	5	7	0	1	450	13	2.89%	97.11%	96.86%	3.14%

TEXAS

All 2003 Texas Collections

Cry1Ac

	----------Sums of All Assays----------								--------Grand Means--------			
Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	6	3	35	17	0	19	80	71	88.75%	11.25%		
1	41	19	19	0	0	1	80	20	25.00%	75.00%	71.83%	28.17%
10	712	23	0	0	0	0	735	0	0.00%	100.00%	100.00%	0.00%

Total Individuals Tested 895

1 03-51 Esperanza

Cry1Ac

Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	4	2	13	12	0	9	40	34	85.00%	15.00%		
1	15	11	14	0	0	0	40	14	35.00%	65.00%	58.82%	41.18%
10	351	9	0	0	0	0	360	0	0.00%	100.00%	100.00%	0.00%

2 03-52 El Paso

Cry1Ac

Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	2	1	22	5	0	10	40	37	92.50%	7.50%		
1	26	8	5	0	0	1	40	6	15.00%	85.00%	83.78%	16.22%
10	361	14	0	0	0	0	375	0	0.00%	100.00%	100.00%	0.00%

NEW MEXICO

03-32 Mesquite

Cry1Ac

Conc	Development						Number of Larvae		Percent	Percent	Corrected	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality	Survival
0	6	0	18	15	0	11	50	44	88.00%	12.00%		
1	28	16	5	0	0	1	50	6	12.00%	88.00%	86.36%	13.64%
10	438	12	0	0	0	0	450	0	0.00%	100.00%	100.00%	0.00%

Total Individuals Tested 550

Table 3. Corrected mortality (±SEM) of the APHIS laboratory strain of pink bollworm evaluated from 1998 through 2004 in diet- incorporation bioassays of 1.0 and 10 µg Cry1Ac toxin per ml of diet. Note that there no survivors of 10 ug/ml bioassays prior to 2004. *(Note: the 2003 collection reported herein were largely evaluated in 2004)*.

	1.0 µg/ml Cry1Ac		***10 µg/ml Cry1Ac***	
Year	**% Mortality**	**SEM (%)**	**% Mortality**	**SEM (%)**
1998	66.2	6.0	100	0.0
1999	61.3	8.0	100	0.0
2000	73.4	23	100	0.0
2001	92.7	5.5	100	0.0
2002	98.6	2.4	100	0.0
2003	48.7	8.9	100	0.0
2004	17.0	3.0	97.7	0.68

Table 4. Susceptibility to Cry2Ab2 of pink bollworm collections made in 2003.

ARIZONA

All 2003 Arizona Collections

Cry2Ab2

----------Sums of All Assays---------- | ----------Grand Means----------

Conc µg/ml	Development 1st and 2nd	3rd	≥4th	Total Subjects	Percent Survival	Percent Mortality	Corrected % Mortality	Corrected % Survival
0	1	5	603	660	91.4	8.64		
1	213	16	28	1152	2.43	97.6	97.3	2.66
10	94	3	1	1290	0.0775	99.92	99.9	0.0848

1 **O3-01-1** **Safford/Thatcher**

Cry2Ab2

Conc µg/ml	Development 1st and 2nd	3rd	≥4th	Total Subjects	Percent survival	Percent mortality	Corrected mortality	Corrected Survival
0	0	0	47	50	94.00	6.00		
1	11	0	0	100	0.00	100	100	0
10	9	0	0	100	0.00	100	100	0

2 **O3-20** **Buckeye**

Cry2Ab2

Conc µg/ml	Development 1st and 2nd	3rd	≥4th	Total Subjects	Percent survival	Percent mortality	Corrected mortality	Corrected Survival
0	0	0	45	50	90.00	10.00		
1	17	3	2	100	2.00	98.00	97.78	2.22
10	8	0	0	120	0.00	100	100	0.00

3 **O3-26** **Mohave Valley**

Cry2Ab2

Conc µg/ml	Development 1st and 2nd	3rd	≥4th	Total Subjects	Percent survival	Percent mortality	Corrected mortality	Corrected Survival
0	0	0	41	50	82.00	18.00		
1	17	0	3	100	3.00	97.00	96.34	3.66
10	7	0	0	100	0.00	100	100	0

4 **O3-27** **Parker Valley**

Cry2Ab2

Conc µg/ml	Development 1st and 2nd	3rd	≥4th	Total Subjects	Percent survival	Percent mortality	Corrected mortality	Corrected Survival
0	0	0	44	50	88.00	12.00		
1	12	1	3	100	3.00	97.00	96.59	3.41
10	12	2	0	120	0.00	100	100	0

5 **O3-28** **Somerton**

Cry2Ab2

Conc µg/ml	Development 1st and 2nd	3rd	≥4th	Total Subjects	Percent survival	Percent mortality	Corrected mortality	Corrected Survival
0	1	2	53	60	88.33	11.67		
1	20	3	0	12	0.00	100	100	0.00
10	8	0	0	130	0.00	100	100	0.00

6 **O3-29** **Texas Hill**

Cry2Ab2

Conc µg/ml	Development 1st and 2nd	3rd	≥4th	Total Subjects	Percent survival	Percent mortality	Corrected mortality	Corrected Survival
0	0	0	55	60	91.67	8.33		
1	21	5	14	100	14.00	86.00	84.73	15.27
10	20	1	1	110	0.90	99.10	99.02	0.98

7 **O3-42** **Salome**

Cry2Ab2

Conc µg/ml	Development 1st and 2nd	3rd	≥4th	Total Subjects	Percent survival	Percent mortality	Corrected mortality	Corrected Survival
0	0	2	37	40	92.50	7.50		
1	12	3	5	100	5.00	95.00	94.59	5.41
10	2	0	0	100	0.00	100	100	0.00

8 **O3-43** **Queen Creek, Site 2**

Cry2Ab2

Conc µg/ml	Development 1st and 2nd	3rd	≥4th	Total Subjects	Percent survival	Percent mortality	Corrected mortality	Corrected Survival
0	0	0	49	50	98.00	2.00		
1	15	0	0	100	0.00	100	100	0.00
10	4	0	0	100	0.00	100	100	0.00

9 **O3-44** **Coolidge**

Cry2Ab2

Conc µg/ml	Development 1st and 2nd	3rd	≥4th	Total Subjects	Percent survival	Percent mortality	Corrected mortality	Corrected Survival
0	0	1	64	70	91.42	8.06		
1	25	0	1	130	0.77	99.23	99.16	0.84
10	12	0	0	110	0.00	100	100	0.00

10 **O3-46** **Duncan**

Cry2Ab2

Conc µg/ml	Development 1st and 2nd	3rd	≥4th	Total Subjects	Percent survival	Percent mortality	Corrected mortality	Corrected Survival
0	0	0	43	50	86.00	14.00		
1	30	1	0	110	0.00	100	100	0.00
10	6	0	0	100	0.00	100	100	0.00

11 **O3-48** **Goodyear**

Cry2Ab2

Conc µg/ml	Development 1st and 2nd	3rd	≥4th	Total Subjects	Percent survival	Percent mortality	Corrected mortality	Corrected Survival
0	0	0	57	60	95.00	5.00		
1	7	0	0	100	0.00	100	100	0.00
10	3	0	0	100	0.00	100	100	0.00

12 **O3-55-1** **Gila Bend**

Cry2Ab2

Conc µg/ml	Development 1st and 2nd	3rd	≥4th	Total Subjects	Percent survival	Percent mortality	Corrected mortality	Corrected Survival
0	0	0	68	70	97.14	2.86		
1	26	0	0	100	0.00	100	100	0.00
10	3	0	0	100	0.00	100	100	0.00

CALIFORNIA

All 2003 Californinia Collections

Cry2Ab2

	----------Sums of All Assays----------				----------Grand Means----------			
Conc µg/ml	Development 1st and 2nd	3rd	≥4th	Total Subjects	Percent Survival	Percent Mortality	Corrected % Mortality	Corrected % Survival
0	0	0	213	240	88.8	11.3		
1	73	1	5	430	1.16	98.8	98.7	1.31
10	36	0	1	430	0.233	99.8	99.7	0.262

1 **O3-34** **Blythe/Palo Verde Site1**

Cry2Ab2

Conc µg/ml	Development 1st and 2nd	3rd	≥4th	Total Subjects	Percent survival	Percent mortality	Corrected mortality	Corrected Survival
0	0	0	48	60	80.00	20.00		
1	21	1	1	110	0.90	99.10	98.88	1.13
10	11	0	1	110	0.90	99.10	98.88	1.13

2 **O3-36** **Blythe/Palo Verde Site 3**

Cry2Ab2

Conc µg/ml	Development 1st and 2nd	3rd	≥4th	Total Subjects	Percent survival	Percent mortality	Corrected mortality	Corrected Survival
0	0	0	50	60	83.33	16.67		
1	19	0	0	120	0.00	100	100	0.00
10	5	0	0	120	0.00	100	100	0.00

3 **O3-37** **Imperial Valley Site 1**

Cry2Ab2

Conc µg/ml	Development 1st and 2nd	3rd	≥4th	Total Subjects	Percent survival	Percent mortality	Corrected mortality	Corrected Survival
0	0	0	56	60	93.33	6.67		
1	17	0	0	100	0.00	100	100	0.00
10	8	0	0	100	0.00	100	100	0.00

4 **O3-39** **Imperial Valley Site 2**

Cry2Ab2

Conc µg/ml	Development 1st and 2nd	3rd	≥4th	Total Subjects	Percent survival	Percent mortality	Corrected mortality	Corrected Survival
0	0	0	59	60	98.33	1.67		
1	16	0	4	100	4.00	96.00	95.93	4.07
10	12	0	0	100	0.00	100	100	0.00

TEXAS

Averages of 2 Texas Collections Made in 2003

Cry2Ab2

----------Sums of All Assays---------- ----------Grand Means----------

Conc µg/ml	Development 1st and 2nd	3rd	≥4th	Total Subjects	Percent Survival	Percent Mortality	Corrected % Mortality	Corrected % Survival
0	0	1	102	120	85.0	15.0		
1	29	1	3	210	1.43	98.6	98.3	1.68
10	22	0	0	200	0.000	100	100	0.000

1 **O3-51** **Esperanza**

Cry2Ab2

Conc µg/ml	Development 1st and 2nd	3rd	≥4th	Total Subjects	Percent survival	Percent mortality	Corrected mortality	Corrected Survival
0	0	1	50	60	83.34	16.66		
1	9	1	3	100	3.00	97.00	96.33	3.67
10	10	0	0	100	0.00	100	100	0.00

2 **O3-52** **El Paso**

Cry2Ab2

Conc µg/ml	Development 1st and 2nd	3rd	≥4th	Total Subjects	Percent survival	Percent mortality	Corrected mortality	Corrected Survival
0	0	0	52	60	86.67	13.33		
1	20	0	0	110	0.00	100	100	0.00
10	12	0	2	100	1.67	98.33	98.10	1.90

NEW MEXICO

1 **O3-32** **Mesquite**

Cry2Ab2

Conc µg/ml	Development 1st and 2nd	3rd	≥4th	Total Subjects	Percent survival	Percent mortality	Corrected mortality	Corrected Survival
0	0	0	44	50	88.00	12.00		
1	18	2	1	100	1.00	99.00	98.86	1.14
10	9	0	2	120	1.67	98.30	98.10	1.90