UPDATE ON PINK BOLLWORM RESISTANCE TO BT COTTON IN THE SOUTHWEST

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Abstract

Monitoring of Arizona pink bollworm (PBW), *Pectinophora gossypiella*, susceptibility to Bt toxin Cry1Ac has been conducted annually since 1997. PBW were collected from cotton fields located throughout the Southwest in 2002, cultured in the laboratory, and tested for susceptibility to Cry1Ac using diet-incorporation bioassays. A total of 13 collections from Arizona were successfully reared and bioassayed. Six California collections and one collection each from New Mexico and Texas were also tested.

Laboratory selection of pink bollworm collected in Arizona in 1997 and exposed to Cry1Ac in diet produced a strain capable of survival on Bollgard cotton. Subsequent studies showed that 10 ug Cry1Ac/ml of insect diet was a reliable diagnostic concentration for detection of pink bollworm that were homozygous for resistance to Cry1Ac. On this basis, resistant PBW were detected in 2002 in only 2 out of 13 Arizona strains. The overall frequency of resistant PBW in 2002 strains tested from Arizona was 0.17% and ranged from 0.0 to 1.7%. One of six California collections evaluated had a single resistant survivor. No resistant pink bollworms were detected in the single New Mexico and Texas collections evaluated.

Resistant PBW were significantly more abundant in Arizona in 2001 and 2002 than they were in 1998, 1999 or 2000. However, the frequency of resistant survivors in bioassays was low in 2001 and 2002, and markedly lower than in 1997.

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. Pink bollworms were found in an average of 23.3% of non-Bt bolls statewide. Bolls from Bt cotton fields yielded an average of 0.144% (range 0 to 1.300%) infested bolls. Of these, all but three of the pink bollworm recovered from plantings of Bt cotton were from bolls that tested negative for Cry1Ac.

We conclude from these findings that there is no indication that pink bollworm resistance to Cry1Ac was a problem at the locations sampled in 2002. Furthermore, Bt cotton continued to exhibit exceptional field performance in Arizona.

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 expressed in 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 decreases in use of conventional insecticides associated with adoption of Bt cotton. In 1995, the year preceding registration of Bt cotton, an average of over 6 insecticide applications were made per acre of cotton in Arizona (Sims et al. 2001). Insecticide use in Arizona cotton has declined each year since 1995, reaching a low of less than 2 treatments per acre in 2000 (Agnew and Baker 2001). These dramatic reductions in insecticide use 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 greater-than 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 resistant and F1 heterozygote individuals were killed by bioassays of 10 ug Cry1Ac/ml diet. Thus 10 ug/ml bioassays provide a reliable diagnostic 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 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 Endotoxin, Cry1Ac

Collection. Collections from Arizona cotton fields commenced in August and continued through December, 2002. Collections were made from 14 sites in Arizona, 6 in California, and two each in New Mexico and Texas (Table 1). The goal was to establish cultures with ≥ 200 PBW from each site. Three collections, noted in Table 1, were not successfully reared and thus are not included in the bioassay results.

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 3 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, 1-5 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 1 oz cups containing approximately 5 g diet each.

Bioassaying PBW Susceptibility to Cry1Ac. Susceptibility of each collection of pink bollworm 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 2-4 liter batches, subdivided by weight into beakers, and held in a water bath 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 then cooled, cubed using a commercial cheese slicer, and ca. 5 g of diet per cup was dispensed into 1 oz medicine cups with tight fitting lids.

Neonate larvae were placed individually in the 1 oz 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 ≥ 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 8 replications of 10 larvae tested at each bioassay concentration. Bioassays normally commenced in the F2 generation and, if necessary to complete the desired number of replicates, continued through the F7 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 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.

Field Efficacy of Bollgard Cotton in Arizona

These studies were conducted by the Arizona Cotton Research and Protection Council, based in Phoenix, Arizona. Forty three pairs of adjacent fields of Bt and non-Bt cotton fields were identified throughout Arizona for estimating efficacy of Bollgard. Fields were grower-managed. From August to November, 2002, 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 visit 150 bolls were collected from the non-Bt field and 500 bolls were sampled from the 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 and samples were distributed from throughout the field edges.

Boll samples were labeled, transported to ACRPC field offices, and placed in boll boxes for 4-6 weeks to permit larvae in bolls to complete development. PBW that cut out of bolls and pupated or webbed up in the paper toweling in the boll boxes were recorded. After 4-6 weeks all bolls were opened and inspected for PBW. Numbers of PBW $\geq 3^{rd}$ instar recovered from each sample were recorded. ANOVA was used to detect differences in mean survival of PBW between sites and years.

When PBW were found to have survived to $\geq 3^{rd}$ instar in bolls from Bt fields, or were found in boll boxes containing bolls from Bt fields, tests were conducted to confirm the presence of Cry1Ac toxin in the damaged boll. When possible, two or three seeds of such bolls were individually tested using 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. Archived samples of Bt and non-Bt cotton provided seeds for internal control groups.

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 trait 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 2) 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.

Survivorship in bioassays of 1.0 µg/ml Cry1Ac has varied greatly between years (Simmons et al. 1998, Patin et al. 1999, Sims et al. 2001). 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 increases in survival of 1.0 µg/ml bioassays are due to increases in pink bollworm that are heterozygous for resistance. However, heterozygote survivors of 1.0 ug/ml bioassays do not survive bioassays of 10 µg ml and would not be capable of surviving on Bt cotton.

Susceptibility of Arizona PBW to the Bt Endotoxin, Cry1Ac

Arizona Collections--2002. Grand mean mortality of 13 Arizona strains of pink bollworm was 15.1%, 87.4.% and 99.8% at concentrations of 0, 1.0 and 10 µg/ml, respectively (Table 3). The lowest mortality observed in discriminating concentration bioassays of 10 µg/ml was 98.3% (corrected = 98.2%); this was observed with the

collection from Goodyear, Arizona. Corrected grand mean mortality for 2002 collections was 85.7% at 1.0 µg/ml and 99.8% at 10 µg/ml. Thus, when corrected for control mortality, an average of 14.3% of individuals bioassayed in 2002 collections survived exposure to 1.0 µg/ml and 0.2% survived 10 µg/ml bioassays.

Changes in AZ Collections 1997-02. We detected resistant PBW in Arizona in both 2001 and 2002 but at much lower frequencies that 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=0.01*5, F*=6.95*, df*=1) or 2000 (P=0.00*7, 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 2002 results showed slightly reduced survivorship in 10 µg/ml bioassays, relative to 2001. However, this reduction is not statistically significant. In conclusion, levels of resistance to Cry1Ac in Arizona pink bollworm remained low in 2002 and were lower than in 1997.

CA, NM and TX Collections--2002. There were no indication of resistance problems in the samples collected from California, New Mexico, or Texas. One of the 8 collections of PBW evaluated from these states had a single survivor of 10 µg/ml bioassays. This represented a single resistant individual found in Imperial Valley, Site 1 (Table 3). Mean mortality was 8.18%, 88.9%, and 99.3% for this collection at concentrations of 0, 1.0 and 10 µg/ml, respectively (Table 3).

Field Efficacy of Bollgard Cotton in Arizona

A total of 10600 non-Bt bolls and 43000 Bt bolls were inspected from 43 paired fields in 2002. Non-Bt bolls yielded 2293 pink bollworm. Bolls from Bt fields yielded 62 PBW. Thus, on a statewide basis, non-Bt fields averaged 23.2% infested bolls while Bt fields averaged 0.144% infested bolls. PBW infestation levels in Bt fields throughout Arizona have averaged ≤ 0.300% over the past 8 years (Dennehy et al. 2003). This amounts to ≤ 3 PBW per 1000 bolls. Thus, efficacy of Bt cotton against PBW in Arizona is exceptional and has changed little since Bollgard was first commercialized in 1996. Moreover, of the 62 PBW that were obtained from Bt fields in 2002, 57 were attributed to bolls that tested negative for Cry1Ac. This means that the true frequency of resistant pink bollworm surviving in Bt cotton is much lower than the average we report of 0.144%.

Conclusions

Monitoring data from the 2002 season confirmed the presence of low frequencies of pink bollworm resistant to Cry1Ac in both Arizona and California. However, contrasts of monitoring results from 1997 to 2002 show that the frequency of resistant pink bollworm was substantially lower than in 1997. Bollgard continued to perform remarkably well against pink bollworm at 43 locations evaluated in 2002. Thus, at this time we have no indications whatsoever of problems with pink bollworm resistance to Cry1Ac in Arizona or elsewhere in the Southwest.

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Table 1. Pink bollworm collections made in 2002.

Location	State	Successfully Cultured	Location	State	Successfully Cultured
Avondale	AZ	yes	Somerton	AZ	yes
Buckeye	AZ	yes	Tacna	AZ	yes
Coolidge	AZ	yes	Blythe/Palo Verde	CA	yes
Goodyear	AZ	yes	Blythe/Palo Verde	CA	yes
Magma	AZ	yes	Blythe/Palo Verde	CA	yes
Marana	AZ	yes	Imperial Valley	CA	yes
Marana Agr. Ctr.	AZ	yes	Imperial Valley	CA	yes
Maricopa	AZ	yes	Imperial Valley	CA	yes
Mohave Val.	AZ	yes	Las Cruces	NM	NO
Parker Val.	AZ	NO	Las Cruces	NM	yes
Pinal Air Park	AZ	yes	Esperanza	TX	yes
Safford	AZ	yes	Tornillo	TX	NO

Table 2. Corrected mortality (±SEM) of the APHIS laboratory strain of pink bollworm evaluated from 1998 through 2003 in diet- incorporation bioassays of 1.0 and 10 µg Cry1Ac toxin per ml of diet. Note that there have been no survivors of 10 ug/ml bioassays.

	1.0 µg/ml Cry1Ac		***10 µg/ml Cry1Ac***	
Year Tested	**% 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
Grand Mean Mortality	*73.5*		*100*	

Table 3. Susceptibility of 2002 Collections of Pink Bollworm to Cry1Ac

Arizona

All 2002 Arizona Collections: Sums and Grand Means
Cry1Ac

	----------Sums of All Assays----------									-------Grand Means----------		
Conc	Development							Number of larvae		Percent	Percent	Corrected
µg/ml	1st	2nd	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	7	3	42	485	374	3	270	1333	1132	84.9%	15.1%	
1	25	233	652	157	0	0	36	1383	193	12.6%	87.4%	85.7%
10	524	784	68	1	1	0	1	1903	3	0.172%	99.8%	99.8%
					Total Individuals Tested			4619				

1 O2-26 Tacna
Cry1Ac

Conc	Development							Number of larvae		Percent	Percent	Corrected
µg/ml	1st	2nd	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	0	0	0	35	20	0	18	80	73	91.3%	8.75%	
1	0	15	66	8	0	0	0	100	8	8.00%	92.0%	91.2%
10	33	47	2	0	0	0	0	123	0	0.00%	100%	100%

2 O2-27 Somerton
Cry1Ac

Conc	Development							Number of larvae		Percent	Percent	Corrected
µg/ml	1st	2nd	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	0	0	1	53	11	0	16	95	80	84.2%	15.8%	
1	0	21	65	0	0	0	0	100	0	0.00%	100%	100%
10	50	43	0	0	0	0	0	135	0	0.00%	100%	100%

3 O2-29 Coolidge
Cry1Ac

Conc	Development							Number of larvae		Percent	Percent	Corrected
µg/ml	1st	2nd	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	0	0	4	39	17	0	24	90	80	88.9%	11.1%	
1	0	6	54	8	0	0	3	90	11	12.2%	87.8%	86.3%
10	34	94	16	0	0	0	0	185	0	0.00%	100%	100%

4 O2-30 Magma
Cry1Ac

Conc	Development							Number of larvae		Percent	Percent	Corrected
µg/ml	1st	2nd	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	0	0	0	35	69	0	36	153	140	91.5%	8.50%	
1	1	11	51	51	0	0	14	160	65	40.6%	59.4%	55.6%
10	23	106	21	1	0	0	0	175	1	0.571%	99.4%	99.4%

5 O2-31 Maricopa
Cry1Ac

Conc	Development							Number of larvae		Percent	Percent	Corrected
µg/ml	1st	2nd	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	2	1	6	37	15	0	21	100	73	73.0%	27.0%	
1	3	22	51	2	0	0	1	100	3	3.00%	97.0%	95.9%
10	58	37	0	0	0	0	0	140	0	0.00%	100%	100%

6 O2-32 Goodyear
Cry1Ac

Conc	Development							Number of larvae		Percent	Percent	Corrected
µg/ml	1st	2nd	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	0	0	1	7	47	3	9	70	66	94.3%	5.71%	
1	0	17	33	9	0	0	2	80	11	13.8%	86.3%	85.4%
10	49	26	0	0	1	0	1	120	2	1.67%	98.3%	98.2%

7 O2-33 Buckeye
Cry1Ac

Conc	Development							Number of larvae		Percent	Percent	Corrected
µg/ml	1st	2nd	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	2	1	7	46	34	0	25	125	105	84.0%	16.0%	
1	4	10	64	24	0	0	1	120	25	20.8%	79.2%	75.2%
10	16	78	8	0	0	0	0	130	0	0.00%	100%	100%

8 O2-35 Pinal Air Park
Cry1Ac

Conc	Development							Number of larvae		Percent	Percent	Corrected
µg/ml	1st	2nd	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	0	0	8	54	10	0	18	110	82	74.5%	25.5%	
1	2	27	56	9	0	0	0	110	9	8.18%	91.8%	89.0%
10	37	64	5	0	0	0	0	150	0	0.00%	100%	100%

9 O2-36 Marana Ag Center
Cry1Ac

Conc	Development							Number of larvae		Percent	Percent	Corrected
µg/ml	1st	2nd	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	0	0	2	34	28	0	40	110	102	92.7%	7.27%	
1	0	16	45	23	0	0	11	110	34	30.9%	69.1%	66.7%
10	36	65	6	0	0	0	0	140	0	0.00%	100%	100%

10 O2-44 Mohave Valley
Cry1Ac

Conc	Development							Number of larvae		Percent	Percent	Corrected
µg/ml	1st	2nd	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	0	1	2	29	54	0	13	105	96	91.4%	8.57%	
1	1	17	40	15	0	0	2	107	17	15.9%	84.1%	82.6%
10	40	44	3	0	0	0	0	130	0	0.00%	100%	100%

11 O2-50 Marana
Cry1Ac

Conc	Development							Number of larvae		Percent	Percent	Corrected
µg/ml	1st	2nd	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	0	0	3	31	32	0	24	105	87	82.9%	17.1%	
1	0	39	32	1	0	0	2	110	3	2.73%	97.3%	96.7%
10	63	40	1	0	0	0	0	140	0	0.00%	100%	100%

12 O2-51 Avondale
Cry1Ac

Conc	Development							Number of larvae		Percent	Percent	Corrected
µg/ml	1st	2nd	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	2	0	2	49	23	0	10	100	82	82.0%	18.0%	
1	3	12	61	0	0	0	0	100	0	0.00%	100%	100%
10	41	107	3	0	0	0	0	220	0	0.00%	100%	100%

13 O2-52 Safford
Cry1Ac

Conc	Development							Number of larvae		Percent	Percent	Corrected
µg/ml	1st	2nd	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	1	0	6	36	14	0	16	90	66	73.3%	26.7%	
1	11	20	34	7	0	0	0	96	7	7.29%	92.7%	90.1%
10	44	33	3	0	0	0	0	115	0	0.00%	100%	100%

California

All 2002 California Collections: Sums and Grand Means

Cry1Ac

	Sums of All Assays									Grand Means		
Conc	Development							Number of larvae		Percent	Percent	Corrected
µg/ml	1st	2nd	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	0	4	11	148	183	0	203	590	534	90.6%	9.38%	
1	9	72	206	120	0	0	36	560	156	29.4%	70.6%	67.5%
10	205	350	49	1	0	0	0	755	1	0.123%	99.9%	99.9%

Total Individuals Tested 1905

1 Blythe Palo Verde Valley, Site 1

Cry1Ac

Conc	Development							Number of larvae		Percent	Percent	Corrected
µg/ml	1st	2nd	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	0	0	0	9	26	0	48	90	83	92.2%	7.78%	
1	0	6	26	19	0	0	3	80	22	27.5%	72.5%	70.2%
10	42	66	3	0	0	0	0	120	0	0.00%	100%	100%

2 Blythe Palo Verde Valley, Site 2

Cry1Ac

Conc	Development							Number of larvae		Percent	Percent	Corrected
µg/ml	1st	2nd	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	0	2	2	24	38	0	20	90	82	91.1%	8.89%	
1	2	5	22	23	0	0	11	80	34	42.5%	57.5%	53.4%
10	30	48	16	0	0	0	0	120	0	0.00%	100%	100%

3 Blythe Palo Verde Valley, Site 3

Cry1Ac

Conc	Development							Number of larvae		Percent	Percent	Corrected
µg/ml	1st	2nd	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	0	0	0	16	20	0	36	80	72	90.0%	10.0%	
1	1	1	11	29	0	0	16	80	45	56.3%	43.8%	37.5%
10	14	61	21	0	0	0	0	120	0	0.00%	100%	100%

4 Imperial Valley Site 1

Cry1Ac

Conc	Development							Number of larvae		Percent	Percent	Corrected
µg/ml	1st	2nd	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	0	1	2	39	12	0	50	110	101	91.8%	8.18%	
1	1	23	39	7	0	0	3	90	10	11.1%	88.9%	87.9%
10	47	52	0	1	0	0	0	135	1	0.741%	99.3%	99.2%

5 Imperial Valley Site 2

Cry1Ac

Conc	Development							Number of larvae		Percent	Percent	Corrected
µg/ml	1st	2nd	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	0	0	1	23	53	0	22	105	98	93.3%	6.67%	
1	5	20	39	20	0	0	3	110	23	20.9%	79.1%	77.6%
10	46	61	7	0	0	0	0	135	0	0.00%	100%	100%

6 Imperial Valley Site 3

Cry1Ac

Conc	Development							Number of larvae		Percent	Percent	Corrected
µg/ml	1st	2nd	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	0	1	6	37	34	0	27	115	98	85.2%	14.8%	
1	0	17	69	22	0	0	0	120	22	18.3%	81.7%	78.5%
10	26	62	2	0	0	0	0	125	0	0.00%	100%	100%

New Mexico

O2-34 Las Cruces, NM
Cry1Ac

Conc	Development							Number of larvae		Percent	Percent	Corrected
µg/ml	1st	2nd	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	0	0	0	34	34	0	23	100	91	91.0%	9.0%	
1	6	20	45	14	0	0	3	110	17	15.5%	84.5%	83.0%
10	34	36	5	0	0	0	0	115	0	0.0%	100.0%	100.0%
					Total Individuals Tested			325				

Texas

O2-53 Esperanza, TX
Cry1Ac

Conc	Development							Number of larvae		Percent	Percent	Corrected
µg/ml	1st	2nd	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	3	2	5	67	34	0	9	140	110	78.6%	21.4%	
1	6	59	45	8	0	0	1	140	9	6.4%	93.6%	91.8%
10	68	61	1	0	0	0	0	180	0	0.0%	100.0%	100.0%
					Total Individuals Tested			460				