The University of Arizona • Extension Arthropod Resistance Management Laboratory

PRELIMINARY REPORT ON SUSCEPTIBILITY OF SOUTHWESTERN PINK BOLLWORM TO *Bt* TOXINS CRY1AC AND CRY2Ab2 IN 2005.

Timothy J. Dennehy, Gopalan C. Unnithan, Sarah Brink, Brook Wood,
Department of Entomology, The University of Arizona, Tucson, AZ

Summary

This preliminary report was compiled in advance of the normal reporting schedule for the project, in order to provide the results for an EPA scientific advisory panel. All bioassays of 2005 collections of pink bollworm have been completed at the time of this writing and statistics and graphics for the final report are scheduled for completion by November, 2006, as previously agreed.

Larvae were collected from cotton fields located throughout the Southwest, cultured in the laboratory, and offspring tested using diet-incorporation bioassays. The total numbers of 2005 collections successfully reared and tested for susceptibility to Cry1Ac were 11 from Arizona and four from California. Success of pink bollworm eradication in New Mexico and Texas precluded successful collection of samples in those states. Susceptibility to was also estimated to Cry2Ab2 for 12 strains from Arizona and four from California, using diet-incorporation bioassays.

Selection of Arizona pink bollworm with Cry1Ac in the laboratory in 1997 produced a resistant 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 larvae homozygous for resistance to Cry1Ac.

No survivors of 10 μg Cry1Ac/ml were detected in any bioassays of 2005 strains (n=5358). Thus, the grand mean frequencies of PBW survival of 10 μg Cry1Ac/ml in 2005 was 0.000%. A susceptible culture, APHIS-S, used each year as an internal control, yielded 100% mortality in tests of 10ug/ml Cry1Ac and 100% mortality in tests of 10 ug/ml Cry2Ab2. All twelve pink bollworm strains collected in 2005 were highly susceptible to Cry2Ab2, based on contrasts with baseline data collected from 2001-2003. There were no survivors of either of our diagnostic concentrations, 1.0 μg/ml (n=1000) and 10 μg/ml (n=3425) Cry2Ab2.

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 44 Arizona locations. Statewide, large pink bollworm larvae were found in an average of 24% of non-Bt bolls sampled from borders of refuge fields. Bolls from adjacent Bt cotton (Bollgard™) fields yielded an average of 0.37% bolls infested with large larvae.

We conclude from these findings that there was no indication of problems with pink bollworm resistance to Cry1Ac or Cry2Ab2 at the locations sampled in 2005. Moreover, Bt cotton continued to exhibit exceptional field performance in Arizona.

Materials and Methods

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

Collections. Collections from cotton fields commenced in August and continued through December, 2005. Over 100 fields of non-Bt cotton were inspected throughout Arizona, California, New Mexico, and Texas. From these, successful collections were obtained from 15 sites in Arizona, two in California, and one in New Mexico. An eradication program underway in New Mexico and Texas resulted in only one collection being obtained from New Mexico and none from Texas. Our objective was to establish laboratory cultures with ≥ 100 PBW from each collection site. At each location 300 to 2,000 bolls were collected from non-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).

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 100 'cut-out' larvae are obtained from boll boxes, bolls were opened manually to collect additional PBW. To prevent or disrupt diapause, larvae that cut out of bolls and webbed up were disturbed 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 and Cry2Ab2 were conducted from November, 2005 through July, 2006.

Bioassay. Susceptibility of each collection of pink bollworm to Cry1Ac and Cry2Ab2 was determined using 21-day diet-incorporation bioassays (Patin et al. 1999). For Cry1Ac, MVP-II® Bioinsecticide obtained from DowAgrosciences was diluted with sterilized, distilled water to produce a stock solution. For Cry2Ab2, the source of toxin was freeze-dried corn leaf powder produced by Monsanto in St. Louis, MO. Our batch of leaf powder was estimated by Monsanto to contain 9.99 mg Cry2Ab2 toxin/g of powder. Toxin was added to liquid wheat germ diet (Adkinson et al. 1960) in amounts necessary to create final concentrations of 1.0, and 10 µg/ml Cry1Ac/ml diet solution, and 1.0 and 10 µg/ml Cry2Ab2/ml diet solution. No toxin was added to control diet groups.

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 was blended thoroughly into the liquid diet. The food coloring was added to ensure thorough mixing of toxin in the diet. Diet was allowed to cool to room temperature and then was refrigerated at 6-8 °C for 48-72 h, after which it was cubed using a commercial cheese slicer. 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 2-3 weeks.

Neonate larvae were placed individually in the one ounce cups and the lids were affixed. For Cry1Ac, subjects from each field strain were assigned to replicates consisting of 10 bioassay cups per replicate of toxin-free controls and 1.0 µg/ml Cry1Ac/ml treatments, and 90 bioassay cups per replicate for 10 µg/ml Cry1Ac/ml treatments. A total of four replications per concentration were conducted, yielding totals for each strain of 40 individuals tested in untreated controls and 1.0 µg/ml Cry1Ac/ml treatments, and 360 individuals tested in treatments of 10 µg Cry1Ac.

Replicates of Cry2Ab2 bioassays consisted of 10 larvae isolated individually in cups containing toxin-free diet, 1.0 µg/ml, or 10 µg/ml Cry2Ab2/ml diet. A total of six replicates of controls and 10 replicates of 1.0 and 10 µg/ml Cry2Ab2 were conducted, yielding totals for each strain of 60, 100, and 100 subjects tested in control, 1.0 and 10 µg/ml treatments.
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 cups contained evidence of feeding consistent with development to 4^{th} instar. Corrected mortality was computed using Abbott's formula (Abbott 1925). Bioassays were conducted on the $F_{2\text{-}8}$ generations. Results obtained from each population were pooled to obtain a single estimate of mortality for each concentration tested.

APHIS-S Laboratory Reference Strain. A laboratory strain susceptible to Cry1Ac and Cry2Ab2, the APHIS-S strain, was bioassayed with both of these Cry toxins at least twice during the period in which we evaluated the 2005 collections. APHIS-S has been used in this manner as an internal control for our bioassays each year since 1998. This laboratory strain

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. Major mutations conferring resistance to Cry1Ac or Cry2Ab2 are rare or absent in this strain.

Interpreting Cry1Ac Bioassay Results

Pink bollworms 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), susceptible laboratory strains (Tabashnik et al. 2000), and individuals heterozygous for the major resistance alleles described from Arizona pink bollworm (Tabashnik et al. 2002) had no survivors of 10 µg/ml Cry1Ac bioassays.

Laboratory selection with Cry1Ac of pink bollworm collected in Arizona in 1997 yielded a strain (AZP-R) with high levels of survival of 10 µg/ml Cry1Ac bioassays (Simmons et al. 1998). Tabashnik et al. (2000) subsequently computed the frequency of resistance in Arizona field populations from statewide monitoring data based of survival of 10 µg/ml bioassays. Greenhouse evaluations showed that the Cry1Ac-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 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.

Interpreting Cry2Ab2 Bioassay Results

Baseline Data. EARML estimates of baseline susceptibility of pink bollworm to Cry2Ab2 were reported previously. Monitoring concentrations of 1.0 and 10 µg/ml Cry2Ab2 were identified from probit responses generated from collections made in 2001 (n=6) and 2002 (Figure 3, n=14). Nineteen pink bollworm strains from throughout the Southwest evaluated in 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 (Figure 4). Only four individuals out of a total of 2040 subjects tested survived bioassays of 10 µg/ml Cry2Ab2/ml. Thus, we have an abundance of evidence that susceptible field populations have very high mortality in bioassays of 1.0 µg/ml Cry2Ab2 and will very rarely have survivors of 10 µg/ml Cry2Ab2/ml treatments. A composite strain established in 2001 and intensively selected with Cry2Ab2 in the laboratory has yielded < 20% mortality in bioassays of 10 µg/ml Cry2Ab2. Studies of the inheritance and cross-resistance in this Cry2Ab2-resistant strain are currently underway.

Field Efficacy of Bollgard Cotton in Arizona

These studies were conducted by the Arizona Cotton Research and Protection Council, based in Phoenix, Arizona. Forty pairs of adjacent commercial fields of Bt and non-Bt cotton fields were evaluated throughout Arizona from August to November, 2005. Each pair was sampled twice, as close as practical to the onset 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 sampled 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 were 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 $\geq 4^{th}$ 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.

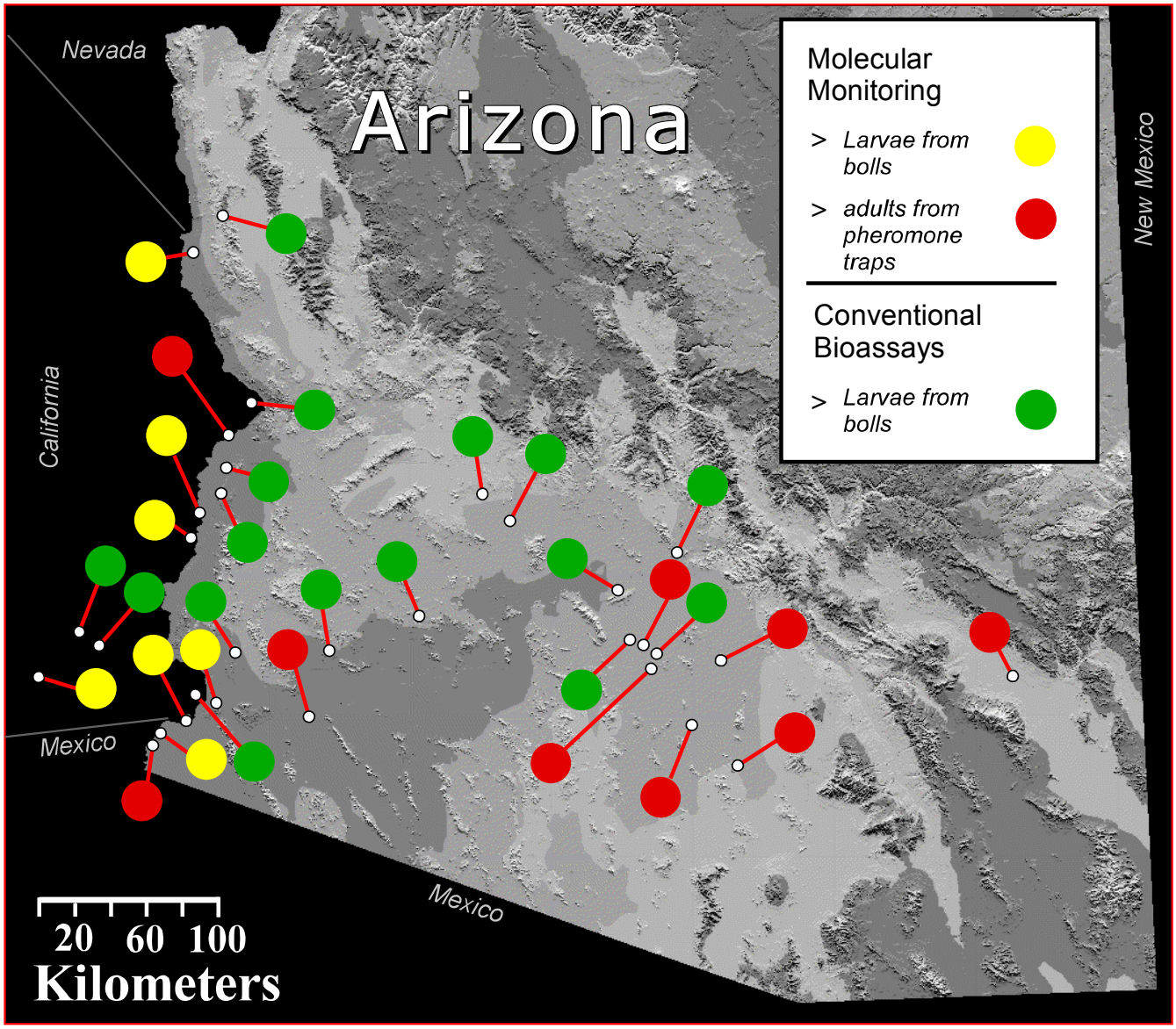

Figure 1. Locations in 2005 at which larval pink bollworm were collected or adult pink bollworm were captured in pheromone traps for the purpose of monitoring for resistance.

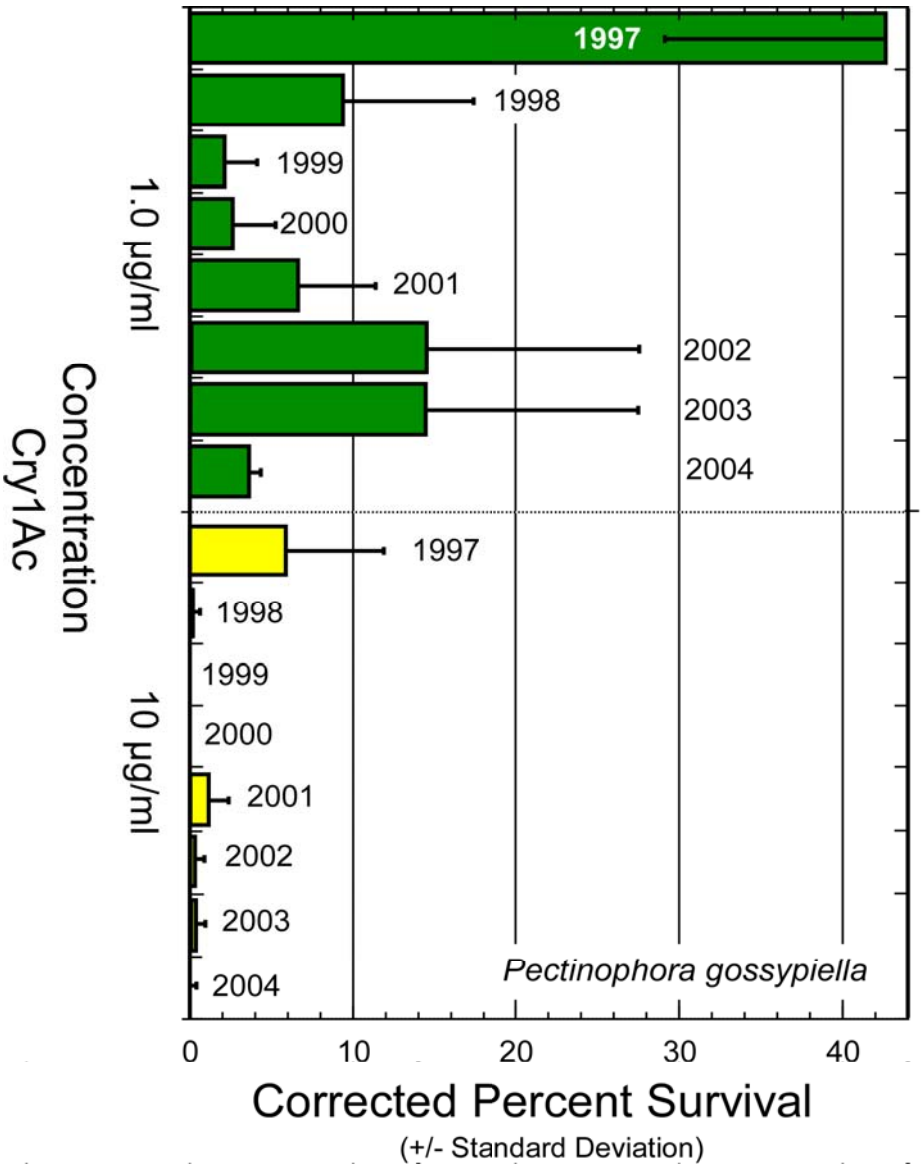

Figure 2. Changes in pink bollworm susceptibility to Cry1Ac in Arizona from 1997 to 2004. Shown are mean values (± standard deviation) for corrected survival observed in replicated 1.0 and 10 µg CrylAc/ml diet bioassays of field collections made throughout Arizona in 1997 (n=9), 1998 (n=12), 1999 (n=14), 2000 (n=17), 2001 (n=17), 2002 (n=13), 2003 (n=16), and 2004 (n=13). See Table 2 for site-specific bioassay results of the Arizona collections made in 2004 and for results from collections made in California, and New Mexico in 2004. **No larvae from any tests of 2005 strains survived treatments of 10 ug/ml Cry1Ac.**

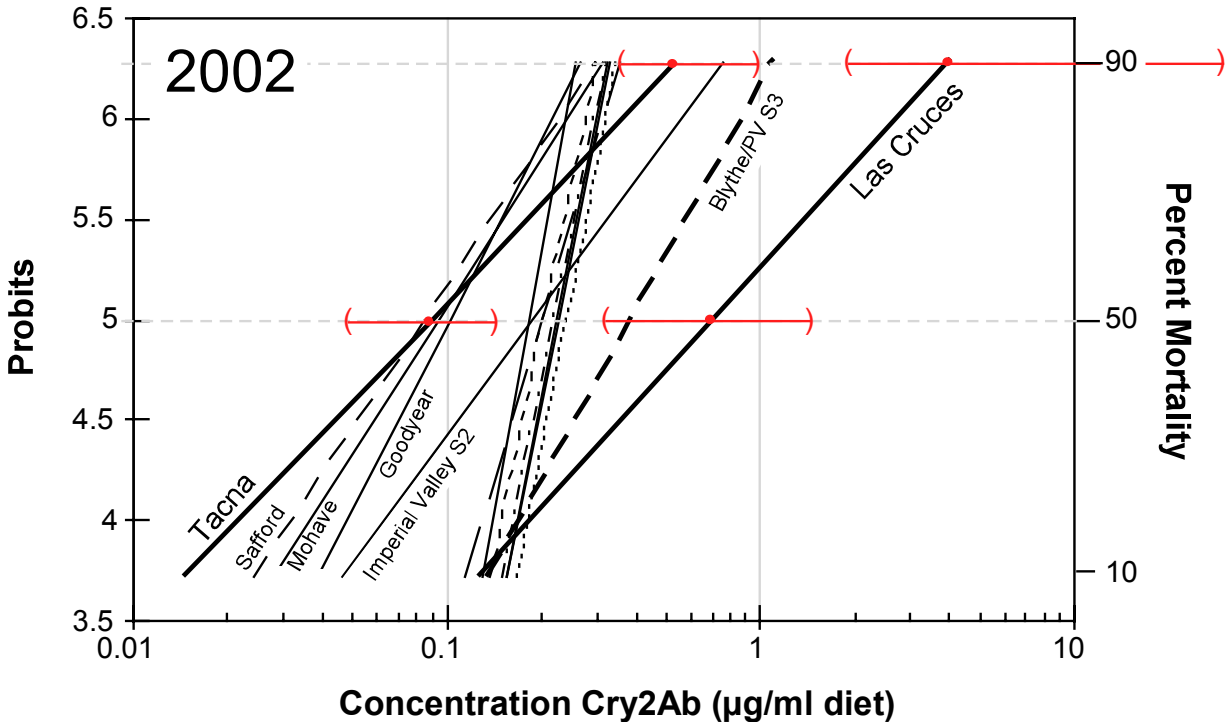

Figure 3. Baseline susceptibility to Cry2Ab2 of pink bollworm as determined by diet-incorporation bioassays. Collections were made in 2002 in Arizona (9 strains), California (3 strains), New Mexico (1 strain), and Texas (1 strain). Probit lines with LC_{50} and LC_{90} estimates (95% F.L.) were generated using the POLO program (Robertson and Preisler 1992).

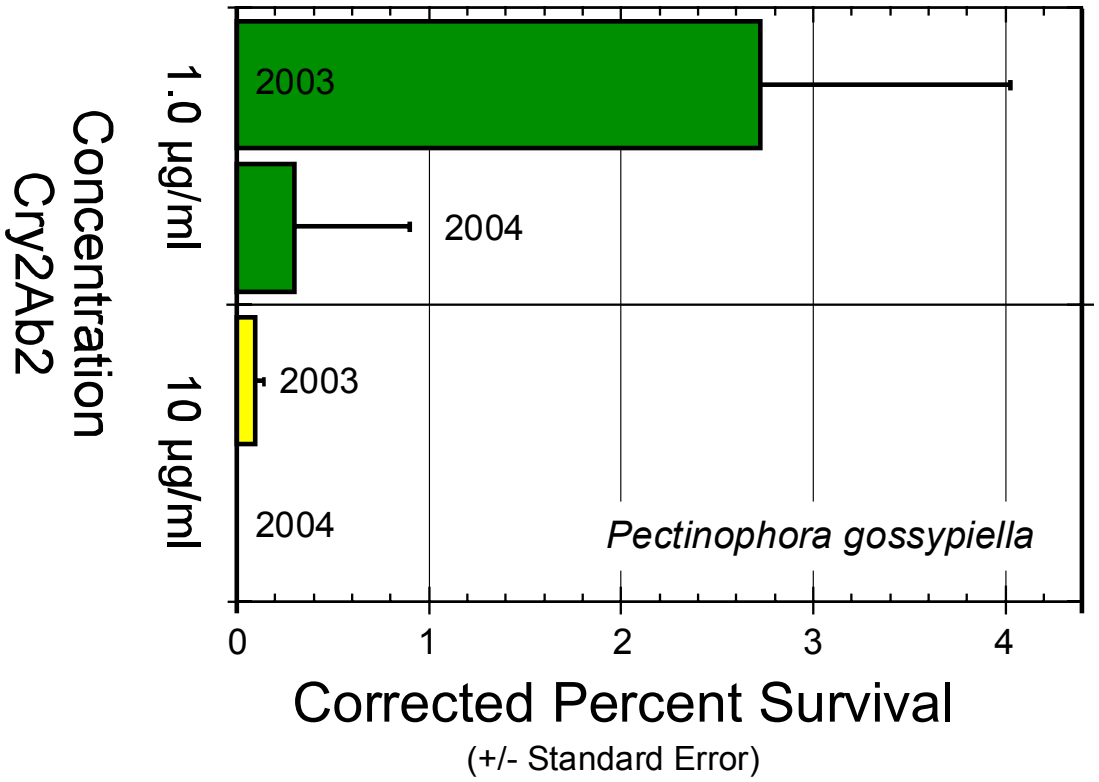

Figure 4. Response of Arizona pink bollworm to diagnostic concentrations of Cry2Ab2. Shown are mean values (± standard deviation) for corrected survival observed in replicated 1.0 and 10 µg Cry2Ab2/ml diet bioassays of field collections made throughout Arizona in 2003 (n=12), and 2004 (n=15). See Table 4 for site-specific bioassay results of the Arizona collections made in 2004 and for results from collections made in California, and New Mexico in 2004. **No pink bollworm evaluated in 2005 survived either the 1.0 or 10 ug/ml treatments.**

A.

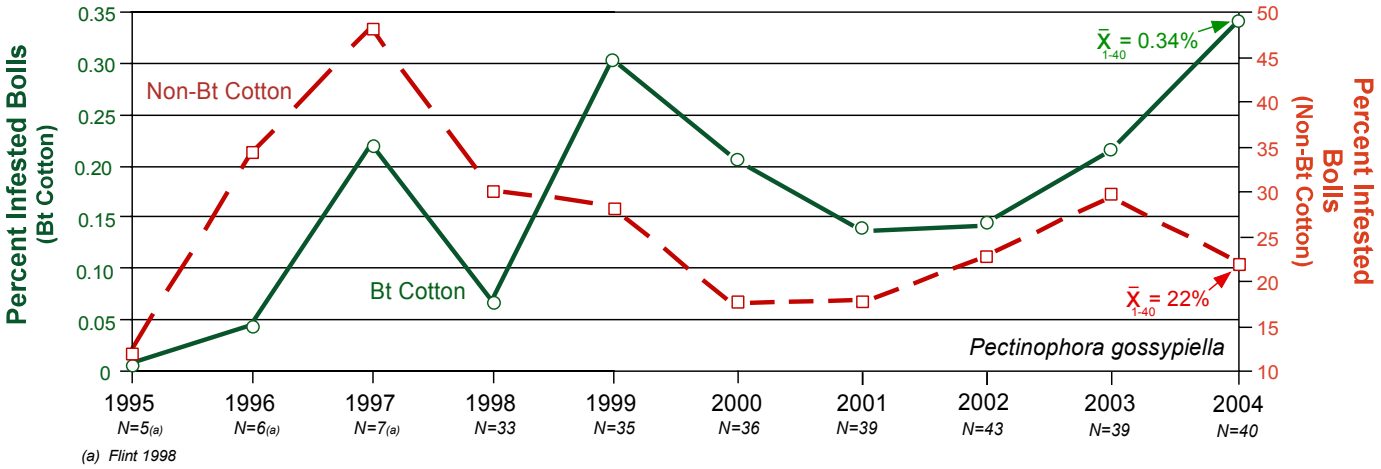

Figure 5. Sustained efficacy of Bt cotton in Arizona: 1995 to 2004. Data from 1995 to 1997 were reported by Flint et al. (1995) and Flint and Park (1996). All other data were collected by the Arizona Cotton Research and Protection Council. Shown are means of percent boll infestation (bolls with ≥ 3rd instar PBW) for pairs of Bt cotton (left axis) and non-Bt cotton fields (right axis) sampled each year from 1995 to 2004. The numbers of pairs of Bt and non-Bt fields (*N*) is indicated for each year. **In 44 pairs of Bt and non-Bt cotton monitored in 2005, the infestation rate in non-Bt cotton was 24% (n=8100 bolls), and the infestation rate in Bt cotton was 0.37% (n=44000 bolls).**

Table 1. Pink bollworm strains evaluated in 2005 for susceptibility to Cry1Ac and/or Cry2Ab2 using diet-incorporation bioassays.

Location	*State*	*Waypoint*	*Cry1Ac*	*Cry2Ab2*
Buckeye	AZ	O5-36	✓	✓
Eloy	AZ	O5-30	no	✓
Maricopa Ag.Ctr.	AZ	O5-33	✓	✓
Mohave Valley	AZ	O5-23	✓	✓
North Gila Valley	AZ	O5-18	✓	✓
Parker Vly	AZ	O5-24	✓	✓
Queen Creek	AZ	O5-32	✓	✓
Sentinel/Agua Caliente	AZ	O5-35	✓	✓
Somerton	AZ	O5-05	✓	✓
Stanfield	AZ	O5-34	✓	✓
Texas Hill	AZ	O5-26	✓	✓
Tonopah	AZ	O5-37	✓	✓
Blythe/Palo Verde	CA	O5-14	✓	✓
Holtville	CA	O5-17	✓	✓
Imperial Valley	CA	O5-16	✓	✓
Palo Verde Vly	CA	O5-15	✓	✓

Table 2. Susceptibility of pink bollworm collected in 2005 to Cry1Ac toxin in diet-incorporation bioassays.

5A (05-05: Somerton, AZ Collected 08/19/2005)

Conc	Development						Number of Larvae		Percent	Percent	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	0	0	4	36	0	10	**50**	**50**	**100.00%**	**0.00%**	
1	5	16	4	2	0	3	**30**	**9**	**30.00%**	**70.00%**	**70.00%**
10	360	16	0	0	0	0	**376**	**0**	**0.00%**	**100.00%**	**100.00%**

5B (05-14: Blythe/Palo Vere Valley, CA Collected 09/12/2005)

Conc	Development						Number of Larvae		Percent	Percent	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	4	1	1	17	1	6	**30**	**25**	**83.33%**	**16.67%**	
10	360	0	0	0	0	0	**360**	**0**	**0.00%**	**100.00%**	**100.00%**

5C (05-16: Imperial Valley, CA Collected 09/12/2005)

Conc	Development						Number of Larvae		Percent	Percent	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	2	1	3	13	0	11	**30**	**27**	**90.00%**	**10.00%**	
10	357	3	0	0	0	0	**360**	**0**	**0.00%**	**100.00%**	**100.00%**

5D (05-18: N. Gila Valley, AZ Collected 09/12/2005)

Conc	Development						Number of Larvae		Percent	Percent	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	4	1	6	18	1	10	**40**	**35**	**87.50%**	**12.50%**	
10	332	0	0	0	0	0	**332**	**0**	**0.00%**	**100.00%**	**100.00%**

5E (05-15: Palo Verde Valley, CA Collected 10/24/2005)

Conc	Development						Number of Larvae		Percent	Percent	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	5	0	2	14	0	9	**30**	**25**	**83.33%**	**16.67%**	
10	368	0	0	0	0	0	**368**	**0**	**0.00%**	**100.00%**	**100.00%**

5F (05-17: Holtville, CA Collected 10/24/2005)

Conc	Development						Number of Larvae		Percent	Percent	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	2	0	2	24	0	7	**35**	**33**	**94.29%**	**5.71%**	
10	370	0	0	0	0	0	**370**	**0**	**0.00%**	**100.00%**	**100.00%**

5G (05-23: Mohave Valley, AZ Collected 10/23/2005)

Conc	Development						Number of Larvae		Percent	Percent	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	3	0	7	19	4	7	**40**	**37**	**92.50%**	**7.50%**	
10	360	0	0	0	0	0	**360**	**0**	**0.00%**	**100.00%**	**100.00%**

5H (05-24: Parker Valley, AZ Collected 10/23/2005)

Conc	Development						Number of Larvae		Percent	Percent	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	3	0	7	22	1	7	**40**	**37**	**92.50%**	**7.50%**	
10	378	0	0	0	0	0	**378**	**0**	**0.00%**	**100.00%**	**100.00%**

5I (05-26: Texas Hill, AZ Collected 10/23/2005)

Conc	Development						Number of Larvae		Percent	Percent	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	4	0	8	19	0	9	**40**	**36**	**90.00%**	**10.00%**	
10	365	1	0	0	0	0	**366**	**0**	**0.00%**	**100.00%**	**100.00%**

5L (05-32: Queen Creek, AZ Collected 12/01/2005)

Conc	Development						Number of Larvae		Percent	Percent	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	2	1	5	24	0	8	**40**	**37**	**92.50%**	**7.50%**	
10	359	0	0	0	0	0	**359**	**0**	**0.00%**	**100.00%**	**100.00%**

5M (05-33: University of Arizona Maricopa Agricultural Center Collected 12/01/2005)

Conc	Development						Number of Larvae		Percent	Percent	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	3	0	5	22	2	8	**40**	**37**	**92.50%**	**7.50%**	
10	360	0	0	0	0	0	**360**	**0**	**0.00%**	**100.00%**	**100.00%**

5N (05-34: Stanfield, AZ Collected 12/01/2005)

Conc	Development						Number of Larvae		Percent	Percent	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	1	1	11	21	0	6	**40**	**38**	**95.00%**	**5.00%**	
10	344	0	0	0	0	0	**344**	**0**	**0.00%**	**100.00%**	**100.00%**

5O (05-35: Sentinel/Agua Caliente, AZ Collected 12/01/2005)

Conc	Development						Number of Larvae		Percent	Percent	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	5	0	9	11	0	15	**40**	**35**	**87.50%**	**12.50%**	
10	360	0	0	0	0	0	**360**	**0**	**0.00%**	**100.00%**	**100.00%**

5P (05-36: Buckeye, AZ Collected 12/01/2005)

Conc	Development						Number of Larvae		Percent	Percent	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	2	1	11	26	0	5	**45**	**42**	**93.33%**	**6.67%**	
10	327	1	0	0	0	0	**328**	**0**	**0.00%**	**100.00%**	**100.00%**

5Q (05-37: Tonopah, AZ Collected 12/02/2005)

	Conc	Development						Number of Larvae		Percent	Percent	Corrected
	µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
Cry1Ac	**0**	4	4	24	5	0	3	**40**	**32**	**80.00%**	**20.00%**	
	10	358	2	0	0	0	0	**360**	**0**	**0.00%**	**100.00%**	**100.00%**

2005 Arizona Totals (12 Sites)

Conc	Development						Number of Larvae		Percent	Percent	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	31	8	97	223	8	88	**455**	**416**	**91.43%**	**8.57%**	
1	5	16	4	2	0	3	**30**	**9**	**30.00%**	**70.00%**	**67.19%**
10	3903	20	0	0	0	0	**3923**	**0**	**0.00%**	**100.00%**	**100.00%**

2005 California Totals (4 Sites)

Conc	Development						Number of Larvae		Percent	Percent	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	13	2	8	68	1	33	**125**	**110**	**88.00%**	**12.00%**	
10	1455	3	0	0	0	0	**1458**	**0**	**0.00%**	**100.00%**	**100.00%**

2005 Totals (16 Sites)

Conc	Development						Number of Larvae		Percent	Percent	Corrected
µg/ml	1st, 2nd, Dead, Missing	3rd	4th	Pupa	Adult	E. Hole	Initial	Survivors	Survival	Mortality	Mortality
0	44	10	105	291	9	121	**580**	**526**	**90.69%**	**9.31%**	
1	5	16	4	2	0	3	**30**	**9**	**30.00%**	**70.00%**	**66.92%**
10	5358	23	0	0	0	0	**5381**	**0**	**0.00%**	**100.00%**	**100.00%**

Table 3. Susceptibility of pink bollworm collected in 2005 to Cry2Ab2 toxin in diet-incorporation bioassays

Arizona Collections

Location: **Buckeye**
Population: **05-36, F1-2**

Conc	# of Larvae	Development									Corrected	Corrected
µg/ml	Initial	1st, 2nd, Dead or Missing	3rd	4th	Pupae	Adult	E. Hole	Survivors	Survival	Mortality	Survival	Mortality
0	80	5	1	31	43	0	0	74	92.5%	7.50%		
1	60	60	0	0	0	0	0	0	0.0%	100%	0.00%	100%
10	210	210	0	0	0	0	0	0	0.0%	100%	0.00%	100%

Location: **Eloy, AZ**
Population: **05-30**

Conc	# of Larvae	Development									Corrected	Corrected
µg/ml	Initial	1st, 2nd, Dead or Missing	3rd	4th	Pupae	Adult	E. Hole	Survivors	Survival	Mortality	Survival	Mortality
0	70	22	0	16	21	0	11	48	68.6%	31.4%		
1	0	0	0	0	0	0	0	0	0.0%	0.00%	0.00%	100%
10	215	215	0	0	0	0	0	0	0.0%	100%	0.00%	100%

Location: **Maricopa Agricultural Center, AZ**
Population: **05-33, F2**

Conc	# of Larvae	Development									Corrected	Corrected
µg/ml	Initial	1st, 2nd, Dead or Missing	3rd	4th	Pupae	Adult	E. Hole	Survivors	Survival	Mortality	Survival	Mortality
0	70	2	0	42	26	0	0	68	97.1%	2.86%		
1	60	60	0	0	0	0	0	0	0.0%	100%	0.00%	100%
10	200	200	0	0	0	0	0	0	0.0%	100%	0.00%	100%

Location: **Mohave Valley, AZ**
Population: **05-23, F2-3**

Conc	# of Larvae	Development									Corrected	Corrected
µg/ml	Initial	1st, 2nd, Dead or Missing	3rd	4th	Pupae	Adult	E. Hole	Survivors	Survival	Mortality	Survival	Mortality
0	70	6	0	21	43	0	0	64	91.4%	8.57%		
1	60	60	0	0	0	0	0	0	0.0%	100%	0.00%	100.0%
10	220	220	0	0	0	0	0	0	0.0%	100%	0.00%	100%

Location: **North Gila valley, AZ**
Population: **05-18, F2-5**

Conc	# of Larvae	Development									Corrected	Corrected
µg/ml	Initial	1st, 2nd, Dead or Missing	3rd	4th	Pupae	Adult	E. Hole	Survivors	Survival	Mortality	Survival	Mortality
0	90	12	1	32	41	0	4	77	85.6%	14.4%		
1	60	60	0	0	0	0	0	0	0.0%	100%	0.00%	100%
10	210	210	0	0	0	0	0	0	0.0%	100%	0.00%	100%

Location: **Parker Valley, AZ**
Population: **05-24, F1-3**

Conc	# of Larvae	Development									Corrected	Corrected
µg/ml	Initial	1st, 2nd, Dead or Missing	3rd	4th	Pupae	Adult	E. Hole	Survivors	Survival	Mortality	Survival	Mortality
0	60	2	0	12	46	0	0	58	96.7%	3.33%		
1	60	60	0	0	0	0	0	0	0.0%	100%	0.00%	100%
10	230	230	0	0	0	0	0	0	0.0%	100%	0.00%	100%

Location: **Queen Creeck, AZ**
Population: **05-32, F2**

Conc	# of Larvae	Development									Corrected	Corrected
µg/ml	Initial	1st, 2nd, Dead or Missing	3rd	4th	Pupae	Adult	E. Hole	Survivors	Survival	Mortality	Survival	Mortality
0	70	6	1	35	28	0	0	63	90.0%	10.0%		
1	60	60	0	0	0	0	0	0	0.0%	100%	0.00%	100%
10	210	210	0	0	0	0	0	0	0.0%	100%	0.00%	100%

Location: **Sentinel/Agua Caliente, AZ**
Population: **05-35, F1-2**

Conc	# of Larvae	Development									Corrected	Corrected
µg/ml	Initial	1st, 2nd, Dead or Missing	3rd	4th	Pupae	Adult	E. Hole	Survivors	Survival	Mortality	Survival	Mortality
0	80	15	0	27	38	0	0	65	81.3%	18.8%		
1	60	60	0	0	0	0	0	0	0.0%	100%	0.00%	100%
10	200	200	0	0	0	0	0	0	0.0%	100%	0.00%	100%

Location: **Sumerton, AZ,**
Population: **05-O5, F1,5-6**

Conc	# of Larvae	Development									Corrected	Corrected
µg/ml	Initial	1st, 2nd, Dead or Missing	3rd	4th	Pupae	Adult	E. Hole	Survivors	Survival	Mortality	Survival	Mortality
0	80	8	0	14	56	2	0	72	90.0%	10.0%		
1	60	60	0	0	0	0	0	0	0.0%	100%	0.00%	100%
10	220	220	0	0	0	0	0	0	0.0%	100%	0.00%	100%

Location: **Stanfield, AZ,**
Population: **05-34, F1-2**

Conc	# of Larvae	Development									Corrected	Corrected
µg/ml	Initial	1st, 2nd, Dead or Missing	3rd	4th	Pupae	Adult	E. Hole	Survivors	Survival	Mortality	Survival	Mortality
0	70	4	0	20	46	0	0	66	94.3%	5.71%		
1	70	70	0	0	0	0	0	0	0.0%	100%	0.00%	100%
10	200	200	0	0	0	0	0	0	0.0%	100%	0.00%	100%

Location: **Texas Hill, AZ**
Population: **05-26, F1-2**

Conc	# of Larvae	Development									Corrected	Corrected
µg/ml	Initial	1st, 2nd, Dead or Missing	3rd	4th	Pupae	Adult	E. Hole	Survivors	Survival	Mortality	Survival	Mortality
0	60	3	0	24	33	0	0	57	95.0%	5.00%		
1	60	60	0	0	0	0	0	0	0.0%	100%	0.00%	100%
10	210	210	0	0	0	0	0	0	0.0%	100%	0.00%	100%

Location: **Tonopah, AZ**
Population: **05-37, F1-2**

Conc	# of Larvae	Development									Corrected	Corrected
µg/ml	Initial	1st, 2nd, Dead or Missing	3rd	4th	Pupae	Adult	E. Hole	Survivors	Survival	Mortality	Survival	Mortality
0	70	4	0	36	30	0	0	66	94.3%	5.71%		
1	60	60	0	0	0	0	0	0	0.0%	100%	0.00%	100%
10	210	210	0	0	0	0	0	0	0.0%	100%	0.00%	100%

California collections

Location: **Blythe/Paloverde, CA**
Population: **05-14, F1, 4-5**

Conc	# of Larvae	Development									Corrected	Corrected
µg/ml	Initial	1st, 2nd, Dead or Missing	3rd	4th	Pupae	Adult	E. Hole	Survivors	Survival	Mortality	Survival	Mortality
0	83	8	1	22	47	5	0	74	89.2%	10.8%		
1	70	70	0	0	0	0	0	0	0.0%	100%	0.00%	100%
10	220	220	0	0	0	0	0	0	0.0%	100%	0.00%	100%

Location: **Holtville, CA**
Population: **05-17, F1-3**

Conc	# of Larvae	Development									Corrected	Corrected
µg/ml	Initial	1st, 2nd, Dead or Missing	3rd	4th	Pupae	Adult	E. Hole	Survivors	Survival	Mortality	Survival	Mortality
0	70	1	0	34	35	0	0	69	98.6%	1.43%		
1	60	60	0	0	0	0	0	0	0.0%	100%	0.00%	100%
10	240	240	0	0	0	0	0	0	0.0%	100%	0.00%	100%

Location: **Imperial Valley, CA**
Population: **05-16, F1, 4-5**

Conc	# of Larvae	Development									Corrected	Corrected
µg/ml	Initial	1st, 2nd, Dead or Missing	3rd	4th	Pupae	Adult	E. Hole	Survivors	Survival	Mortality	Survival	Mortality
0	80	8	1	27	44	0	0	71	88.8%	11.3%		
1	60	60	0	0	0	0	0	0	0.0%	100%	0.00%	100%
10	220	220	0	0	0	0	0	0	0.0%	100%	0.00%	100%

Location: **Paloverde Valley, CA**
Population: **05-15, F1-3**

Conc	# of Larvae	Development									Corrected	Corrected
µg/ml	Initial	1st, 2nd, Dead or Missing	3rd	4th	Pupae	Adult	E. Hole	Survivors	Survival	Mortality	Survival	Mortality
0	70	4	0	14	52	0	0	66	94.3%	5.71%		
1	140	139	1	0	0	0	0	0	0.0%	100%	0.00%	100%
10	210	210	0	0	0	0	0	0	0.0%	100%	0.00%	100%

Location: Arizona

No. of populations: 12

Conc	# of Larvae	Development									Corrected	Corrected
µg/ml	Total	1st, 2nd, Dead or Missing	3rd	4th	Pupae	Adult	E. Hole	Total surv.	Survival	Mortality	Survival	Mortality
0	870	89	3	310	451	2	15	778	89.4%	10.6%		
1	670	670	0	0	0	0	0	0	0.0%	100%	0.00%	100%
10	2535	2535	0	0	0	0	0	0	0.0%	100%	0.00%	100%

Location: California

No. of populations: 4

Conc	# of Larvae	Development									Corrected	Corrected
µg/ml	Total	1st, 2nd, Dead or Missing	3rd	4th	Pupae	Adult	E. Hole	Total surv.	Survival	Mortality	Survival	Mortality
0	303	21	0	97	178	0	0	280	92.4%	7.59%		
1	330	329	1	0	0	0	0	0	0.0%	100%	0.00%	100%
10	890	890	0	0	0	0	0	0	0.0%	100%	0.00%	100%

2005 Totals for all Field Collections

No. of populations: 16

Conc	# of Larvae	Development									Corrected	Corrected
µg/ml	Total	1st, 2nd, Dead or Missing	3rd	4th	Pupae	Adult	E. Hole	Total surv.	Survival	Mortality	Survival	Mortality
0	1173	110	3	407	629	2	15	1058	90.2%	9.80%		
1	1000	999	1	0	0	0	0	0	0.0%	100%	0.00%	100%
10	3425	3425	0	0	0	0	0	0	0.0%	100%	0.00%	100%