The University of Arizona • Extension Arthropod Resistance Management Laboratory

SUSCEPTIBILITY TO *Bt* TOXINS CRY1AC AND CRY2Ab2 OF SOUTHWESTERN PINK BOLLWORM IN 2004.

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Summary

Monitoring of Arizona pink bollworm (PBW), Pectinophora gossypiella, susceptibility to Bt toxin Cry1Ac has been conducted annually from 1997-2004. Parallel studies with Cry2Ab2 have been conducted since 2001. 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 2004 collections successfully reared and tested for susceptibility to Cry1Ac were: 13 from Arizona, two from California, one from New Mexico. A pink bollworm eradication program limited collections to one from New Mexico and none from Texas. Susceptibility to Cry2Ab2 was estimated for 15 strains from Arizona, two from California, and one from New Mexico.

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 pink bollworm homozygous for resistance to Cry1Ac. Survivors of 10 µg Cry1Ac/ml were detected from only one collection made in 2004, Casa Grande Site 1, and this represented only a single survivor. The grand mean frequencies of PBW survival of 10 µg Cry1Ac/ml in 2004 collections were: Arizona 0.0262% (range 0.000-0.300%), California 0.000% and New Mexico 0.000%. A susceptible culture, APHIS-S, used each year as an internal control, yielded 100% mortality in tests of 10ug/ml Cry1Ac and in tests of 10 ug/ml Cry2Ab2. Eighteen pink bollworm strains collected in 2004 were highly susceptible to Cry2Ab2, based on contrasts with baseline data collected from 2001-2003. Mean corrected mortality ranged from 95.2 to 100% in bioassays of 1.0 µg/ml Cry2Ab2 and was 100% in all bioassays of 10 µg/ml 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 40 Arizona locations. Statewide, large pink bollworm larvae were found in an average of 21.7% (range 0 to 100%) of non-Bt bolls sampled from borders of refuge fields. Bolls from adjacent Bt cotton (Bollgard™) fields yielded an average of 0.340% (range 0 to 4.69%) bolls infested with large larvae. Of 35 PBW-infested bolls collected from Bt cotton fields, all but two tested negative for Cry1Ac.

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 2004. Moreover, 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 toxins produced by Bt cotton. 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 has declined to historic low levels since 1995, falling to less than two treatments per acre in 2000. These dramatic reductions are attributable, in large measure, 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 was anticipated to be the greatest biological limitation of transgenic insecticidal crops (Mellon and Rissler 1998). This was anticipated as a result of the many months each year that pests are exposed to toxins in plants producing Bt toxins. Resistance seemed all the more probable in Arizona cotton following the selection in our laboratory of high levels of resistance of Arizona 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). Greenhouse trials showed that Arizona pink bollworm exposed to Cry1Ac in the laboratory were able to survive on Bollgard plants. This resistance was 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, genetic analysis confirmed that 10 μg/ml provided a reliable diagnostic concentration for monitoring resistant pink bollworm to Cry1Ac. The frequency of pink bollworm resistance to Cry1Ac, although unexpectedly high in 1997 collections, declined from 1998 to 2004 (Patin et al. 1999, Tabashnik et al. 2000, Tabashnik et al. 2005).

Herein, we report the 2004 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 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, 2004 through July, 2005.

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 $\geq 4^{th}$ 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 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 2004 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, 2004. 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.

Results and Discussion

Susceptibility of Southwestern PBW to Bt Toxin Cry1Ac

Arizona Collections--2004. Grand mean mortality of 13 Arizona strains of pink bollworm was 11.3%, 96.5% and 99.9% at concentrations of 0, 1.0 and 10 µg/ml, respectively (Table 3). The lowest mortality (corrected) observed in discriminating concentration bioassays of 10 µg/ml was 99.7%. This was observed with a collection from Casa Grande (Site 1), Arizona, from which a single individual, representing 0.0262% of individuals tested, survived in bioassays of 10 µg/ml Cry1Ac.

Change in AZ Collections 1997-03. Figure 2 shows change in corrected survivorship in bioassays of 1.0 and 10 µg/ml Cry1Ac from 1997 to 2004. Survivors of 10 µg/ml bioassays of PBW strains were detected in Arizona from 2001-2004, 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=0.01*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 98.9-100% in 1998-2004 (Figure 2). Thus, survivorship in discriminating concentration bioassays of Cry1Ac in Arizona pink bollworm remained low in 2004 and was lower than in 1997.

CA, NM and TX Collections--2004. Multiple collection trips to the El Paso, Texas, and southern New Mexico regions yielded only a single sample of pink bollworm in 2004. The pink bollworm eradication program underway in these areas is credited with greatly reducing larval populations in cotton. Two collections from the Palo Verde Valley of California and one collection from the New Mexico State University, Leyendecker Farm near Las Cruces, New Mexico, yielded no survivors of 10 µg/ml Cry1Ac bioassays (Table 2).

Susceptibility of Southwestern PBW to Bt Toxin Cry2Ab2

Eighteen pink bollworm strains collected in 2004 were highly susceptible to Cry2Ab2, based on contrasts with probit responses obtained from 2002 collections (Figure 3) and discriminating concentration bioassays of 2003 collections (Figure 4). Mean corrected mortality ranged from 95.2 to 100% in bioassays of 1.0 µg and was 100% in all bioassays of 10 µg/ml Cry2Ab2 (Table 3).

Field Efficacy of Bollgard Cotton in Arizona

A total of 10,375 non-Bt bolls and 39,500 Bt bolls were inspected from a total of 40 pairs of fields in 2004. Non-Bt bolls yielded 2,082 pink bollworms. Bolls from Bt fields yielded 133 PBW. The grand mean of boll infestation was 0.340% for Bt fields and 21.7% for non-Bt fields (Figure 5a). Only two of the 40 Bt fields sampled had >2% boll infestation (Figure 5b)

and the median value for boll infestation in Bt fields was 0.000%. Contrasts of 2004 results with data from previous years revealed a significant increase in the percent infested bolls in Bt cotton from 2003 to 2004 (Figure 6). However, 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 have Cry1Ac toxin in the seeds. Of 35 infested bolls collected from Bt fields in 2004 and tested for Cry1Ac, only two were positive for toxin. This means that the true frequency of resistant pink bollworm surviving in Bt cotton is substantially lower than the boll infestation rate of 0.340% we observed in Bt bolls. 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.

Irrespective of the increased numbers of bolls from Bt fields that produced pink bollworm, infestation levels in Bt fields have averaged ≤ 0.350% over the past ten years (Figure 6). This amounts to < four PBW per 1000 bolls. Thus, the efficacy of Bt cotton against pink bollworm in Arizona continues to be exceptional and has changed little since Bollgard was first commercialized in 1996.

Conclusions

Extensive monitoring of pink bollworm from throughout the Southwestern US in 2004 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 40 Arizona locations evaluated in 2004. At this time we have no indications of imminent problems with pink bollworm resistance to either Cry1Ac or Cry2Ab2 in Arizona or elsewhere in the Southwest.

Acknowledgement

We recognize and thank the numerous contributors to this collaborative effort. Jerry Kerr, Penny Malone, Bobby Soto, Don Struckmeyer, Bill Thompkins, Rick Webb, and Mike Woodward of the Arizona Cotton Research and Protection Council supported the field components of the studies. Bob van Deven (deceased), Pria Jood, Grace Hoben, Jamie Jennette, Melanie Meyers, Tory Foster, Anna Fulford, Josh Garcia, Priya Jood, Marlene Yafuso provided technical assistance in the EARML laboratories. Joseph Ellington and Tracy Carillo of New Mexico State University, Dan Keaveny and Jodi Brigman of the California Department of Food and Agriculture, and Robert Staten, Joe Friesen and Edward Herrere of the USDA-APHIS assisted with collections of pink bollworm. Christa Ellers-Kirk and Robert Biggs assisted with rearing and molecular genotyping of putative resistant moths. EARML facilities are provided and maintained by the University of Arizona. Diet for rearing of pink bollworm was provided by Thomas Henneberry, Director of the USDA Western Cotton Research Laboratory. Funding for this project was provided by the USDA-IFAFS program, Cotton Incorporated, the Arizona Cotton Research and Protection Council, and Monsanto Life Sciences.

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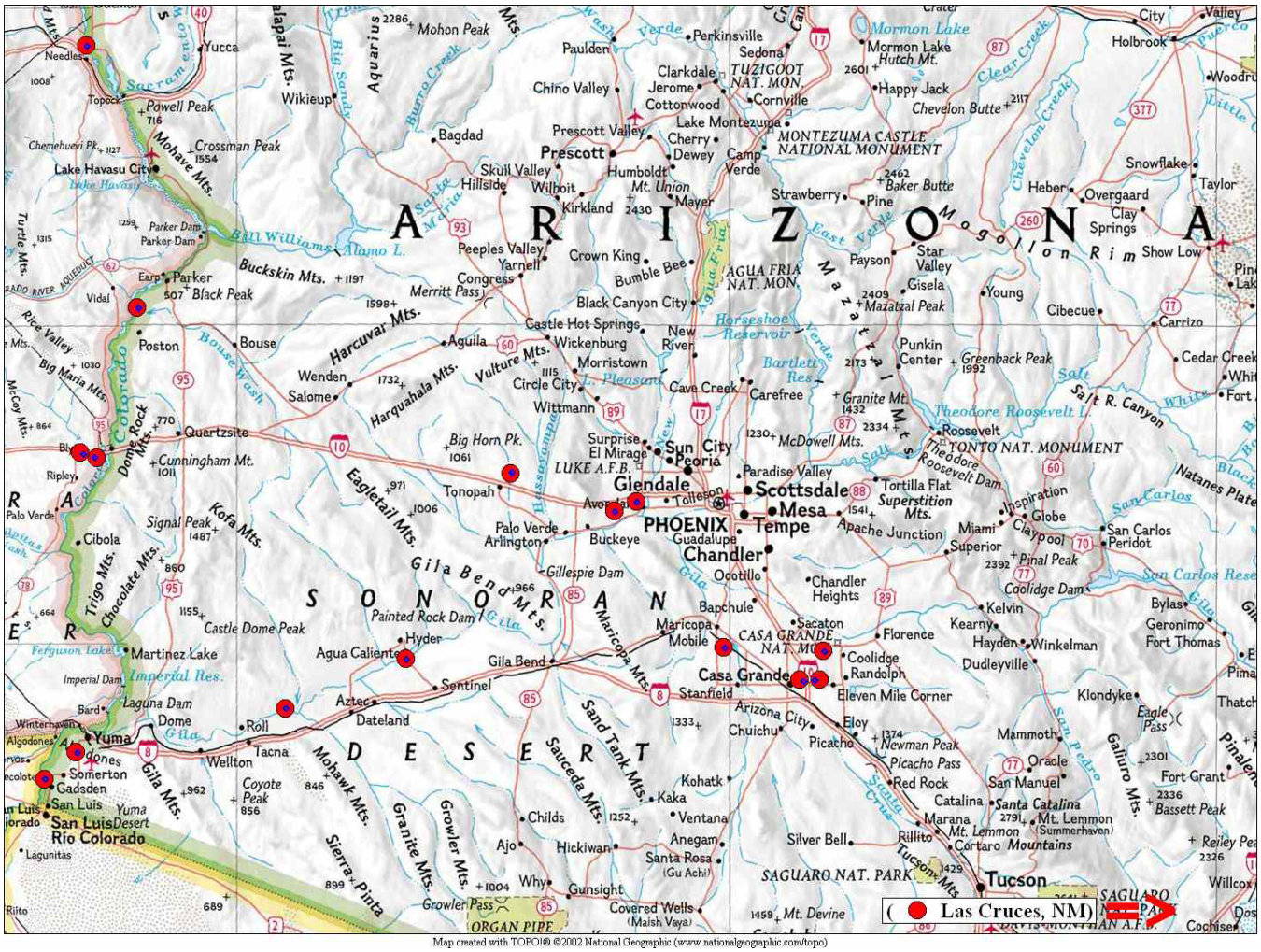

Figure 1. Locations from which pink bollworm were collected in 2004 and bioassayed for susceptibility to Cry toxins.

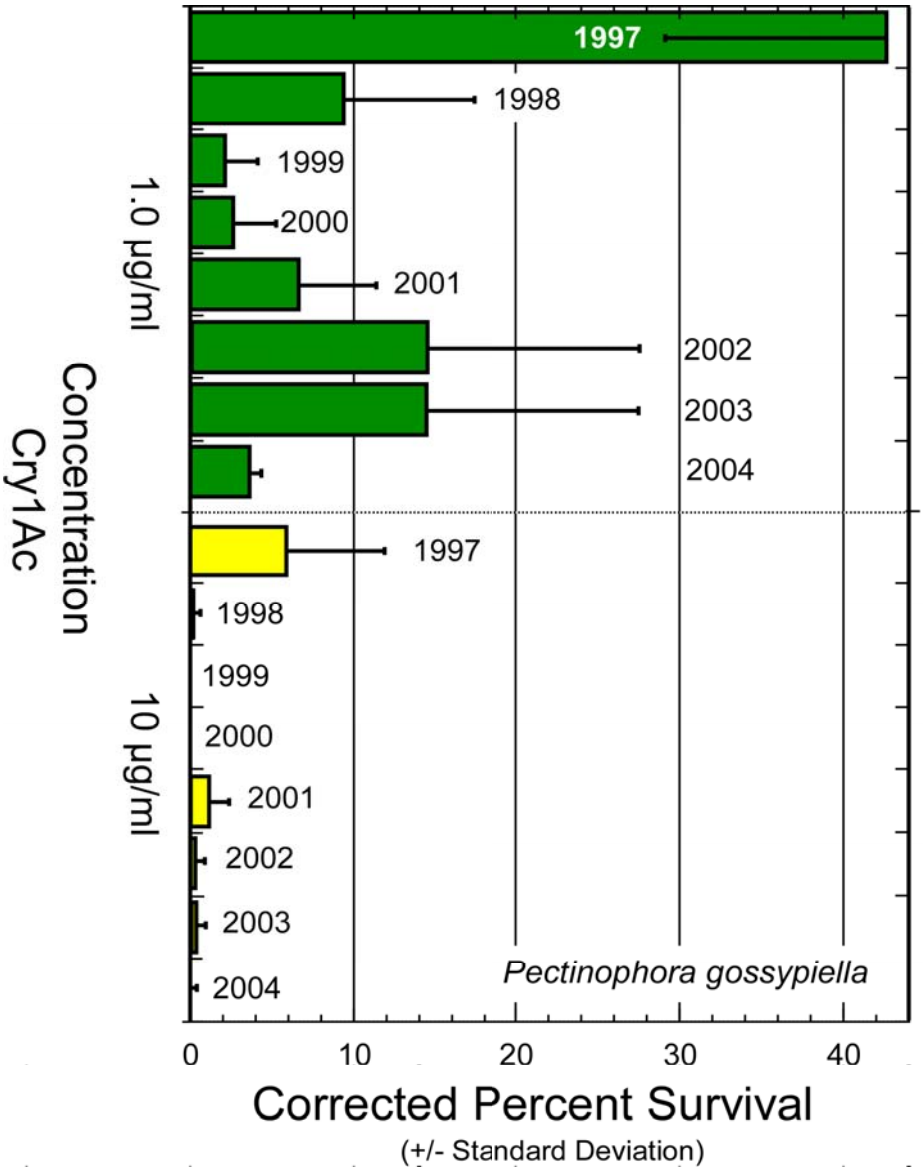

Figure 2. Changes in pink bollworm susceptibility to Cry1Ac in Arizona from 1997 to 2004. Shown are mean values (± standard deviation) for corrected survival observed in replicated 1.0 and 10 µg CrylAc/ml diet bioassays of field collections made throughout Arizona in 1997 (n=9), 1998 (n=12), 1999 (n=14), 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.

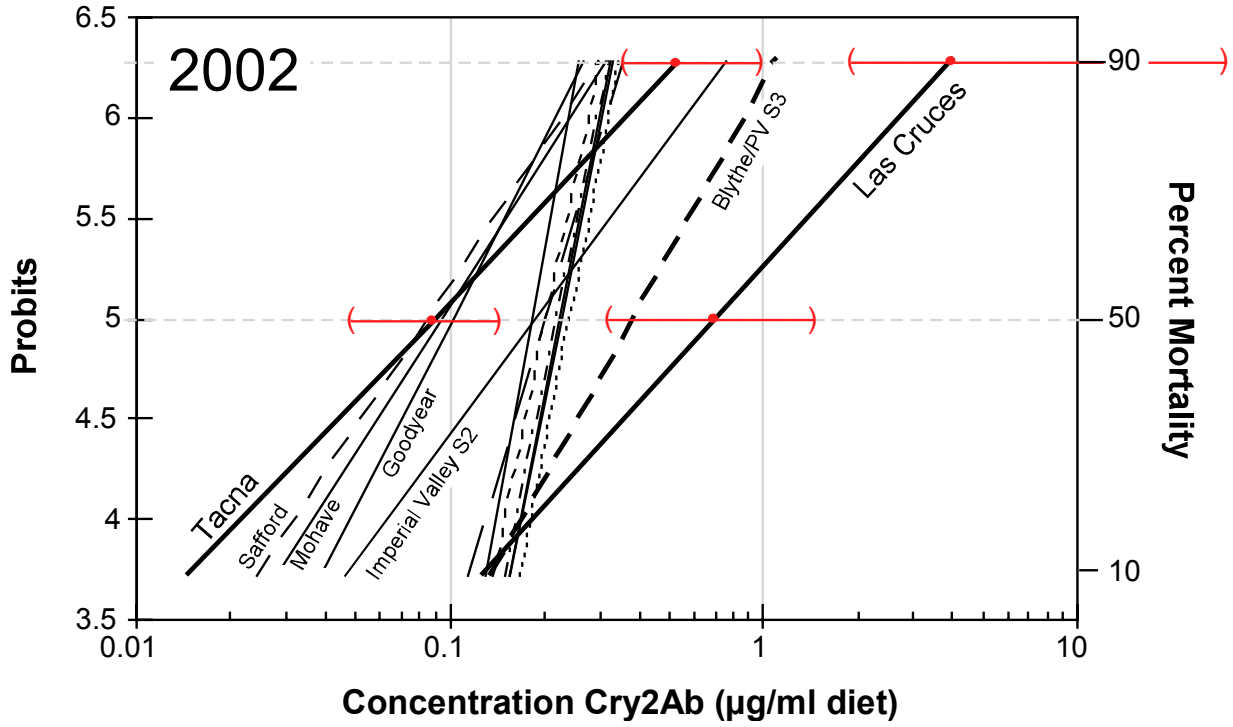

Figure 3. Baseline susceptibility to Cry2Ab2 of pink bollworm as determined by diet-incorporation bioassays. Collections were made in 2002 in Arizona (9 strains), California (3 strains), New Mexico (1 strain), and Texas (1 strain). Probit lines with LC_{50} and LC_{90} estimates (95% F.L.) were generated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

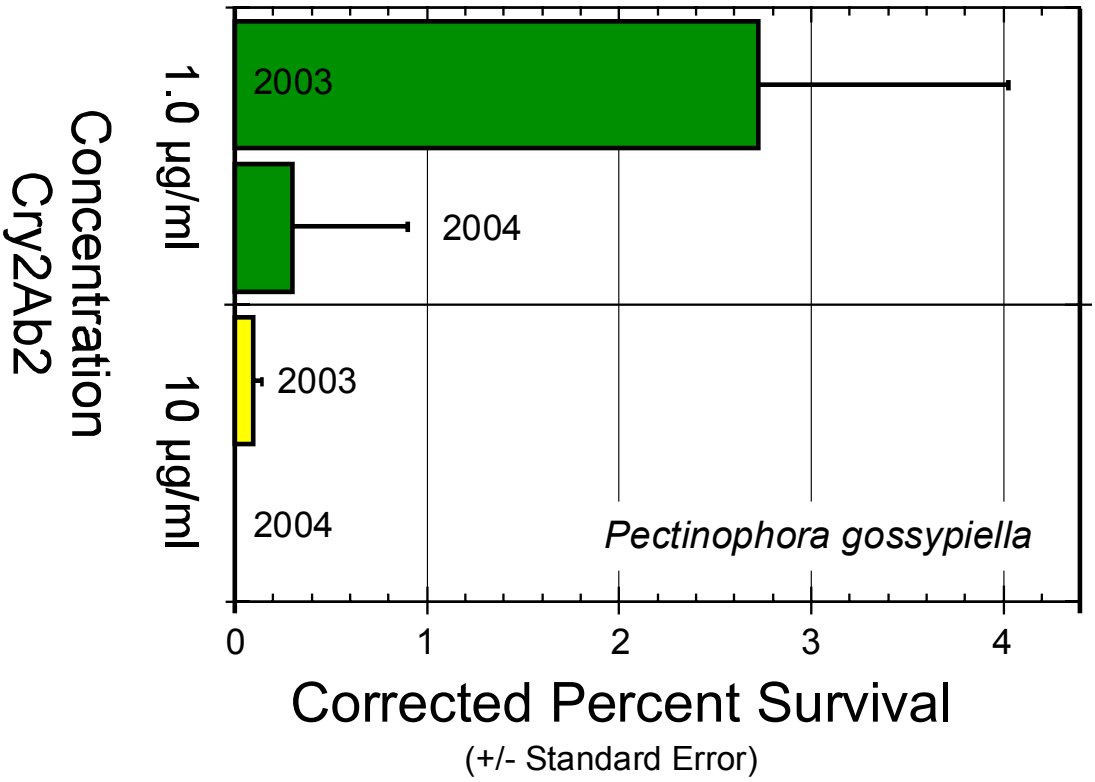

Figure 4. Response of Arizona pink bollworm to diagnostic concentrations of Cry2Ab2. Shown are mean values (± standard deviation) for corrected survival observed in replicated 1.0 and 10 µg Cry2Ab2/ml diet bioassays of field collections made throughout Arizona in 2003 (n=12), and 2004 (n=15). See Table 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.

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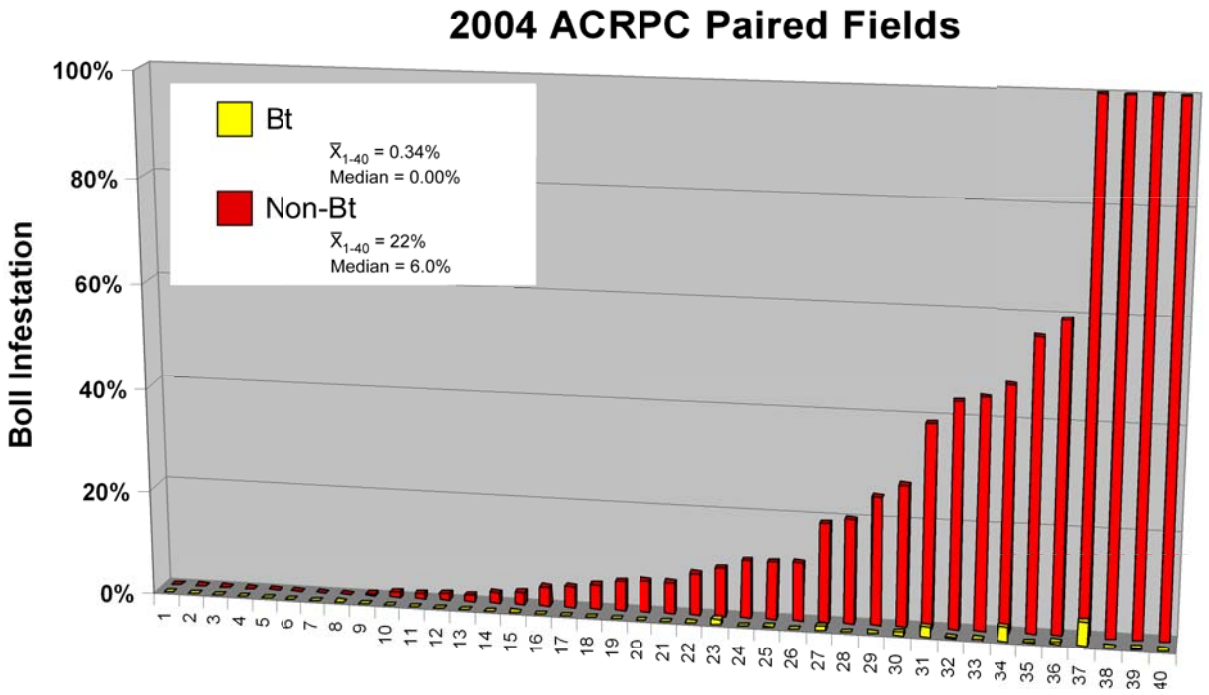

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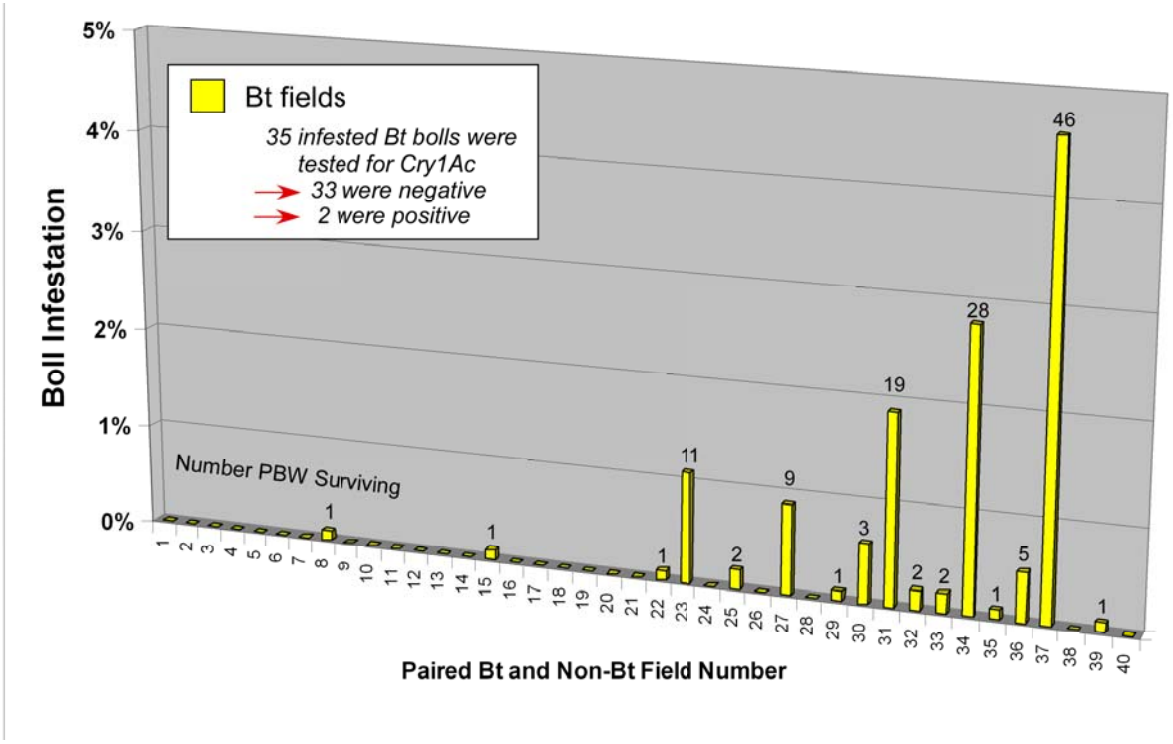

Figure 5A, B. Field performance of Bt cotton at 40 locations throughout Arizona in 2004. Adjacent exterior rows of pairs of Bt and non-Bt cotton were sampled. A) Mean percent boll infestation (≥ 3rd instar PBW) in Bt and non-Bt fields. B) A total of 133 pink bollworm were recovered from bolls collected in Bt fields. Thirty-five of these infested bolls from Bt fields were tested for presence of Cry1Ac toxin. Only 2 yielded positive results for Cry1Ac. Thus, we estimate that over 90% of pink bollworm surviving in Bt fields in Arizona were in bolls that did not produce Cry1Ac toxin.

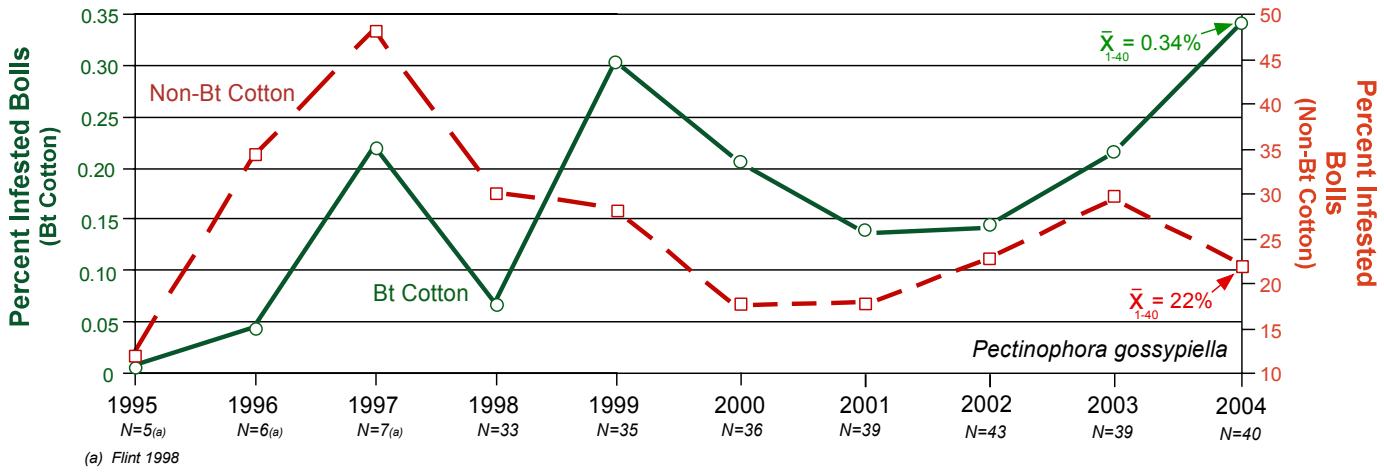

Figure 6. Sustained efficacy of Bt cotton in Arizona: 1995 to 2004. Data from 1995 to 1997 were reported by Flint et al. (1995) and Flint and Park (1996). All other data were collected by the Arizona Cotton Research and Protection Council. Shown are means of percent boll infestation (bolls with ≥ 3rd 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.

Table 1. Pink bollworm strains evaluated in 2004 for susceptibility to Cry1Ac and/or Cry2Ab2.

Location	State	Waypoint	Cry1Ac	Cry2Ab2
Avondale	AZ	O4-24	✓	✓
Casa Grande, Site 1	AZ	O4-12	✓	✓
Casa Grande, Site 2	AZ	O4-27	✓	✓
Coolidge	AZ	O4-13	✓	✓
Dome Valley	AZ	O4-18	✓	✓
Goodyear	AZ	O4-23	✓	✓
Hyder	AZ	O4-40	✓	✓
Maricopa	AZ	O4-28	✓	✓
Mohave Valley	AZ	O4-14	✓	✓
Parker Valley	AZ	O4-15	✓	✓
Somerton	AZ	O4-16	✓	✓
Stanfield	AZ	O4-21		✓
Tacna	AZ	O4-17	✓	✓
Tonopah	AZ	O4-32		✓
Yuma	AZ	O4-118	✓	✓
Blythe/Palo Verde, Site 1	CA	O4-20	✓	✓
Blythe/Palo Verde, Site 2	CA	O4-35	✓	✓
NMSU, Leyendecker Farm	NM	O4-30	✓	✓

Table 2. Susceptibility of pink bollworm collected in 2004 to Cry1Ac toxin in diet-incorporation bioassays.

Location: **Avondale, AZ**
Population: **04-24**

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	40	3	2	10	22	0	3	35	87.5%	12.0%		
1	40	33	6	1	0	0	0	1	2.50%	97.5%	2.86%	97.1%
10	330	330	0	0	0	0	0	0	0.000%	100%	0.00%	100%

Location: **Casa Grande, AZ, Site 1**
Population: **04-12**

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	40	2	1	22	11	0	4	37	92.5%	7.50%		
1	40	38	2	0	0	0	0	0	0.000%	100%	0.000%	100%
10	360	359	0	1	0	0	0	1	0.278%	99.7%	0.300%	99.7%

Location: **Casa Grande , AZ, Site 2**
Population: **04-27**

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	40	1	0	3	32	0	4	39	97.5%	2.50%		
1	40	28	12	0	0	0	0	0	0.000%	100%	0.000%	100%
10	360	360	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Coolidge, AZ**
Population: **04-13**

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	40	4	0	16	4	0	16	36	90.0%	10.0%		
1	40	35	5	0	0	0	0	0	0.000%	100%	0.000	100%
10	349	349	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Goodyear, AZ**
Population: **04-23**

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	40	1	0	0	34	1	4	39	97.5%	2.50%		
1	40	20	17	3	0	0	0	3	7.50%	92.5%	7.69%	92.3%
10	390	390	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Hyder, AZ**
Population: **04-40**

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	40	5	2	3	19	0	11	33	82.5%	17.5%		
1	40	16	17	7	0	0	0	7	17.5%	82.5%	21.2%	78.7%
10	360	360	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Maricopa, AZ**
Population: **04-28**

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	40	5	0	4	23	2	6	35	87.5%	12.5%		
1	40	26	12	2	0	0	0	2	5.00%	95.0%	5.71%	94.2%
10	360	360	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Mohave Valley, AZ**
Population: **04-14**

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	40	4	0	20	12	0	4	36	90.0	10.0%		
1	40	35	5	0	0	0	0	0	0.000%	100%	0.000%	100%
10	348	348	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Parker Valley, AZ**
Population: **04-15**

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	40	4	2	12	15	0	7	34	85.0%	15.0%		
1	40	26	13	1	0	0	0	1	2.50%	97.5%	2.94%	97.0%
10	360	360	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Somerton, AZ**
Population: **04-16**

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	40	6	0	19	12	0	3	34	85.0%	15.0%		
1	40	34	6	0	0	0	0	0	0.000%	100%	0.000%	100%
10	360	360	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Tacna, AZ**
Population: **04-17**

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	40	6	0	2	30	1	1	34	85.0%	15.0%		
1	40	24	14	2	0	0	0	2	5.00%	95.0%	5.88%	94.1%
10	360	360	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Tonopah, AZ**
Population: **04-32**

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	40	4	1	9	15	0	11	35	87.50%	12.50%		
1	40	26	12	1	0	0	1	2	5.00%	95.00%	5.71%	94.29%
10	360	360	0	0	0	0	0	0	0.00%	100.00%	0.00%	100.00%

Location: **Yuma, AZ**
Population: **04-118**

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	40	2	4	23	9	0	2	34	85.00%	15.00%		
1	40	37	3	0	0	0	0	0	0.00%	100.00%	0.00%	100.00%
10	360	360	0	0	0	0	0	0	0.00%	100.00%	0.00%	100.00%

Location: **Blythe/Palo Verde, CA, Site 1**
Population: **04-20**

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	40	6	0	5	23	1	5	34	85.0%	15.0%		
1	40	31	5	4	0	0	0	4	10.0%	90.0%	11.8%	88.2%
10	360	360	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Blythe/ Palo Verde, CA, Site 2**
Population: **04-35**

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	40	4	0	10	18	0	8	36	90.0%	10.0%		
1	40	30	10	0	0	0	0	0	0.000%	100%	0.000%	100%
10	360	358	2	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **NMSU Leyendecker Farm, NM**
Population: **04-30**

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	40	1	0	7	25	1	6	39	97.5%	2.50%		
1	40	27	9	3	0	0	1	4	10.0%	90.0%	10.2%	89.7%
10	360	360	0	0	0	0	0	0	0.000%	100%	0.00%	100%

2004 Arizona Totals (N=13)

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	520	47	12	143	238	4	76	461	88.6%	11.3%		
1	520	378	124	17	0	0	1	18	3.46%	96.5%	3.90%	96.1%
10	4308	4307	0	1	0	0	0	1	0.0232%	99.9%	0.0262%	99.9%

5348

2004 California Totals (N=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	10	0	15	41	1	13	70	87.5%	12.5%		
1	80	61	15	4	0	0	0	4	5.00%	95.0%	5.71%	94.3%
10	720	718	2	0	0	0	0	0	0.00%	100%	0.000%	100%

880

2004 New Mexico Totals (N=1))

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	40	1	0	7	25	1	6	39	97.5%	2.50%		
1	40	27	9	3	0	0	1	4	10.0%	90.0%	10.3%	89.7%
10	360	360	0	0	0	0	0	0	0.000%	100%	0.000%	100%

440

Susceptible Laboratory Strain (APHIS-S)

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	100	17	1	7	8	64	3	82	82.0%	18.0%		
1	100	39	27	27	7	0	0	34	34.0%	66.0%	41.5%	58.5%
10	750	728	22	0	0	0	0	0	0.000%	100%	0.000%	100%

950

2004 Totals for all Field Collections (N=16)

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	640	58	12	165	304	6	95	570	89.0%	10.9%		
1	640	466	148	24	0	0	2	26	4.06%	95.9%	4.56%	95.4%
10	5388	5385	2	1	0	0	0	1	0.0185%	99.9%	0.0208%	99.9%

6668

Table 3. Susceptibility of pink bollworm collected in 2004 to Cry2Ab2 toxin in diet-incorporation bioassays

Location: **Avondale, AZ**
Population **04-24**

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	50	19	0	13	18	0	0	31	62.0%	38.0%		
1	110	110	0	0	0	0	0	0	0.000%	100%	0.000%	100%
10	100	100	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Casa Grande, AZ, Site 1**
Population **04-12**

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	11	0	8	41	0	0	49	81.7%	18.3%		
1	100	100	0	0	0	0	0	0	0.000%	100%	0.000%	100%
10	100	100	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Casa Grande, AZ, Site 2**
Population **04-27**

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	10	0	17	32	1	0	50	83.3%	16.7%		
1	102	102	0	0	0	0	0	0	0.000%	100%	0.000%	100%
10	100	100	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Coolidge, AZ**
Population **04-13**

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	22	41	1	0	64	91.4%	8.57%		
1	100	100	0	0	0	0	0	0	0.000%	100%	0.000%	100%
10	100	100	0	0	0	0	0		0.000%	100%	0.000%	100%

Location: **Dome Valley,AZ**
Population **04-18**

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	12	0	14	31	3	0	48	80.0%	20.0%		
1	100	99	0	1	0	0	0	1	1.00%	99.0%	1.25%	98.8%
10	110	110	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Goodyear, AZ**
Population **04-23**

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	5	0	19	36	0	0	55	91.7%	8.33%		
1	102	102	0	0	0	0	0	0	0.000%	100%	0.000%	100%
10	90	90	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Hyder, AZ**
Population **04-40**

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	13	0	19	28	0	0	47	78.3%	21.7%		
1	100	100	0	0	0	0	0	0	0.000%	100%	0.000%	100%
10	100	100	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Maricopa, AZ**
Population **04-28**

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	11	0	20	25	1	3	49	81.7%	18.3%		
1	100	100	0	0	0	0	0	0	0.000%	100%	0.000%	100%
10	100	100	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Mohave Valley, AZ**
Population **04-14**

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	13	0	15	32	0	0	47	78.3%	21.7%		
1	103	103	0	0	0	0	0	0	0.000%	100%	0.000%	100%
10	108	108	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Parker Valley, AZ**
Population **04-15**

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	31	33	0	0	64	91.4%	8.57%		
1	100	100	0	0	0	0	0	0	0.000%	100%	0.000%	100%
10	110	110	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Somerton, AZ**
Population **04-16**

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	9	1	13	57	0	0	70	87.5%	12.5%		
1	114	107	4	3	0	0	0	3	2.63%	97.4%	0.000%	100%
10	110	110	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Stanfield, AZ**
Population **04-21**

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	6	0	34	20	0	0	54	90.0%	10.0%		
1	110	110	0	0	0	0	0	0	0.000%	100%	0.000%	100%
10	100	100	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Tacna, AZ**
Population **04-17**

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	5	0	18	46	1	0	65	92.9%	7.14%		
1	102	100	0	2	0	0	0	2	1.96%	98.0%	2.11%	97.9%
10	110	110	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Tonopah, AZ**
Population **04-32**

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	15	0	15	30	0	0	45	75.0%	25.0%		
1	100	100	0	0	0	0	0	0	0.000%	100%	0.000%	100%
10	100	100	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Yuma, AZ**
Population **04-118**

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	9	0	15	46	0	0	61	87.1%	12.9%		
1	100	100	0	0	0	0	0	0	0.000%	100%	0.000%	100%
10	100	100	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Blythe/Palo Verde, CA, Site 1**
Population **04-20**

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	78	4	0	16	58	0	0	74	94.9%	5.13%		
1	110	97	8	5	0	0	0	5	4.55%	95.5%	4.79%	95.2%
10	100	100	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **Blythe/ Palo Verde, CA, Site 2**
Population **04-35**

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	10	1	13	36	0	0	49	81.7%	18.3%		
1	106	106	0	0	0	0	0	0	0.000%	100%	0.000%	100%
10	100	100	0	0	0	0	0	0	0.000%	100%	0.000%	100%

Location: **NMSU Leyendecker Farm, NM**
Population **04-30**

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	60	3	0	16	41	0	0	57	95.0%	5.00%		
1	140	135	1	4	0	0	0	4	2.86%	97.1%	3.01%	97.0%
10	100	100	0	0	0	0	0	0	0.000%	100%	0.000%	100%

2004 Arizona Totals (N=16)

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	950	150	1	273	516	7	3	799	84.1%	15.9%		
1	1543	1533	4	6	0	0	0	6	0.389%	99.6%	0.280%	99.8%
10	1538	1538	0	0	0	0	0	0	0.0000%	100%	0.0000%	100%

4031

2004 California Totals (N=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	138	14	1	29	94	0	0	123	89.1%	10.9%		
1	216	203	8	5	0	0	0	5	2.31%	97.7%	2.40%	97.6%
10	200	200	0	0	0	0	0	0	0.000%	100%	0.000%	100%

554

2004 New Mexico Totals (N=1)

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	16	41	0	0	57	95.0%	5.00%		
1	140	135	1	4	0	0	0	4	2.86%	97.1%	3.01%	97.0%
10	100	100	0	0	0	0	0	0	0.000%	100%	0.000%	100%

300

Susceptible Laboratory Strain (APHIS-S)

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	125	11	0	0	12	102	0	114	91.2%	8.80%		
1	203	168	3	7	23	2	0	32	15.8%	84.2%	17.3%	82.7%
10	120	120	0	0	0	0	0	0	0.000%	100%	0.000%	100%

448

Cry1Ac-Resistant Laboratory Strain (AZP-R)

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	128	2	4	0	28	94	0	122	95.3%	4.69%		
1	204	145	2	33	22	2	0	57	27.9%	72.1%	29.3%	70.7%
10	130	130	0	0	0	0	0	0	0.000%	100%	0.000%	100%

462

2004 Totals for all Field Collections (N=18)

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	1148	167	2	318	651	7	3	979	85.3%	14.7%		
1	1899	1879	13	15	0	0	0	15	0.79%	99.2%	0.926%	99.1%
10	1838	1838	0	0	0	0	0	0	0.000%	100%	0.000%	100%

4885