Susceptibility of Arizona Pink Bollworm to Cry1Ac Following Six Years of Intensive Use of Transgenic Bt Cotton in Arizona

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Abstract

We summarize information on the performance of Bt cotton against pink bollworm (PBW), Pectinophora gossypiella, in Arizona following six years of use of this new technology. Monitoring of PBW susceptibility to Bt toxin Cry1Ac was conducted annually since 1997 by collecting insects from ten to 17 cotton fields, culturing strains in the laboratory, and measuring susceptibility to Cry1Ac in diet-incorporation bioassays. Based on survival in discriminating concentration bioassays of 10 µg Cry1Ac/ml of insect diet, resistant PBW were detected in low frequencies at 10 out of 17 Arizona locations sampled in 2001 and ranged from 0.0 to 4.0%. Though significantly more abundant than in the previous three seasons, resistant PBW were statistically less abundant in 2001 than they were in 1997. One collection from Paloma, AZ, had 4.0% survivors (uncorrected, actual survival) in bioassays of 10 µg/ml and samples from Coolidge, Maricopa, and Parker Arizona yielded • 1.0% survivors of this concentration. Susceptibility of a limited numbers of 2001 collections of PBW from California, New Mexico and Texas is also reported. Bioassays of 2002 collections are underway at the time of this writing.

In a parallel effort, field efficacy of Bt cotton against PBW was documented at five to 39 Arizona locations per year since 1995 by collecting cotton bolls at seasons' end and counting PBW and exit holes. In 39 pairs of adjacent Bt and non-Bt fields evaluated in 2001 by the Arizona Cotton Research and Protection Council, mean end-of-season pink bollworm infestation levels were > 15% for non-Bt fields and were < 0.15% in adjacent Bt fields. Thus, field observations indicated that performance of Bt cotton continued to be excellent throughout Arizona in the 2002 season.

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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 new technology.

Additionally, the environment and integrated pest management have benefited demonstrably from decreases in use of broad-spectrum insecticides associated with use of Bt cotton in Arizona. In 1995, the year preceding registration of Bt cotton, an average of over 6 insecticide applications was made per acre of cotton in Arizona (). Insecticide use in Arizona cotton has declined each year since 1995, reaching a low of less than 2 treatments per acre in 2000 (Sims et al. 2002, Ellsworth and Naranjo 2000). These dramatic reductions in insecticide use are attributable in large part to Bt cotton used to control pink bollworm and to improved management of whiteflies with insect growth regulators (Dennehy and Williams 1997, Dennehy et al. 2002, Ellsworth and Martinez-Carrillo 2001).

Optimism stemming from benefits of Bt cotton in Arizona has been tempered by the many gloomy predictions from scientists regarding the feasibility of preserving insect susceptibility to toxins expressed for long periods of time in genetically modified plants. Such concerns seemed particularly relevant in Arizona following the discovery in 1997 of pink bollworm highly resistant to Cry1Ac, the Bt toxin produced by Bollgard® cotton (Bartlett 1995, Simmons et al. 1998, Patin et al. 1999, Liu et al. 1999, Tabashnik et al. 2000, Sims et al. 2001). Thus it was both the current benefits of, and the specter of future problems with Bt cotton that prompted establishment in Arizona of the multi-agency collaboration that generated the results described herein. In this paper we provide data from 1997-2001 statewide monitoring of pink bollworm resistance, based at the UA Extension Arthropod Resistance Laboratory, and 1995-2001 evaluations of the field performance of Bt cotton, conducted by the Arizona Cotton Research and Protection Council. We conclude that Bt cotton, now entering its eighth year of intensive use in Arizona, continues to provide excellent efficacy against this severe cotton pest, despite the longstanding and irrefutable presence of low frequencies of resistance to Cry1Ac. Thus use of Bt cotton in Arizona continues with cautious optimism.

I. Statewide Monitoring of Susceptibility of Arizona PBW to Cry1Ac

Materials and Methods

Collection. Collections from Arizona cotton fields commenced as early as August and continued through as late as December. In 1997, collections were made from 9 sites: Coolidge, Eloy (2 samples), Marana, Mohave Valley, Paloma, Parker, Safford, Solomon, and Stanfield. In 1998, boll collections were made at 12 sites. These sites were in the vicinities of Buckeye, Casa Grande, Coolidge, Cotton Center (North), Cotton Center (South), Eloy, Hopeville, Marana, Mohave Valley, Parker, and Safford. Additionally, there was one collection from the Palo Verde Valley in California. Fourteen boll collections were made in the 1999 season in proximity of Buckeye, Coolidge (2 samples), Cotton Center South, Eloy, Harquahala Valley, Marana, Maricopa, Mohave Valley (2 samples), North Gila Valley, Parker, Safford, and Stanfield. Collections in 2000 were made at 17 Arizona sites. These were in the vicinity of Buckeye, Cotton Center, Eloy, North Gila Valley, Laveen, Marana, Maricopa, Mesa, Mohave Valley, Paloma, Parker, Queen Creek, Safford, Stanfield, Tacna, and Yuma. Collections in 2001 were made at 17 sites in Arizona. These were in the vicinity of Casa Grande, Coolidge, Cotton Center, Eloy, Laveen, Marana, Maricopa (2 samples), Mesa, Mohave Valley, North Gila Valley, Paloma Ranch, Parker Valley, Queen Creek, Tacna, Tonopah, and Yuma. Additional samples were collected from the Blythe/Palo Verde Valley areas of California (3 samples), Las Cruces, New Mexico (2 samples), and Tornillo, Texas (1 sample). Some collections were not successfully reared and thus are not included in the results.

A total of 14 pink bollworm collections were made in Arizona in 2002 plus 6 Palo Verde Valley, CA collections, and two collections each from the Las Cruces, NM and El Paso regions. The Arizona collections were made in the following areas: Buckeye (2 collections), Litchfield Park, Magma (2 collections), Marana (3 collections), Maricopa, Mohave Valley, Parker, Safford, Somerton, and Tacna. We anticipate completion of bioassays of these collections by June 2003.

At each location 300 to 2,000 bolls were collected, mainly from non-Bt cotton fields in areas adjacent to Bt fields. In three instances it was possible to collect pink bollworm from the upper bolls (second fruiting cycle) of Bt cotton:

Eloy Bt in 1997, and Coolidge Bt and Mohave Valley Bt in 1999. 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 floor of the boxes. Fourth instar larvae cut out of infested bolls and dropped onto the paper toweling on the bottom of the boxes. Larvae were transferred to pupation boxes, consisting of tightly sealed, 1.7 liter rectangular Rubbermaid® containers enclosing sheets of paper towel. To prevent or disrupt diapause, larvae that had cut out of bolls and webbed up were disturbed, twice per week, by pulling the paper toweling apart and spraying it lightly with water. After being held in boxes for • 30 days, bolls were opened ("cracked") to recover larvae that had diapaused within.

Rearing. We reared PBW using a modified version of the method of Bartlett and Wolf (1985). $F_{1\text{-}2}$ offspring of field-collected PBW were reared singly in 1 oz cups containing approximately 9 g diet. Subsequent generations were reared in 16 oz cups containing approximately 158 g of diet, as described by Patin et al. (1999).

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 (Mycogen, San Diego, CA) was mixed into 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 necessary to create final concentrations of 0 (control), 1.0, 10, and 100 µg Cry1Ac/ml diet solution. In 1997, concentrations of 0.1 and 3.2 µg/ml were also evaluated but no bioassays of 100 µg/ml were conducted. Solutions were blended thoroughly into liquid diet at 50-60°C. Diet was made in 1 liter batches of each concentration. It was then cooled, shredded and ca. 9.0 g of diet per cup dispensed into 1 oz medicine cups with tight fitting lids. Neonate larvae were placed individually in each 1 oz cups and the tops were affixed. Subjects were assigned to replicates consisting of 10 bioassay cups for each concentration. Bioassay cups were incubated in darkness at 29±2 °C for 21 days, after which mortality and developmental stage of survivors (Watson and Johnson 1974) were recorded. Live fourth instar larvae and pupae were scored as alive. Corrected mortality was computed using Abbott's formula (Abbott 1925).

For each population our goal was to complete 8 replications of 10 larvae tested at each bioassay concentration. In 1997, a limited number of bioassays of F1 larvae were included in analyses. Thereafter, bioassays 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 1540, 3140, 3406, 3336, and 9480 in 1997, 1998, 1999, 2000, and 2001, respectively. These comprised averages of 60 to 70 larvae tested per concentration for each collection evaluated.

Differences between years in susceptibility of pink bollworm collected from Arizona cotton were analyzed with one-way analysis of variance statistical software (Minitab, State College, PA). To do this corrected mean mortality in bioassays of 0.1 and 10 µg/ml Cry1Ac was transformed using $\arcsin\sqrt{x}$ for each collection made in 1997, 1998, 1999, 2000, and 2001. ANOVA tests were then used to contrast: 1) mean mortality of 1997 through 2000; 2) mean mortality of 1998 versus 2001; and, 1997 versus 2001. Response to Cry1Ac of the APHIS-S laboratory culture is reported as an internal standard (control) each year.

Results and Discussion

1997. Mean mortality of the 10 pink bollworm populations collected in 1997 was 30.2%, 70.2% and 95.9% at concentrations of 0, 1.0 and 10 µg/ml, respectively (Table 1). The lowest mortality observed in discriminating concentration bioassays of 10 µg/ml was 82.5; this was observed with the collection from the Mohave Valley. Corrected mean mortality for 1997 collections was 57.4% at 1.0 µg/ml and 94.1% at 10 µg/ml. Thus, when corrected for mortality in controls, ca. 50% of 1997 collections survived exposure to 1.0 µg/ml.

Five of the ten collections made in 1997 had survivors of 10 µg/ml bioassays. These survivors were subsequently bulked and resistance was amplified in the laboratory by rearing them on diet into which 10 µg/ml Cry1Ac was incorporated (Patin et al. 1999). Subsequent genetic studies (Tabashnik et al. 2000, Liu et al. 2001) showed that the survivors of 10 µg/ml were homozygous for a major factor conferring resistance to Cry1Ac. Greenhouse evaluations on Bollgard® plants revealed that this resistance permitted approximately 40% survival of resistant PBW on Bt cotton (Liu et al. 1999), relative to survival on non-Bt cotton. These findings heightened concerns in

Arizona regarding the threat that resistance posed to transgenic Bt cotton technology because they demonstrated that genes conferring resistance to Bt cotton were present at detectable frequencies in Arizona populations of PBW.

1998. A total of twelve populations collected in 1998 were successfully reared and evaluated, including one from California (Table 2). These had grand mean mortality of 19.8%, 92.5%, 99.9%, and 99.9% in bioassays of 0, 1.0, 10 and 100 µg/ml, respectively. The lowest mortality observed in discriminating concentration bioassays of 10 µg/ml in 1998 was 98.6%; this was observed with the collection from the Palo Verde Valley of CA. Corrected grand mean mortality was 90.6% at 1.0 µg/ml, 99.9% at 10 µg/ml, and 99.9% at 100 µg/ml. Thus, when corrected for mortality in controls, ca. 9% of 1998 collections survived exposure to 1.0 µg/ml Cry1Ac, and 0.2% and 0.1% survived the 10 and 100 µg/ml assays, respectively (Table 2).

1999. Fourteen collections of Arizona pink bollworm were successfully reared and evaluated in 1999 (Table 3). Mean mortality was 22.3%, 98.4%, 100% and 100% in bioassays of 0, 1.0, 10, and 100 µg/ml, respectively. When corrected for mortality in controls, ca. 2% of the 1999 collections survived exposure to 1.0 µg/ml and none of the 14 collections survived 10 µg/ml bioassays (Table 3).

2000. Grand mean mortality of the 17 pink bollworm populations collected in 2000 was 27.7%, 98.1% and 100% at concentrations of 0, 1.0 and 10 µg/ml, respectively (Table 4). Corrected mortality for these collections was 97.4% at 1.0 µg/ml and 100% at 10 µg/ml. Thus, when corrected for mortality in controls, only ca. 2% of the 2000 year collections survived exposure to 1.0 µg/ml and none of the 17 collections evaluated had survivors of 10 µg/ml bioassays.

2001. Grand mean mortality of 17 Arizona collections of pink bollworm evaluated from 2001 collections was 21.7%, 95.9% and 99.6% at concentrations of 0, 1.0 and 10 µg/ml, respectively (Table 5). The lowest mortality observed with 2001 Arizona PBW in discriminating concentration bioassays of 10 µg/ml was 96.0% (corrected = 94.8%); this was observed with the collection from the Paloma Ranch. Corrected grand mortality for 2001 collections was 93.4% at 1.0 µg/ml and 98.9% at 10 µg/ml. Thus, when corrected for control mortality, an average of 6.6% of individuals bioassayed in 2001 collections survived exposure to 1.0 µg/ml and 1.1% survived 10 µg/ml bioassays.

Changes 1997-01. Arizona pink bollworm were significantly less susceptible to Cry1Ac in 1997 than 1998 (P=0.031, F=5.36, df=1), 1999 (P=0.015, F=6.95, df=1) or 2000 (P=0.007, F=8.52, df=1)) in bioassays of 10 µg Cry1Ac/ml. 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. Similarly significant statistical increases in susceptibility of Arizona PBW were detected in bioassays of 1.0 ug/ml (statistics not shown). Grand mean mortality (corrected) in bioassays of 1.0 µg/ml increased from 57.4% in 1997 to 90.6%, 97.9%, and 97.4% in 1998, 1999, and 2000, respectively. In the 2001 season, mortality in bioassays of 1.0 ug/ml dropped to 93.4%.

The California samples yielded no survivors of 10 µg/ml. However, one of the Las Cruces collections had > 9.0% survivors of bioassays of 10 µg/ml Cry1Ac and the single collection from Texas had > 10% survivors of bioassays of this concentration. Baseline data are inadequate at this time to reliably interpret the resistance status of these New Mexico and Texas collections with respect to survivorship of bioassays of 10 µg/ml Cry1Ac. We do not know that survivors of 10 µg/ml Cry1Ac from the Texas and New Mexico cultures will possess the same recessive resistance factor found in Arizona collections that confers the ability to survive on Bt cotton expressing Cry1Ac.

Local authorities monitoring PBW in the Las Cruces and El Paso areas observed no indications of field problems with performance of Bt cotton in 2001 or 2002. Prior use of Bt cotton is reported to have been moderate in the Texas and New Mexico areas sampled. Studies are currently underway to isolate resistance from Texan and New Mexico strains. This will permit us to contrast resistance expression with the highly characterized resistant strains from Arizona, and to determine if resistant pink bollworm from Texas and New Mexico can survive on Bt plants in the greenhouse.

In summary, in 2001, for the first time since 1997, we found low but statistically significant increases in Cry1Ac-resistant pink bollworm in Arizona cotton fields. One-way ANOVA showed that resistance levels in 1997 were significantly higher than in 2001, based on survivorship in bioassays of 10 ug/ml (P=0.037, F=4.85, df=1). That is, in the sixth year of widespread use of Bt cotton in Arizona, 2001, resistance levels increased but were still lower

than they were in the second year of use of Bt cotton, 1997. Subsequent to the initial isolation of Cry1Ac-resistant PBW from 1997 collections (Patin et al. 1999), we showed that the frequency of resistance declined abruptly in field populations in 1998 and remained undetectable in 1999 and 2000 (Sims et al. 2001, Tabashnik et al. 2000). Indeed, we were unable to isolate resistant strains from any of the field collections made in 1998, 1999 or 2000. For this reason we are reluctant to interpret the 2001 findings to be an indication of impending problems with pink bollworm resistance to Bt cotton, especially in light of the absence of any unusual survival on Bt plants in the field (see below). Figure 5 illustrates cumulative changes in the frequency of Arizona PBW surviving bioassays of 1 and 10 µg/ml from 1997 to 2001.

II. Field Performance of Bt Cotton in Arizona

Documenting the field performance of Bt cotton is a critical aspect of the multi-agency collaboration underway in Arizona to manage resistance to Bt cotton. This work is based at the Arizona Cotton Research and Protection Council. Pink bollworm infestations at the interface of adjacent Bt cotton and non-Bt cotton (refuge) fields have been measured at 33 to 39 locations throughout Arizona since 1998. Dr. Hollis Flint of the USDA-ARS Western Cotton Research Laboratory evaluated field efficacy of Bt cotton at five to seven locations per year from 1995 to 1997 (Flint et al. 1996, Flint and Parks 1997).

Materials and Methods

Methods for collection of bolls from paired Bt and non-Bt fields are described in Antilla et al. 2001. At each field location 400 bolls were collected from the designated non-Bt field and 800-1000 were collected from the adjacent Bt field in the pair. Bolls were brought to the ACRPC in Phoenix, where they were placed in boll boxes. During the subsequent 30 days, all bolls were cracked to record large pink bollworm (> 3rd instar) or exit holes.

Results and Discussion

Bt cotton continued to perform extremely well in Arizona through the 2001 season. Levels of pink bollworm infestations varied widely across all 39 non-Bt fields evaluated (Figure 4); 16 non-Bt fields had • 20% infested bolls. Whereas mean percent infested bolls was > 15% across all non-Bt fields evaluated, mean percent infested bolls was < 0.15% in collections from the adjacent Bt fields (Figure 5). Thus, after six years of intensive use, there are no indications of resistance reducing the exceptionally high efficacy of Bt cotton against pink bollworm in Arizona.

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Table 1. Mortality (±SEM) of pink bollworm collected from Arizona cotton in 1997 and tested in diet bioassays incorporating Cry1Ac toxin.

	Concentration Cry1Ac µg/ml diet		
Collection Site	**0**	**1**	**10**
Coolidge	35.5 (8.5)	74.5 (12)	100 (0.0)
Eloy	41.7 (4.4)	82.8 (4.1)	100 (0.0)
Eloy Bt	32.0 (6.0)	67.4 (8.6)	92.0 (5.8)
Marana	46.3 (5.6)	96.8 (3.7)	100 (0.0)
Mohave Valley	22.0 (4.7)	59.0 (7.8)	82.5 (4.8)
Paloma	22.9 (6.0)	55.7 (9.7)	100 (0.0)
Parker	24.5 (6.0)	82.1 (5.6)	100 (0.0)
Safford	22.7 (5.6)	58.2 (11)	98.0 (2.0)
Solomon	17.3 (3.6)	59.6 (8.4)	90.0 (10)
Stanfield	37.5 (8.2)	66.3 (9.4)	96.0 (2.4)
Total AZ Collections	*10*	*10*	*10*
Grand Mean Mortality	*30.2*	*70.2*	*95.9*
Corrected Grand Mean Mortality		*57.4*	*94.1*
Median Mortality	*28.3*	*66.9*	*99.0*
Minimum Mortality	*17.3*	*55.7*	*82.5*
Std. Dev. Mortality	*9.73*	*13.5*	*5.94*
Corrected Grand Mean Survivorship		*42.6*	*5.90*
APHIS-S	*22.0 (8.3)*	*73.6 (6.0)*	*100 (0.0)*

Table 2. Mortality (±SEM) of pink bollworm collected from Arizona cotton in 1998 and tested in diet bioassays incorporating Cry1Ac toxin.

	Concentration Cry1Ac µg/ml diet			
Collection Site	**0**	**1**	**10**	**100**
Buckeye	17.5 (6.2)	98.8 (1.3)	100 (0.0)	100 (0.0)
Casa Grande	14.6 (3.1)	89.4 (4.1)	99.6 (0.4)	99.6 (0.4)
Coolidge	14.4 (4.4)	93.3 (3.3)	100 (0.0)	100 (0.0)
Cotton Center South	18.0 (3.9)	75.0 (5.0)	100 (0.0)	100 (0.0)
Cotton Center North	21.8 (4.6)	90.0 (3.1)	100 (0.0)	100 (0.0)
Eloy	30.0 (4.5)	*	100 (0.0)	100 (0.0)
Hopeville	28.6 (3.5)	98.8 (1.3)	100 (0.0)	100 (0.0)
Marana	30.0 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)
Mohave Valley	20.8 (2.6)	85.0 (12)	100 (0.0)	100 (0.0)
Parker	4.00 (2.4)	*	100 (0.0)	100 (0.0)
Safford	19.2 (3.4)	100 (0.0)	100 (0.0)	100 (0.0)
Palo Verde, CA	18.6 (2.9)	95.0 (5.0)	98.6 (1.0)	99.2 (0.8)
Total AZ Collections	*12*	*10*	*12*	*12*
Grand Mean Mortality	*19.8*	*92.5*	*99.9*	*99.9*
Corrected Grand Mean Mortality		*90.6*	*99.9*	*99.9*
Median Mortality	*18.9*	*94.1*	*100*	*100*
Minimum Mortality	*4.00*	*75.0*	*98.6*	*99.2*
Std. Dev. Mortality	*7.42*	*7.99*	*0.419*	*0.250*
Corrected Grand Mean Survivorship		*9.40*	*0.100*	*0.100*
APHIS-S	6.25(2.3)	63.7(8.0)	100(0.0)	*

Table 3. Mortality (±SEM) of pink bollworm collected from Arizona cotton in 1999 and tested in diet bioassays incorporating Cry1Ac toxin.

	Concentration Cry1Ac µg/ml diet			
Collection Site	**0**	**1**	**10**	**100**
Buckeye	20.0 (8.0)	100 (0.0)	100 (0.0)	*
Coolidge	16.0 (3.7)	100 (0.0)	100 (0.0)	*
Coolidge Bt	28.6 (5.1)	98.6 (1.4)	100 (0.0)	*
Cotton Center S	21.7 (4.3)	98.3 (2.0)	100 (0.0)	*
Eloy	19.0 (4.1)	99.0 (0.8)	100 (0.0)	100 (0.0)
Harquahala	18.8 (3.6)	98.8 (0.9)	100 (0.0)	100 (0.0)
Marana	32.7 (3.3)	100 (0.0)	100 (0.0)	100 (0.0)
Maricopa	17.6 (5.1)	95.3 (2.4)	100 (0.0)	100 (0.0)
Mohave Valley	20.0 (2.6)	93.3 (6.7)	100 (0.0)	100 (0.0)
MohaveValley Bt	33.3 (12)	100 (0.0)	100 (0.0)	*
North Gila Valley	17.5 (4.9)	96.2 (1.8)	100 (0.0)	100 (0.0)
Parker	28.7 (3.0)	98.7 (1.3)	100 (0.0)	100 (0.0)
Safford	22.0 (9.2)	100 (0.0)	100 (0.0)	100 (0.0)
Stanfield	16.7 (6.7)	100 (0.0)	100 (0.0)	100 (0.0)
Total AZ Collections	*14*	*14*	*14*	*9*
Mean Mortality	*22.3*	*98.4*	*100*	*100*
Corrected Grand Mean Mortality		*97.9*	*100*	*100*
Median Mortality	*20.0*	*98.9*	*100*	*100*
Minimum Mortality	*16.0*	*93.3*	*100*	*100*
Std. Dev. Mortality	*5.95*	*2.08*	*0.00*	*0.00*
Corrected Grand Mean Survivorship		*2.10*	*0.00*	*0.00*
APHIS-S	3.21 (4.3)	74.3 (23)	100 (0)	*

Table 4. Mortality (±SEM) of pink bollworm collected from Arizona cotton in 2000 and tested in diet bioassays incorporating Cry1Ac toxin.

	Concentration Cry1Ac µg/ml diet		
Collection Site	**0**	**1**	**10**
Buckeye	48.9 (10)	98.9 (1.1)	100 (0.0)
Cotton Center N	25.4 (5.3)	100 (0.0)	100 (0.0)
Cotton Center S	24.3 (4.2)	99.3 (0.71)	100 (0.0)
Eloy	10.0 (5.7)	95.0 (2.8)	100 (0.0)
Gila Valley	16.0 (6.5)	99.0 (1.0)	100 (0.0)
Laveen	27.7 (4.8)	99.2 (0.76)	100 (0.0)
Marana	30.0 (10)	95.0 (5.0)	100 (0.0)
Maricopa	34.3 (5.7)	99.3 (0.71)	100 (0.0)
Mesa	21.2 (5.5)	99.4 (0.59)	100 (0.0)
MohaveValley	20.0 (9.4)	98.6 (1.5)	100 (0.0)
Paloma	31.7 (9.0)	100 (0.0)	100 (0.0)
Parker	23.7 (5.9)	94.5 (2.8)	100 (0.0)
Queen Creek	17.1 (4.6)	100 (0.0)	100 (0.0)
Safford	26.7 (8.6)	98.3 (1.2)	100 (0.0)
Stanfield	34.3 (8.2)	100 (0.0)	100 (0.0)
Tacna	40.0 (11)	100 (0.0)	100 (0.0)
Yuma Valley Ag. Center	38.8 (7.2)	90.6 (3.7)	100 (0.0)
Total AZ Collections	*17*	*17*	*17*
Grand Mean Mortality	*27.7*	*98.1*	*100*
Corrected Grand Mean Mortality		*97.4*	*100*
Median Mortality	*26.7*	*99.2*	*100*
Minimum Mortality	*10.0*	*90.6*	*100*
Std. Dev. Mortality	*9.78*	*2.67*	*0.00*
Corrected Grand Mean Survivorship		*2.60*	*0.00*
APHIS-S	*11.5 (8.4)*	*93.5 (5.5)*	*100 (0.0)*

Table 5. Mortality (±SEM) of pink bollworm collected from Arizona, California, New Mexico, and Texas cotton in 2001 and tested in diet bioassays incorporating Cry1Ac toxin.

	Concentration Cry1Ac µg/ml diet		
Collection Site	**0**	**1**	**10**
AZ-Casa Grande	23.4 (9.3)	100 (0.0)	100 (0.0)
AZ-Coolidge	20.0 (3.9)	97.0 (2.1)	97.2 (1.0)
AZ-Cotton Center	11.7 (2.3)	85.6 (6.8)	99.1 (0.67)
AZ-Eloy	14.0 (2.2)	93.0 (2.6)	99.6 (0.40)
AZ-Laveen	20.0 (*)	100 (*)	100 (*)
AZ-Marana Agric. Ctr.	13.0 (4.6)	94.0 (2.4)	100 (0.0)
AZ-Maricopa	11.0 (2.4)	90.0 (6.3)	98.4 (1.6)
AZ-Maricopa Agric. Ctr.	26.7 (4.4)	96.7 (3.3)	100 (0)
AZ-Mesa	27.5 (5.9)	97.5 (2.5)	99.0 (1.0)
AZ-Mohave Valley	25.0 (4.6)	90.0 (3.6)	100 (0.0)
AZ-North Gila Valley	33.3 (8.8)	100 (0.0)	100 (0.0)
AZ-Paloma Ranch	22.5 (5.2)	97.5 (2.5)	96.0 (2.3)
AZ-Parker	17.1 (2.1)	97.1 (1.2)	97.1 (1.2)
AZ-Queen Creek	24.6 (5.4)	99.2 (0.83)	99.3 (0.45)
AZ-Tacna	20.0 (6.3)	85.0 (7.6)	100 (0.0)
AZ-Tonopah	21.1 (4.0)	95.6 (2.9)	99.6 (0.44)
AZ-Yuma Agric. Ctr.	17.9 (4.7)	92.9 (4.2)	99.4 (0.57)
Total AZ Collections	*17*	*17*	*17*
Grand Mean Mortality	*20.5*	*94.7*	*99.1*
Corrected Grand Mean Mortality		*93.4*	*98.9*
Median Mortality	*20.0*	*96.7*	*99.6*
Minimum Mortality	*11.0*	*85.0*	*96.0*
Std. Dev. Mortality	*6.05*	*4.77*	*1.23*
Corrected Grand Mean Survivorship		*6.60*	*1.10*
CA-Blythe/Palo Verde-1	21.4 (1.8)	98.6 (1.4)	100 (0.0)
CA-Blythe/Palo Verde-3	36.7 (6.7)	100 (0.0)	100 (0.0)
NM-Las Cruces-1	16.7 (6.7)	96.7 (3.3)	100 (0.0)
NM-Las Cruces-4	30.6 (4.5)	86.1 (5.8)	90.2 (5.5)
TX-Tornillo	26.1 (5.1)	76.9 (5.8)	89.2 (2.8)
••			
APHIS-S	8.75 (9.5)	98.7 (2.4)	100 (0.0)

* = No variance computed--only one bioassay replication completed

Figure 1. Locations where pink bollworm collections were made from 1997 through 2001 for assessing susceptibility to the Bt toxin Cry1Ac. Pink bollworm were not obtained from each location each year.

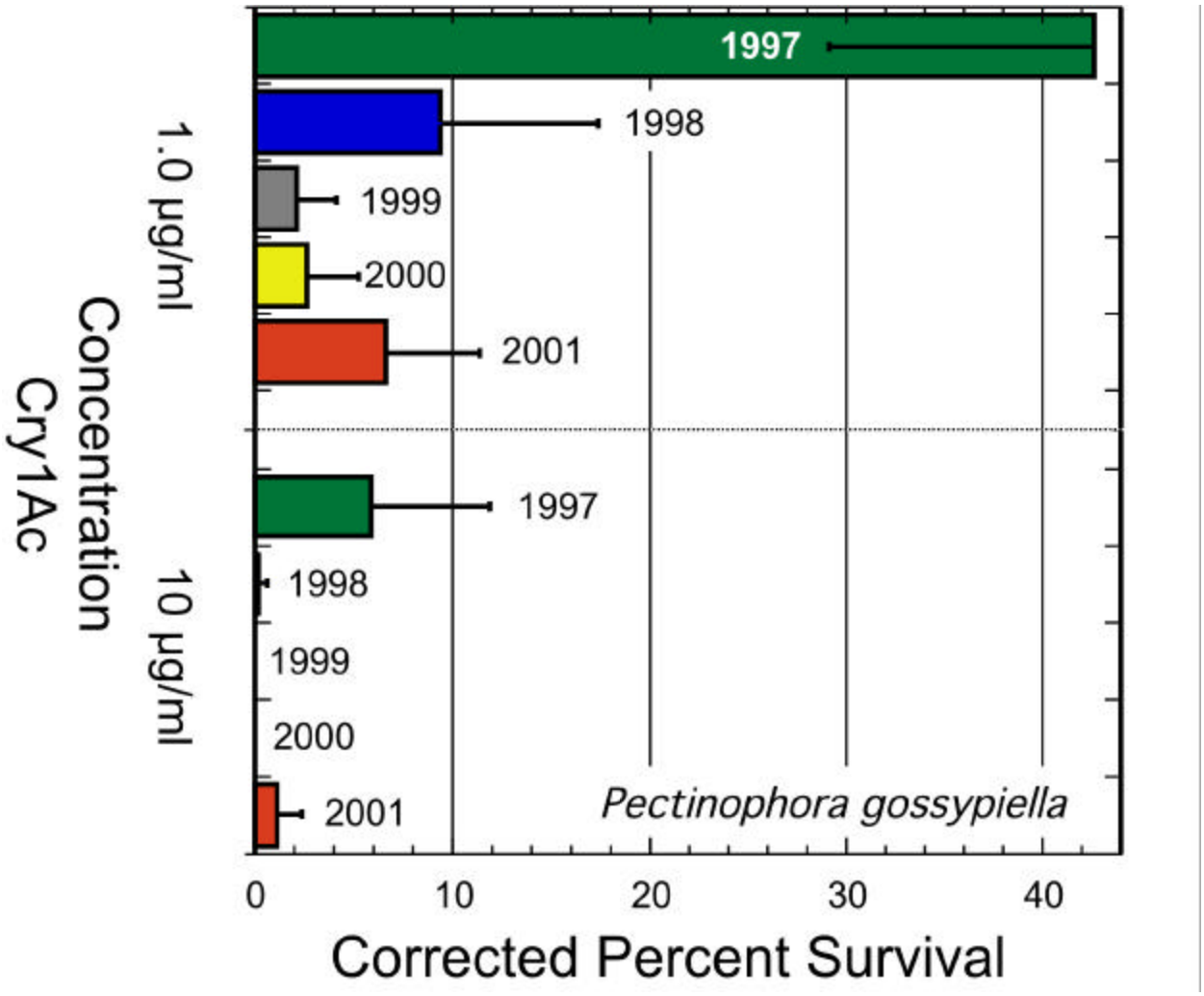

Figure 2. Changes in Arizona pink bollworm susceptibility to Cry1Ac in Arizona from 1997 to 2001. Shown are mean values (± standard deviation) of corrected survival observed in replicated bioassays of 1.0 and 10 µg CrylAc/ml diet of field collections made throughout Arizona in 1997 (n=9), 1998 (n=12), 1999 (n=14), 2000 (n=17) and 2001 (n=17).

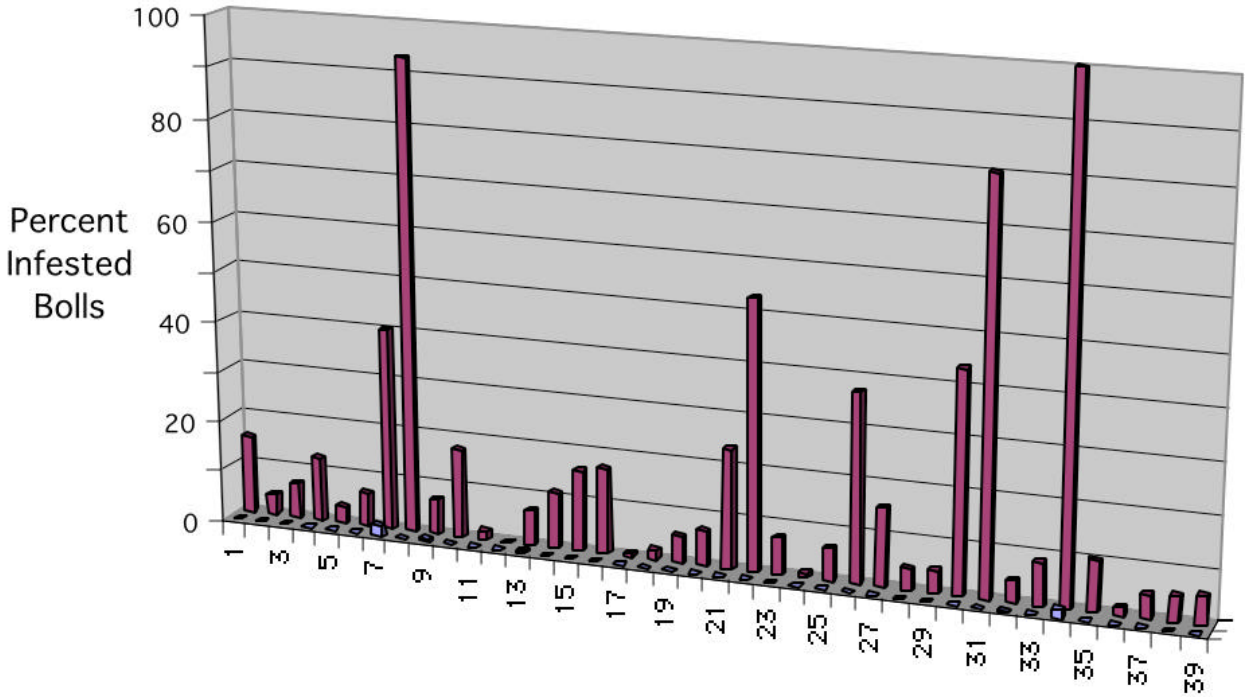

Figure 3. Bt cotton continued to be highly efficacious against pink bollworm in 2001 field evaluations in Arizona. The Arizona Cotton Research and Protection Council sampled bolls from 39 pairs of adjacent Bt (front row of bars) and non-Bt (rear row of bars) cotton fields and recorded bolls as infested that contained large (>3rd instar) pink bollworm larvae, pupae, or exit holes.

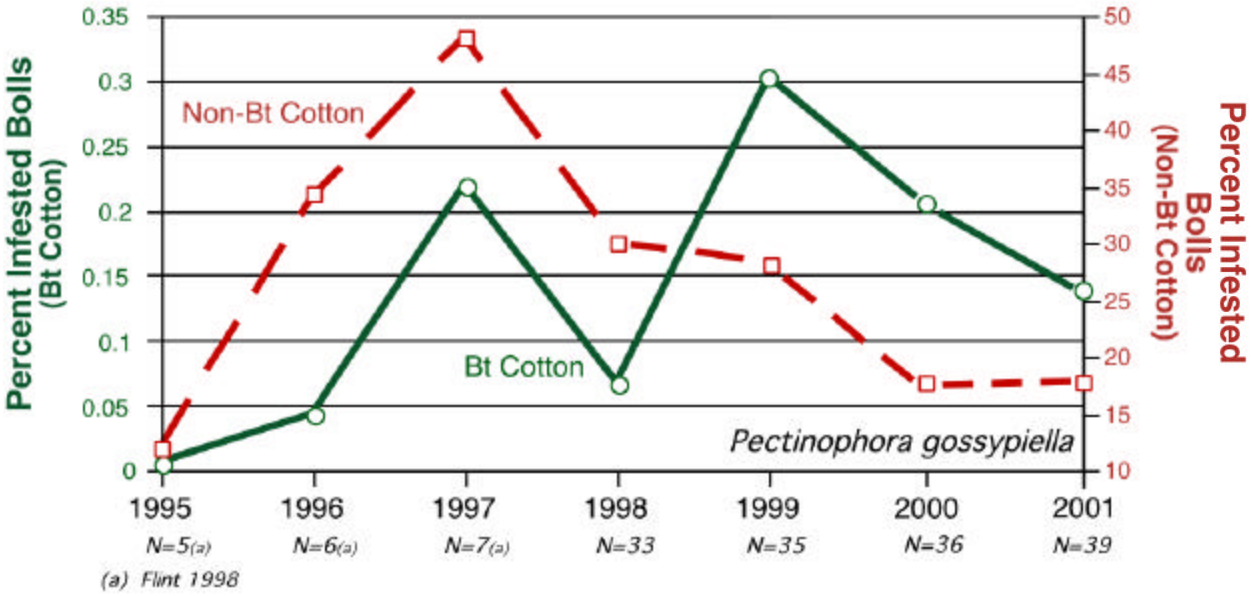

Figure 4. Efficacy of Bt cotton in Arizona: 1995 to 2001. Data from 1995 to 1997 were reported by Flint et al. 1995 and Flint and Park 1996. All other data were collected by the Arizona Cotton Research and Protection Council. Shown are grand means of the percentage infested bolls (>3rd instar larvae, pupae or exit holes) for Bt cotton fields (O—O, left axis) and non-Bt cotton fields (☐—☐, right axis) sampled each year from 1995 to 2001.