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SUSCEPTIBILITY OF ARIZONA PINK BOLLWORM TO CRY1AC

Maria A. Sims, Timothy J. Dennehy, Laura Shriver, Danny Holley,
Yves Carrière and Bruce Tabashnik
Department of Entomology
The University of Arizona
Tucson, AZ
Larry Antilla and Mike Whitlow
Arizona Cotton Research and Protection Council
Phoenix, AZ

Abstract

Genetically modified cotton expressing the Cry1Ac toxin has been used in Arizona since 1996 with exceptionally positive results in terms of economic returns to growers and reductions in insecticide use in cotton. Since 1995, average insecticide use in Arizona cotton has declined from greater than six applications per acre to less than two in 2000. Bt cotton has contributed greatly to these savings to growers, as have insect growth regulators used for whitefly control. Collections of pink bollworm, *Pectinophora gossypiella*, made in 1997 and subsequently exposed to Cry1Ac in the laboratory from 1998 to 2000, yielded a laboratory strain with susceptibility to Cry1Ac reduced 1,000 to 3,000-fold, relative to highly susceptible field populations. Unparalleled measures have been taken to detect and manage this resistance. In this report we summarize results of statewide monitoring of pink bollworm susceptibility to Cry1Ac conducted from 1997 to 2000, and results of field evaluations of the effectiveness of Bt cotton from 1995 to 2001. Susceptibility of Arizona pink bollworm to Cry1Ac, increased from 1997 to 2000. Mean corrected mortality in 1µg/ml Cry1Ac assays was 57.4% in 1997, 90.6% in 1998, 97.9% in 1999 and 97.4% in 2000. Mean corrected mortality in bioassays of 10 µg/ml also increased: it was 94.1% in 1997, 99.9% in 1998, 100% in 1999 and 100 % in 2000. Field performance of Bt cotton in 2000 continued to be excellent at 39 locations throughout Arizona cotton at which paired Bt and non-Bt fields were evaluated. Whereas non-Bt cotton fields had mean infestations of over 15% infested bolls, Bt cotton fields averaged less than 0.15% infested bolls. Thus, after six years of intensive use of Bt cotton in Arizona, pink bollworm populations show no signs of being resistant to Bollgard cotton. Indeed, for reasons that are not understood at this time, they have been found to be significantly more susceptible to the Bt toxin in Bollgard cotton at the end of the 2000 season than they were in 1997.

Introduction

Registration of Bt cotton in the US in 1996 marked the beginning of the era of genetically modified agricultural plants expressing biopesticides. The major insect pest of Arizona cotton, pink bollworm (*Pectinophora gossypiella*) 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 (Table 1, Figure 1). Additionally, the environment and integrated pest management have benefitted from the associated decreases in use of conventional insecticides. In 1995, the year preceding registration of Bt cotton, an average of over 6 insecticide applications were made per acre of cotton in Arizona (Figure 2). Insecticide use in Arizona cotton has declined each year since 1995, reaching a low of less than 2 treatments per acre in 2000. These dramatic reductions in insecticide use are attributable to both insect growth regulators used for whitefly control and to Bt cotton used to control pink bollworm.

Industry, academics, governmental regulators, and environmental groups have given unprecedented attention to insect resistance to transgenic Bt crops (Mellon and Rissler 1998, Carrière et al 2001). The strategy for thwarting pink bollworm resistance to Bt cotton is based on 1) plants expressing Bt toxin levels sufficiently high to result in extreme mortality of resistant heterozygotes and 2) label regulations requiring planting of refuges of non-Bt cotton to preserve susceptible pink bollworms. However, with the benefit of the hindsight afforded from six years of relatively intensive use of Bt cotton in many areas of central Arizona, it is now possible to resonably conclude that our worst fears regarding rapid resistance development have not materialized. No failures of Bt cotton have been observed in Arizona, nor have resistance-related failures been reported with other target pests elsewhere in the US. Thus our perspective on the feasibility of sustaining this powerful new technology has brightened considerably over the past six years and we are now asking questions about the feasibility of sustaining activity of the first generation of Bt cotton for a decade or more. There are, however, no grounds for complacency regarding monitoring and proactive management of resistance to Bt cros. Resistance continues to be the greatest single threat to the future of transgenic insecticidal plants.

Preserving insect susceptibility to Bt crops is judged by many to be the greatest challenge ever addressed by resistance management. This is due, in large measure, to the many months that insecticidal toxins are produced in plants. Managing

pink bollworm resistance to Bt cotton seemed all the more unlikely in Arizona cotton following the discovery of pink bollworm highly resistant to the Bt toxin produced by Bollgard® cotton, Cry1Ac (Bartlett 1995, Patin et al. 1999, Sims et al. 2001). It was with the many benefits and high resistance risks of Bt cotton in mind, that we established the multi-agency collaboration that produced the results described herein. In this paper we summarize 1997-2000 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.

I. Statewide Monitoring of Resistance

Materials and Methods

Susceptibility of Arizona PBW to the Bt Endotoxin, Cry1Ac

Collection. Collections from Arizona cotton fields (Figure 3) commenced as early as August and continued through as late as December each year. 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 from 12 sites 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 North, Cotton Center South, Eloy, North Gila Valley, Laveen, Marana, Maricopa, Mesa, Mohave Valley, Paloma, Parker, Queen Creek, Safford, Stanfield, Tacna, and Yuma.

At each location 300 to 2,000 bolls were collected, from non-Bt cotton fields in areas adjacent to Bt fields. In only three instances was it possible to collect pink bollworm from Bt cotton. In these three cases (Eloy Bt 1997, Coolidge Bt 1999, Mohave Valley Bt 1999) pink bollworm were found surviving in the upper bolls (second fruiting cycle) of Bt cotton. 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). F1 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. No bioassays of 100 µg Cry1Ac/ml were conducted in either 1997 or 2000. 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 g of diet per cup dispensed into 1 oz medicine cups with tight fitting lids. Neonate larvae were placed individually in the 1 oz cups and the tops were affixed. Subjects were assigned to replicates consisting of 10 bioassay cups for each concentration. Bioassay cups were placed in egg cartons and 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 per replication for each concentration tested. 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 F4-F5 (rarely 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, and 3336 in 1997, 1998, 1999, and 2000, respectively. These comprised averages of 60 to 70 larvae tested per concentration for each collection evaluated.

Results and Discussion

1997 Collections. 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 2). Corrected mortality for these collections was 57.4% at 1.0 µg/ml and 94.1% at 10 µg/ml. Thus, when corrected for mortality in controls, ca. 43% of 1997 collections survived exposure to 1.0 µg/ml. More importantly, five of the nine collections had survivors of 10 µg/ml bioassays. These survivors were bulked and resistance was selected in the laboratory by rearing them on diet into which Cry1Ac concentrations beginning at 10 µg/ml and increasing to as high as 320 ug/ml was incorporated form 1998 to 2000. As of 2000, this resistant laboratory culture, AZP-R, is 1000- to 3000-fold less susceptible to Cry1Ac than susceptible field collections of pink bollworm (Sims et al 2001, Tabashnik et al. 2000, Dennehy unpublished results). In greenhouse trials, AZP-R had approximately 40-50% survival on Bt cotton, relative to survival on non-Bt cotton. Importantly, resistant heterozygotes were shown not to survive on Bt plants (Liu et al 1999, Liu et al 2002). Thus, resistance to Cry1Ac in AZP-R is functionally recessive.

1998 Collections. A total of twelve populations collected in 1998 were successfully reared and evaluated, including one from California (Table 3). These had mean mortality of 19.8%, 92.5%, 99.9%, and 99.9% in bioassays of 0, 1.0, 10 and 100 µg/ml, respectively. Corrected 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. 9.0% of 1998 collections survived exposure to 1.0 µg/ml Cry1Ac, and 0.1% survived the 10 and 100 µg/ml assays (Table 3).

1999 Collections. Fourteen collections of Arizona pink bollworm were successfully reared and evaluated in 1999 (Table 4). Mean mortality was 22.3%, 98.4%, 100% and 100% in bioassays of 0, 1.0, 10, and 100 µg/ml, respectively. Thus, when corrected for mortality in controls, ca. 2% of the 1999 collections survived exposure to 1.0 µg/ml Cry1Ac and there were no survivors of 10 or 100 µg/ml assays (Table 4).

2000 Collections. 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 5). 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 2000 collections survived exposure to 1.0 µg/ml and none of the 17 collections evaluated had survivors of 10 µg/ml bioassays.

Changes 1997-00. Arizona pink bollworm were significantly less susceptible to Cry1Ac in 1997 than 1998, 1999 or 2000 in bioassays of 1.0 µg Cry1Ac/ml (Tables 2-4). 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. Survivorship of pink bollworm was also significantly greater in bioassays of 10 µg/ml in 1997 versus 1998, 1999 , or 2000. Analysis of the 1997 data by Tabashnik et al. (2000) yielded an average proportion of 0.16 (95% confidence interval = 0.05-0.26) for a recessive resistance allele conferring resistance to Cry1Ac. At this time we cannot explain why the frequency of alleles for this resistance have decreased, rather than increased, in the face of intensive use of Bt cotton in some areas of Arizona (Figure 1).

II. Field Performance of Bt Cotton in Arizona

Documenting the field performance of Bt cotton is an important objective of our multi-agency collaboration. 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. Prior to that Dr. Hollis Flint of the USDA-ARS Western Cotton Research Laboratory evaluated baseline efficacy of Bt cotton at five to seven locations per year from 1995 to 1997 (Flint et al. 1996, Flint and Parks 1999).

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 non-Bt field and 800-1000 were collected from the Bt field in the pair. Boll were brought to the ACRPC in Phoenix, where they were placed in boll boxes. As time allowed during the subsequent 30 days, all bolls were cracked within the subsequent 30 days and the number of large ($\geq 3^{rd}$ instar) pink bollworm or exit holes were recorded for each boll. Mean percent boll infestation was computed for each Bt and non-Bt pair of fields. A grand mean of boll infestation was then computed from the total number of pairs of fields evaluated each year.

Results and Discussion

Bollgard cotton continued to perform extremely well in Arizona through the 2001 season. Pink bollworm infestations varied widely across all 39 non-Bt fields evaluated (Figure 4). Sixteen non-Bt fields had $\geq$ 20% infested bolls in 2001. The maximum infestation level observed in Bt cotton was 2.2 % in 2001 and this was found in a Bt field in which the adjacent non-Bt cotton had 40% infestated bolls. Importantly, all putatively Bt bolls in which large larvae were found tested negative for the presence of Cry1Ac toxin (R. Webb, unpublished data).

The grand mean pink bollworm infestation rate in 2001 (*N*=39 pairs) was 17.8% infested bolls in non-Bt cotton and 0.136% in Bt cotton. In other words, a sample of 1000 bolls from a Bt field averaged only 1 large pink bollworm larva while a sample of 1000 bolls from a non-Bt (refuge) field averaged 178 large larvae. Thus, efficacy of Bt cotton in Arizona in the 2001 season was exceptional and indistinguishable from the previous five seasons during which Bt cotton has been used commercially in Arizona.

Conclusions

In this report we summarize results of statewide monitoring of pink bollworm susceptibility to the Bt toxin, Cry1Ac, conducted from 1997 to 2000 and field evaluations of the effectiveness of Bt cotton from 1995 to 2001. Susceptibility of Arizona pink bollworm to the Bt toxin in Bollgard cotton, Cry1Ac, increased from 1997 to 2000. Mean corrected mortality in 1µg/ml Cry1Ac assays was 57.4% in 1997, 90.6% in 1998, 97.9% in 1999 and 97.4% in 2000. Mean corrected mortality in bioassays of 10 µg/ml also increased; it was 94.1% in 1997, 99.9% in 1998, 100% in 1999 and 100 % in 2000. Field performance of Bt cotton in 2000 continued to be excellent at 39 locations throughout Arizona cotton at which paired Bt and non-Bt fields were evaluated by the Arizona Cotton Research and Protection Council. Whereas non-Bt cotton fields had mean infestations of over 15% infested bolls, Bt cotton fields averaged less than 0.15% infested bolls. Thus, after six years of intensive use of Bt cotton in Arizona, pink bollworm populations show no signs of being resistant to Bollgard cotton.

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Table 1. Estimated use of *Bt* cotton in Arizona 1996 to 2000.

Year	***Percent Bt Cotton***	***Total Cotton Acreage***
1996	<20	355,900
1997	50	341,000
1998	61	253,688
1999	55	278,745
2000	62[a]	285,000

[a] Preliminary estimate

Table 2. 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 collections	*10*	*10*	*10*
Mean mortality	*30.2*	*70.2*	*95.9*
Corrected mortality		*57.4*	*94.1*
Median	*28.3*	*66.9*	*99.0*
Minimum Value	*17.3*	*55.7*	*82.5*
Std. Dev.	*9.73*	*13.5*	*5.94*
APHIS-S	22.0 (8.3)	73.6 (6.0)	100 (0.0)

Table 3. 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 S	18.0 (3.9)	75.0 (5.0)	100 (0.0)	100 (0.0)
Cotton Center N	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 collections	*12*	*10*	*12*	*12*
Mean mortality	*19.8*	*92.5*	*99.9*	*99.9*
Corrected mortality		*90.6*	*99.9*	*99.9*
Median	*18.9*	*94.1*	*100*	*100*
Minimum Value	*4.00*	*75*	*98.6*	*99.2*
Std. Dev.	*7.42*	*7.99*	*0.419*	*0.25*
APHIS-S	6.25(2.3)	63.7(8.0)	100(0.0)	*

Table 4. 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)	*
N 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 collections	*14*	*14*	*14*	*9*
Mean mortality	*22.3*	*98.4*	*100*	*100*
Corrected mortality		*97.9*	*100*	*100*
Median	*20.0*	*98.9*	*100*	*100*
Minimum Value	*16.0*	*93.3*	*100*	*100*
Std. Dev.	*5.95*	*2.08*	*0.00*	*0.00*
APHIS-S	3.21 (4.3)	74.3 (23)	100 (0)	*

Table 5. 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 (5.55)	99.4 (0.588)	100 (0.0)
MohaveValley	20.0 (9.43)	98.6 (1.54)	100 (0.0)
Paloma	31.7 (9.03)	100 (0.00)	100 (0.0)
Parker	23.7 (5.92)	94.5 2.82)	100 (0.0)
Queen Creek	17.1 (4.62)	100 (0.00)	100 (0.0)
Safford	26.7 (8.67)	98.3 (1.23)	100 (0.0)
Stanfield	34.3 (8.24)	100 (0.00)	100 (0.0)
Tacna	40.0 (11.0)	100 (0.00)	100 (0.0)
Yuma Ag. Center	38.8 (7.17)	90.6 (3.69)	100 (0.0)
Total collections	*17*	*17*	*17*
Mean mortality	*27.7*	*98.1*	*100*
Corrected mortality		*97.4*	*100*
Median	*26.7*	*99.2*	*100*
Minimum Value	*10.0*	*90.6*	*100*
Std Dev.	*9.78*	*2.67*	*0.00*
APHIS-S	11.5 (8.4)	93.5 (5.5)	100 (0.0)

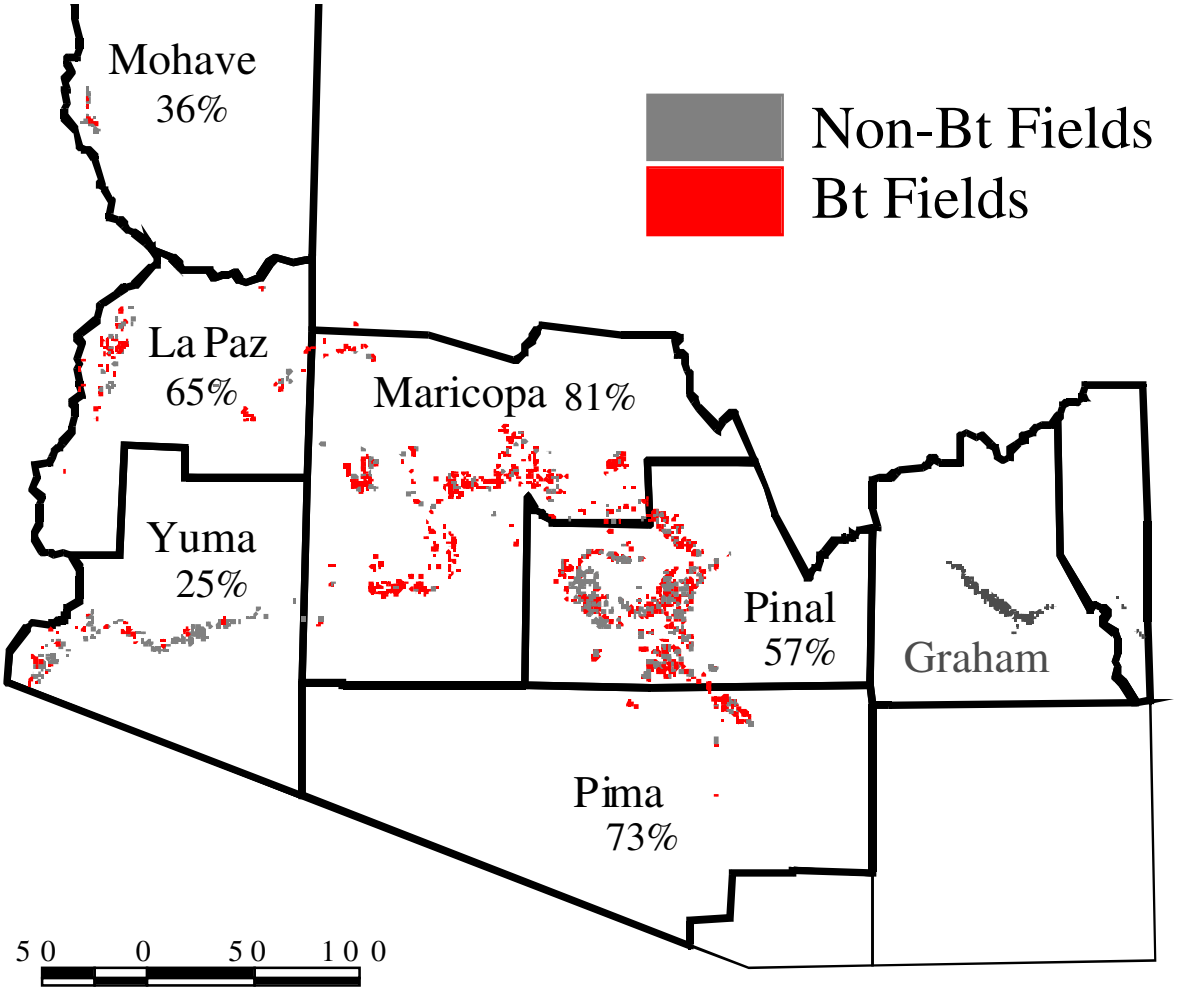

Figure 1. Census information collected by the Arizona Cotton Research & Protection Council identifying the location of Bt cotton (red) and non-Bt cotton (gray) throughout Arizona in 2000. Analysis of use patterns will permit characterization of conditions that give rise to resistance and evaluation of compliance with refuge requirements. Note the large differences between counties in use of Bt county (values shown). Scale is in kilometers.

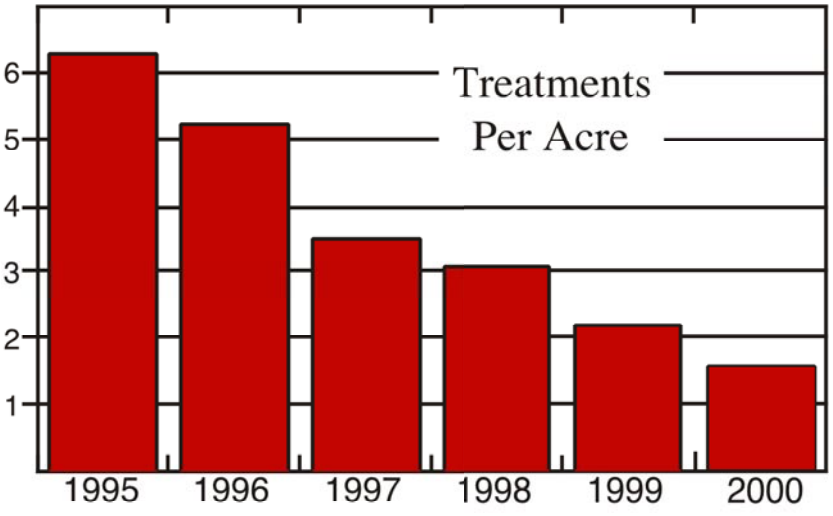

Figure 2. Estimated numbers of insecticide treatments applied to Arizona cotton, 1995-00. Adapted from: Agnew et al. 2000, and L. Shanley, personal communication.

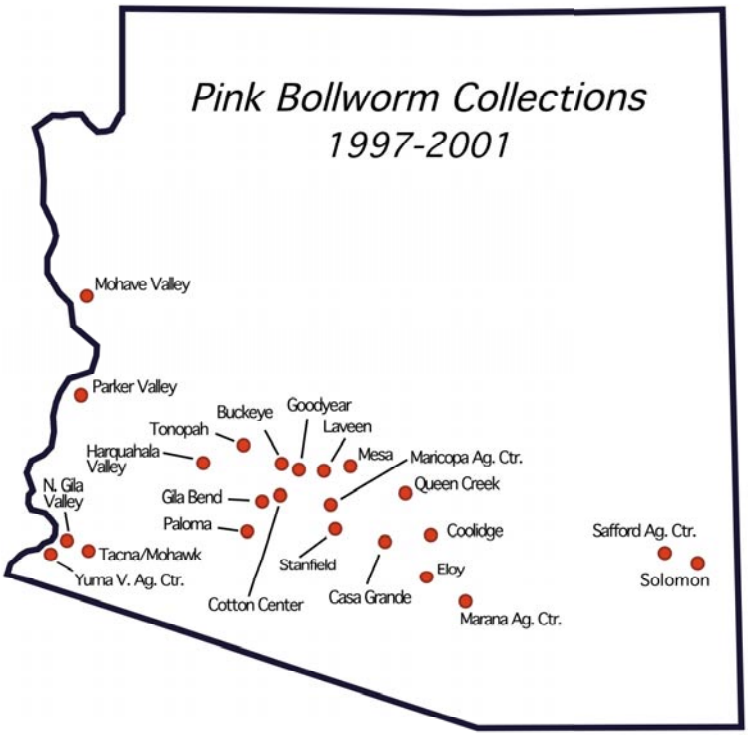

Figure 3. Locations where pink bollworm collections were made from 1997 through 2001 for assessing susceptibility to the Bt toxin, Cry1Ac. Pink bollworm were not found at each location each year.

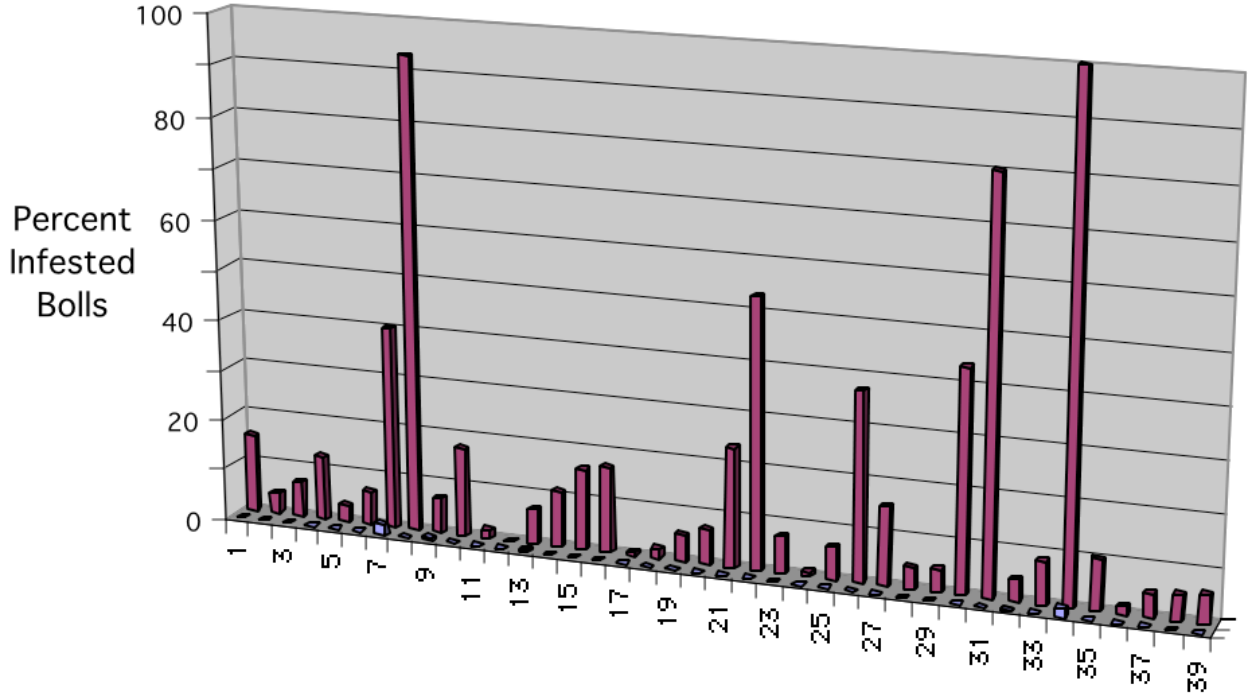

Figure 4. Evaluations of the efficacy of Bt cotton in Arizona conducted by the ACRPC in 2001. Bolls were sampled from 39 pairs of adjacent Bt and non-Bt cotton fields. Shown is the ratio of large (3rd instar or larger) pink bollworm and exit holes per boll in each field.