EVALUATION OF B.T. COTTON DEPLOYMENT STRATEGIES AND EFFICACY AGAINST PINK BOLLWORM IN ARIZONA

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Abstract

A multi-agency team in Arizona in 1997 evaluated B.t. cotton deployment strategies in a large field trial; conducted statewide monitoring of pink bollworm (PBW) susceptibility to the Cry1Ac endotoxin, and established a Rapid Response Team that investigated claims of unacceptable performance of B.t. cotton. Though needing further evaluation, in-field refuges of one row of non-B.t. cotton for each five rows of B.t. cotton showed promise as an alternative to the current recommendation of external refuges for planting B.t. cotton. Preliminary results of statewide monitoring showed that four field populations were more susceptible to Cry1Ac than were two reference susceptible laboratory strains. A strain of PBW previously reported to be resistant to Cry1Ac was confirmed to be significantly less susceptible to this toxin than were the two susceptible laboratory strains or the four field populations tested. The Rapid Response Team, based at the Arizona Cotton Growers Association, investigated nine reports of unusual larval survivorship in B.t. cotton. Only one of these, which has been placed in culture, was confirmed to have resulted in substantial numbers of large larvae surviving in bolls of putatively B.t. cotton. Further investigations of this population and the plants from which it was derived are underway.

Introduction

Cotton varieties that express insecticidal proteins derived from Bacillus thuringiensis, B.t. cotton (Perlak et al. 1990), offer many benefits including: reduced environmental and worker exposure to conventional insecticides, reduced selection for resistance to conventional insecticides, and improved conservation of natural enemies. Cotton accounts for nearly half of all the insecticide use in U.S. row crops. In Arizona alone, the annual cost of insecticide treatments in cotton can exceed 60 million dollars and up to $25 per acre can be spent on controlling pink bollworm (PBW), Pectinophora gossypiella. Thus, reducing the use of conventional, broadly toxic insecticides in cotton through the use of the biological insecticide, B.t., could have a major impact on worker safety and environmental pesticide load in the desert Southwest. Indeed, genetically engineered B.t. cotton has already dramatically decreased conventional insecticide treatments in Arizona cotton and is anticipated to be planted on as much as 80% of Upland cotton acreage in the coming season. However, this benefit will be short-lived if key pests such as PBW develop resistance to B.t. toxin.

Considerable controversy surrounds predictions about development of resistance to genetically engineered plants that express insecticidal toxins. On the most optimistic side is the view that refuges will thwart resistance development in many pests. The pessimistic view is that our current technology results in such prolonged and intense selection that severe resistance problems are assured in key pests. Findings from diamondback moth (Tabashnik et al. 1990), Indianmeal moth (McGaughey and Johnson 1987) and other insects (Stone et al. 1989) confirm that resistance can develop to B.t. and that some cases of broad cross-resistance extend to many of the B.t. toxins (Gould et al. 1992). Although sustainable deployment of B.t. cotton offers significant benefits to consumers, farm workers, and growers, the scientific foundation for managing resistance to B.t. cotton is weak. In particular, rigorous data from the field are sorely lacking.

The resistance management strategy for B.t. cotton in the U.S. is derived from theoretical models that have not been tested in the field. The models use biological parameters for bollworm (Helicoverpa zea (Boodie)) and budworm (Heliothis virescens (F.)), not Arizona's key lepidopteran pest, PBW. Therefore, while all areas of the cotton belt urgently need to test resistance management strategies, the need in Arizona is arguably greatest because our key pest has not been the focus of most of the mathematical models and simulations. We are conducting a long-term field evaluation of the most promising options for deploying B.t. cotton in Arizona. With these contrasts we hope to improve deployment of future genetically-engineered products and to respond most appropriately to manage resistance once it occurs in Arizona. Therefore, the objectives of this project are to foster the goal of sustainable use of this powerful new technology, in Arizona. To that end we are: 1) evaluate deployment strategies for B.t. cotton in the field, 2) establish statewide monitoring of PBW susceptibility to B.t., and 3) establish a multi-agency Rapid Response Team to investigate unusual survivorship of PBW in B.t. cotton.

Materials and Methods

Field Trial to Evaluate B.t. Deployment Strategies

A 200-acre field trial was established with the cooperation of cotton growers Lacho and Arnold Burruell in Eloy,

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1025-1030 (1998)
National Cotton Council, Memphis TN

Arizona. These growers agreed to conduct a three year evaluation of contrasting B.t. deployment strategies. Each of six treatments were replicated twice and comprised ca. 20 acres each (Figure 1). The treatments were: 1) In-field refuges- (5 rows B.t.: 1 row non-B.t.); 2) Rotation in-Years (all B.t. in '97, non-B.t. in '98, and '99 predicated on field pressure in '98); 3) Biointensive (B.t. + parasitic nematodes); 4) External refuges (Monsanto strategy -20% non- B.t. refuge); 5) 100% B.t.; and 6) Control (100% non-B.t.). The varieties of cotton grown in the different treatments were determined based on grower preference and restrictions imposed by seed production. The in-field refuge treatment (treatment 1) was 5 rows of NuCotn 33B to each row of SureGrow 125 (non-B.t). This was accomplished by designating one hopper of a six row planter for non-B.t. seed. Treatments 2, 3, and 5 were planted to Hartz 1560BG. Treatment 4 was of 80% B.t. (Hartz 1560BG) and 20% non-B.t. (Hartz 1560). Treatment 6 was planted with the non-B.t. seed (Hartz 1560). The field trial was planted on April 7th-15th, 1997.

An insecticide spray, of 2 1/2 pints Penncap-M and 1/4 lb Lannate was applied on August 1st for lygus control. A second insecticide spray of, 2 1/2 pints Penncap-M and 1 1/2 lb Lannate was applied on August 20th for lygus control. Both applications were made across all treatments. Two insecticide sprays of 2 1/2 pints Penncap-M were applied on August 12th and August 19th to control plots only, for PBW control.

Monitoring PBW Infestations

Delta pheromone traps were used to monitor adult PBW populations in all plots throughout the season. Traps were serviced and pheromone septa were replaced weekly.

Throughout July rosetted blooms were censured once per week. In the center 20% of each plot, 40 paces were walked along a single row located in the middle of each plot. Rosetted bloom counts were made between 10 AM and 2 PM.

PBW levels were monitored weekly in all plots beginning the 3rd of July. Fifty 'susceptible' bolls approximately 2.5 cm in diameter, were sampled from the central 20% of each replication. The bolls were transported back to the laboratory where they were refrigerated until the bolls could be cracked and the number and instar of the infesting PBW found could be recorded. Bolls were cracked within one week of collection. In Treatment 1 (in-field refuge) and Treatment 4 (external refuge) collections were made independently from the B.t. and non-B.t. cotton rows on each sampling date.

Biointensive Treatment-Parasitic Nematode Applications

The parasitic nematode, Steinernema carpocapsae is an effective biological control of PBW (Gouge et. al, 1997). It attacks the larvae of PBW once they cut out from bolls and enter the soil to pupate. The biointensive treatment involved the combined effects of B.t. cotton and parasitic nematodes.

Forty cassettes containing cut-out stage PBW larvae were buried a few inches below the surface to estimate the mortality imposed on PBW by the parasitic nematode treatments. These were arranged in four lines of five cassettes each, on the top of the furrows and four lines of five cassettes each, at the bottom of the furrows. The nematodes were obtained from Thermo Trilogy as a water dispersible granule. During irrigation on August 11, a nurse tank containing 950 gallons of water and six billion nematodes was emptied into the irrigation canal. Continuous agitation in the nurse tank maintained a nematode suspension that was released into the irrigation canal. This dispersed the nematodes throughout the treatment. Forty-eight hours after irrigation the cassettes were collected and the percentage parasitism was scored. Treatment 3 Replicate 2 was the only biointensive plot treated due to heavy rains which prevented further controlled irrigation of the treatment fields.

Bioassaying PBW Susceptibility to the B.t. Endotoxin [Cry1A(c)]

We intended to bioassay PBW from each of the six treatments and, where relevant, from B.t. and non-B.t. plants of treatments. This proved impractical due to the very low numbers of larvae surviving in all but two of the B.t. cotton treatments. By late September we detected areas of 2-4% survivorship of late instar PBW ($\geq$ 3rd instar) in replicates of B.t. cotton of Treatments 3 and 4. On October 14-16 2000 bolls were collected from Treatment 4 and a like number from the non-B.t. portion of Treatment 4 and from our control plots (non-B.t., Treatment 6). Bolls were cracked and larvae were placed into culture and bioassayed, as detailed below, for susceptibility to the Cry1Ac endotoxin expressed in Bollgard® cotton.

Statewide Monitoring of PBW Susceptibility to the B.t. Endotoxin [Cry1A(c)]

PBW collections commenced on August 20th and continued through November. Collections were made at Parker, Safford, Mohave Valley, Coolidge, Marana, Paloma, Stanfield and Eloy, Arizona. At the time of reporting four of the eight field populations have been evaluated.

Boll Collection

Bolls infested with PBW were collected from non-B.t. cotton fields adjacent to B.t. fields. At each location, 1,000 to 2,000 bolls were sampled and transported to UA Extension Arthropod Resistance Management Laboratory (EARML) in Tucson, where they were placed in boll boxes. The boll boxes suspend infested bolls approximately 3 cm above sheets of paper towel. Just prior to pupation the larvae cut out of infested bolls, drop down onto the toweling, and commence pupating. Fourth instars which had not pupated were transferred to pupation boxes, tightly sealed 2 quart Rubbermaid containers enclosing sheets of

paper towel. The PBW in the pupation boxes were disturbed by pulling paper towels apart. This was done twice per week to promote pupation and deter diapause. After 14 days, if a minimum of 200 larvae had not exited from a sample of bolls in boll boxes, the bolls were cracked and the remaining larvae collected. These larvae were then put into culture.

Rearing of PBW

Rearing method of Bartlett and Wolf (1985) was followed. Twice a week pupae collected from boll and pupation boxes were transferred to 8 oz. disposable containers. Adults emerged in the containers, fed on a 5% sucrose solution, and began laying eggs within 3-4 days. Eggs were deposited onto 2.5 cm x 2.5 cm sheets of paper towel placed over the nylon screening of the container lids. Eggs were harvested twice per week. Groups of egg sheets were stapled to the lids of 16 oz paper containers half filled with wheat germ diet obtained from the USDA Western Cotton Research Laboratory Phoenix, AZ. These containers were then placed atop two hexcel, honey combed cardboard sheets, within plastic boxes that had been fitted with screened lids. After 21 days, PBW burrowed out of the paper containers and pupated within the hexcel sheets. Pupae were gathered and placed into 8 oz containers to emerge as adults, initiating another cycle of egg laying.

Bioassaying PBW Susceptibility to B.t.

Bioassays involved placing neonate larvae on a wheat germ diet. into which the Cry1Ac toxin had been incorporated, and evaluating mortality after 21 days. MVP® II Bioinsecticide (Mycogen, San Diego, CA) was mixed into sterilized distilled water to produce a stock solution of toxin. The stock was then added to liquid wheat germ diet in amounts necessary to create final concentrations ranging of 0.001 to 100 µg/ml Cry1Ac. Solutions were blended thoroughly into hot (not exceeding 60°C) liquid PBW diet. Concentrations of toxin used in 1997 for routine monitoring were 0, 0.001, 0.01, 0.1, 1, and 3.2 µg Cry1Ac toxin /ml of diet. Diet was made in 1 liter batches of each concentration evaluated. It was then shredded and dispensed into 1oz cups (Bartlett, 1995). One neonate larva was transferred with a fine brush into each cup. Using this procedure we aimed to test four replicates of twenty five larvae for each concentrations, i.e., 100 subjects per concentration. Bioassays were incubated in darkness at 29+/-2° C for 21 days, after which survivorship and developmental stage (Watson and Johnson 1974) were recorded.

Rapid Response Team

Under the guidance of the Arizona Cotton Research and Protection Council (ACRPC) a Rapid Response (RR) Team was established to investigate swiftly and systematically reports of problems with PBW control in B.t. cotton in Arizona. To inform the growers of this development an educational bulletin was produced. It provides the telephone number of the ACRPC office to report unusual PBW survivorship, details a boll sampling procedure, and the criterion for determining what constitutes unusual PBW survivorship in cotton. The receptionist at ACRPC was provided a standard questionnaire for handling calls about unusual survivorship of PBW in B.t. cotton. The first response was for an ACRPC District Supervisor to meet with the grower/PCA at the field location to diagnose the problem. The supervisor sampled at least 100 bolls in the putative problem area and forwarded an affirmative or negative report to ACRPC. When an affirmative report was filed, RR Team member visited the site within the same week to obtain a second opinion. If confirmatory evidence of unusual survivorship was obtained, then a 2,000 boll sample was collected and transported to EARML in Tucson.

Results and Discussion

Evaluation of B.t. Deployment Strategies: Eloy Trial

Pheromone Trap Catch

PBW trap catches ranged from moderate to high throughout the season (Figure 2) and were relatively similar across treatments during any given week. These results show that all treatments were subjected to PBW throughout the season and that late-season numbers increased sharply.

Rosetted Blooms

No rosetted blooms were observed in any plots.

Larval Infestations

No large PBW larvae (≥ 3rd instar) were found prior to September 14 in any of the six treatments evaluated at the Eloy location. On the 14 September sampling, five larvae were detected in the non-B.t. portion of Treatment 4, the external refuge treatment. No large larvae were detected in any other treatments on this date.

September 20 was the final date on which bolls were sampled for the purpose of estimating PBW densities. This was because the process of defoliation had begun; irrigation had been previously terminated and plants had begun to wilt and drop leaves. Collections made on September 20 yielded large larvae (≥ 3rd instar) in both B.t. and non-B.t. plots (Figure 3). As anticipated, high rates of damage were found in the control plots (non-B.t., Treatment 1), as well as in the non-B.t. portions of Treatment 4, the external refuge plots (Figure 3).

In-Field Refuges Vs. External Refuges

The in-field refuge plots (Treatment 1), for which one of every six rows was non-B.t. cotton with adjacent rows of B.t. cotton, revealed strikingly lower damage to non-B.t. plants, presumably from being interspersed within the B.t. cotton. Whereas non-B.t. plants of the control and external refuge plots had 47 and 20 large larvae per 100 bolls, respectively, on September 20, non-B.t. rows of the in-field refuge plots had only 1 large larva per 100 bolls (Figure 3).

Though systematic sampling of plots was terminated on September 20 for the reasons previously detailed, subsequent collections of bolls in October from the in-field refuge plots revealed a top crop very highly infested with large PBW larvae in the non-B.t. plants. We concluded that non-B.t. plants in the in-field refuge produced substantial numbers of PBW in the top crop but, along with the non-B.t. plants of the control and external refuge treatments, had non-detectable infestations prior to mid-September.

Science currently cannot provide precise answers regarding the optimal size, configuration and placement of refuges for thwarting resistance to B.t. cotton. We do know, however, that for refuges to be effective they must produce susceptible adult moths in sufficiently high numbers and in sufficiently close proximity to areas of B.t. cotton to ensure that survivors of B.t. plants mate with susceptible individuals. In-field refuges of non-B.t. cotton offer the advantage over external refuges of placing susceptible PBW systematically throughout treated fields. They also simplify some of the complex decisions that growers must make regarding placement of external refuges, and in doing so could potentially reduce problems with non-compliance with the current external refuge strategy. What we do not know is whether single-row in-field refuges produce enough susceptible moths. Additional trials of in-field refuges will be evaluated in the coming year.

Homogeneous mixtures of B.t. and non-B.t. cotton are an alternative to the systematic placement of rows of non-B.t. within B.t. fields. Homogeneous mixtures comprising 10-20% non-B.t. seed were evaluated previously in Arizona (Watson, 1995) and were judged to be promising. However, a significant limitation of seed mixtures stems from not being able to know which plants in a field are non-B.t. When large larvae are found, systematic placement of non-B.t. rows allows scouts to estimate their densities independently in the non-B.t. and B.t. plants. This will be especially important for diagnosing putative resistance problems. Placing visible leaf markers (e.g., leaf color or shape) in the non-B.t. variety used would simplify management of in-field refuges. Only through additional field contrasts of B.t. deployment strategies, whether they be heterogeneous mixtures of B.t. or non-B.t. seed or systematic placement of rows of non-B.t. within B.t. fields, will we be able to discern whether production of susceptible moths within in-field refuges is likely to thwart PBW resistance to B.t. more effectively than the currently required external refuges. Growers acceptance of in-field refuges is most likely to hinge on yield and quality of the crop. This will be the focus of further field evaluations in the coming year.

Biointensive Treatment: Application of Parasitic Nematodes

Field bioassays of efficacy of the nematode treatment, using 4th instar PBW larvae buried in cassettes, showed that the nematodes killed an estimated 87% of this stage.

Unusual PBW Survivorship in B.t. Plots

On September 20, four and one large PBW larvae were discovered in samples of 100 bolls collected from B.t. plants in the biointensive (Treatment 3) and external refuge (Treatment 4) plots (Figure 3). The larvae found in the biointensive treatment were from replicates treated and untreated with parasitic nematodes. Collections of 200 and 1000 bolls from B.t. plants of the biointensive (Treatment 3) and external refuge (Treatment 4) were subsequently made on October 13, 1997. Treatment 3, yielded 3% and Treatment 4, yielded 4.1% bolls infested with large larvae. Care was taken to sample only a single boll from any given plant. Surviving large larvae were placed into culture and are presently being tested for susceptibility to B.t. Plant samples were lyophilized and are being tested for presence of the Cry1Ac toxin. Results of these evaluations should allow us to determine whether the observed survivorship reflected reduced susceptibility of PBW or a quality control problem with the B.t. cotton seed used to plant the field. A third possibility is that late-season expression of toxin was sufficiently reduced to allow PBW to survive on plants on which they would have been killed earlier in the season.

In intensive, season-long sampling of bolls from B.t. and adjacent non-B.t. cotton at five Arizona locations in 1995 and 1996, Flint has observed PBW survivorship ranging from 0 to 0.1% in B.t. plants (Flint and Parks 1997, Flint et al. 1996). Given our findings of 3-4% infested B.t. bolls we are inclined to attribute our observations of PBW survivorship at Eloy to a quality control problem with the seed lot used in our experiment.

Statewide Monitoring of PBW Susceptibility to B.t.

Susceptible and Resistant Reference Strains

Reference strains provide a basis for contrasting the susceptibility of field strains in bioassays. Our susceptible reference strains comprised a population that had been in culture for over two decades, APHIS-S, and one that we have had in culture for less than two years, Marana-S. These populations responded comparably to B.t. toxin (Figure 4).

The resistant reference strain was developed by selection of a laboratory strain of PBW with lyophilized B.t. plant material (Bartlett 1993). We confirmed Bartlett's findings; this strain was significantly less susceptible to Cry1Ac toxin than were our two susceptible reference strains. Future field studies are planned to evaluate the degree to which this level of resistance enables survival on B.t. cotton.

Field Populations: Preliminary Findings

At the time of this writing evaluations are underway of susceptibility to B.t. of eight field populations collected throughout Arizona cotton in 1997. The four populations for which evaluations have been completed (Figure 5) were more susceptible to Cry1Ac than were the susceptible reference strains (Figure 4). Whereas the Bartlett-R and APHIS-S populations had 70 and 4% survival at

concentrations of 3.2 µg/ml Cry1Ac, respectively, few F1 offspring of field populations survived bioassays of 1.0 µg/ml (Figure 5).

Survival of F1 offspring from field populations in control treatments ranged from ca. 40-70% (Figure 5), whereas it was uniformly around 90% for the laboratory strains (Figure 4). We attribute this difference to the fact that the F1 generation of field strains had not previously adapted to living on meridic diet, whereas the laboratory stains had done so. Evaluations of future generations of these field populations is expected to yield better control survivorship. However, because resistance to B.t. has been found in some cases to decline rapidly in laboratory cultures of heterogeneous populations (Tabashnik et al. 1994a b), it is important that we test field strains as soon after collection as possible. Our findings are generally consistent with the baseline susceptibility data for PBW reported by Watson and Kelly-Johnson (1995) and Bartlett et al. (1997), though differences in methodology preclude us from being able to compare mortality on a concentration-by-concentration basis.

Rapid Response Team

Investigations by team members were made of 9 reports of putatively unusual larval survivorship on B.t. cotton. Two of these cases involved boll damage by Helicoverpa zea in the 2-10% range. No unusual PBW survivorship was found at any location prior to mid-September. Subsequently, only two late-season events were verified. The first, described above, occurred at the Eloy test plot. The second occurred in the Harquahala Valley in early December. At this site team members found high proportions of bolls with single lochules that had been damaged by PBW larvae but in which there were no surviving larvae. Exit holes were found on some putatively B.t. plants. A sample of 10,000 bolls was collected and placed in boll boxes at the USDA-APHIS Phoenix Methods Development Laboratory. These yielded fewer than 20 pupae. Therefore, we are unable to eliminate the possibility that the observed exit holes and collected pupae originated from non-B.t. plants. According to seed producers, up to 2% of non-B.t. plants (rogues) can occur in B.t. fields. Plantings of B.t cotton at this location will be observed more intensively in the 1998 season.

Acknowledgments

We thank A. Bartlett for assistance and counsel on all aspects of this project and for providing the resistant strain that he successfully produced. Similarly, we recognize and thank H. Flint and T.J. Henneberry for support of this project. For assistance with field work we thank M. Whitlow, R. Webb, and the staff of the Arizona Cotton Research and Protection Council. For assistance in the field and laboratory we recognize M. Sims, M. Zaborac, A. Patin and the staff of the UA Extension Arthropod Resistance Management Laboratory.

Funding for this project was provided by the Arizona Cotton Growers Association, Cotton Incorporated, and Monsanto. These studies would not have been possible were it not for the willingness of L. and A. Burruell, Eloy, Arizona, to contribute their land and assistance for three-year duration of this project. For this we are very grateful.

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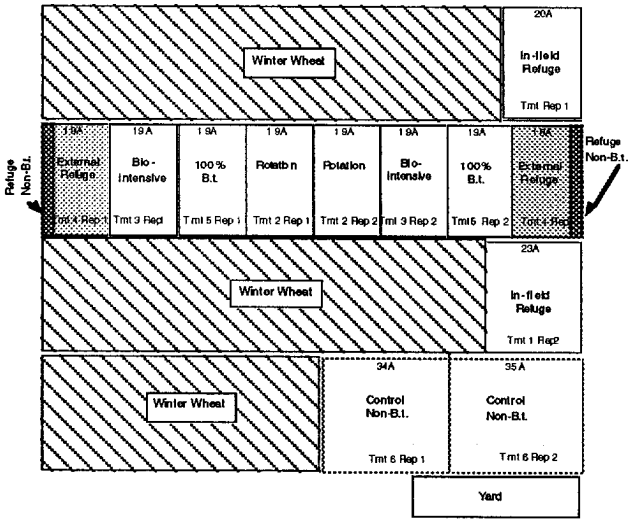

Figure 1: Arrangement of plots at the Eloy, Arizona, experiment to evaluate B.t. deployment strategies. Six treatments were replicated twice and comprised a minimum of 19 acres per block.

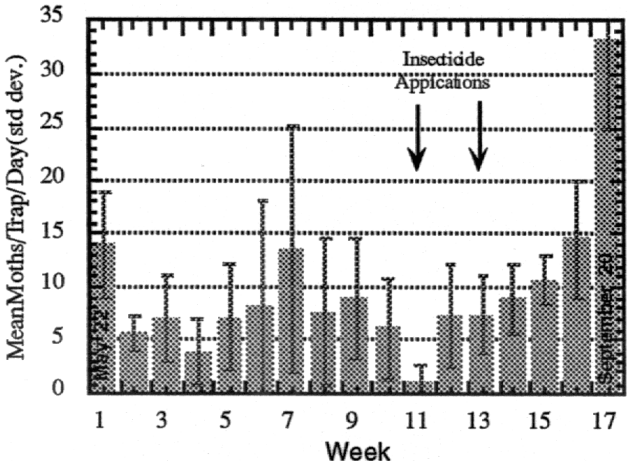

Figure 2: Pheromone traps at the Eloy test site captured relatively high numbers of pink bollworm throughout the 1997 season. Values shown are mean trap catches across all treatments.

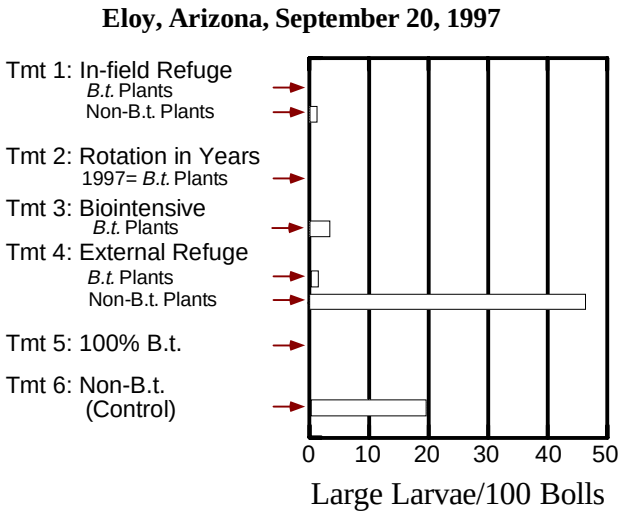

Figure 3. Large larvae (≥3rd instar) of pink bollworm detected in 100 boll samples (50 bolls per replicate) from the B.t. deployment treatments at Eloy, Arizona

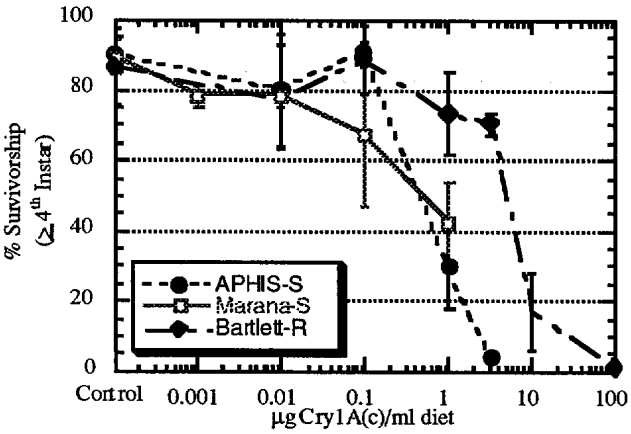

Figure 4: Response to B.t. endotoxin, Cry1Ac, of resistant and susceptibile laboratory populations of pink bollworm. The Bartlett-R strain was previously described Bartlett (1995).

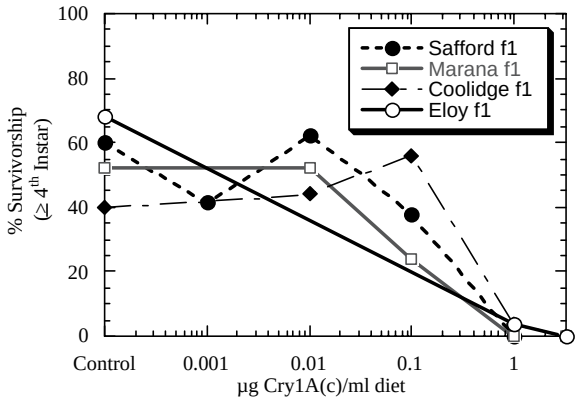

Figure 5: Preliminary results of the Arizona statewide monitoring of PBW susceptibility to the B.t. endotoxin, Cry1Ac, in 1997.