

# BENEFITS OF MULT-LEVEL MONITORING ACTIVITIES FOR A PINK BOLLWORM RESISTANCE MANAGEMENT PROGRAM IN TRANSGENIC (Bt) COTTON IN ARIZONA

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## Abstract

The advent of Bt cotton has revolutionized the control of pink bollworm, the most serious pest of cotton in the desert southwest. Widespread adoption of Bt technology by Arizona growers has increased the risk of PBW resistance development. To address this issue a multi-level approach to resistance monitoring has been adopted by the Arizona Cotton Research and Protection Council including:

1. Monitoring of over wintering PBW moth population emergence. This long-term survey provides insight into the suppressive effects of Bt cotton on PBW areawide.
2. Paired Field Studies-Boll sampling of 36 pairs of adjacent Bt/non-Bt fields statewide measures the continued efficacy of Bt in areas of high PBW pressure.
3. Embedded Refuge Studies-Sampling studies involving a single row embedded non-Bt refuge strategy provide an effective alternative to external refuge scenarios.
4. Rapid Response Team-A standardized approach utilizing trained personnel to rapidly investigate reported cases of inadequate field performance of Bt significantly increases the scope of monitoring statewide.

## Introduction

For more than thirty-five years the pink bollworm, *Pectinophora gossypiella*, has been the most serious cotton pest in Arizona, California, and Northwestern Mexico. Arizona and California growers alone have applied more than seventy-two million acre-equivalents of harsh pesticides at an estimated cost of 1.3 billion dollars. On a farm-by-farm basis pink bollworm (PBW) can have a direct impact upon grower costs and returns by adding \$50 to \$120 per acre per year for chemical control depending on the severity of the infestation. Crop values may be reduced by an estimated ten percent or higher per acre when populations are not controlled. Considerable secondary losses are also likely to occur including the buildup of other pests, honeybee losses, and the adverse impact on other integrated pest management systems.

The introduction of commercially available transgenic cotton varieties containing the Bt endotoxin revolutionized pink bollworm control in the desert southwest. Arizona growers rapidly integrated the technology into their farming practices and by 1998 Bt varieties accounted for more than sixty percent of the cotton growing in Central And Northwestern Arizona. Because of its high degree of efficacy and widespread adoption by producers, it is widely accepted that Bt cotton is at a very high risk of PBW resistance development. In order to address the issue of resistance, the Arizona Cotton Research and Protection Council is working closely with the staff of the University of Arizona, Extension Arthropod Resistance Management Laboratory (EARML), to sustain the effectiveness of Bt cotton for as long as possible.

## Program Objectives

Activities on the part of the Arizona Cotton Research and Protection Council focus on the utilization of a multi-level approach to resistance monitoring of pink bollworm populations across Arizona. Objectives also include close collaboration with the University of Arizona and the Arizona Cotton Growers Association to produce coordinated efforts in the arena of resistance management.

## Materials and Methods

### Population Monitoring

Annually, beginning in 1992 ACRPC field personnel have conducted a statewide pheromone trapping survey designed to monitor spring emergence of over wintering pink bollworm moth populations. Standard Delta sticky traps are used (Foster et al 1977). Traps are placed adjacent to cotton fields beginning at the accumulation of 500 heat units from January 1<sup>st</sup> of each year according to the Huber heat unit model. This represents the earliest emergence point for pink bollworm. Traps are run weekly through the accumulation of 2200 heat units, which normally coincides with the ending of over wintering PBW emergence. Approximately 1000 traps are utilized for the survey and are deployed at the rate of one trap per quarter section (160 acres) of cotton on a grid pattern. Pheromone lure consists of red rubber septa impregnated with a 4mg load of gossypure, a USDA standard.

### Paired Field Studies

Thirty-six sites have been selected throughout Arizona's cotton growing regions for comparative studies. At each site an adjacent pair of fields (one Bt and one non-Bt) is selected for sampling. The assumption is that the interface of these two disparate fields represents an area of high PBW pressure on the Bt component of the pair. On each of two sampling dates (one in September-October and one in October-November), a minimum of 50 and 500 susceptible bolls are harvested from the edge rows of non-Bt and Bt fields respectively. These bolls are then placed in ventilated incubation boxes for approximately three weeks. Following incubation, exit holes, exited larvae and/or adults are counted and recorded.

### Embedded Refuge Studies

Studies originated by the Extension Arthropod Resistance Management Laboratory group at the University of Arizona demonstrated as early as 1997 that infield or embedded refuges of one row of non-Bt cotton for every 5 rows of Bt cotton showed promise as an alternative to the standard external refuge scenario (Simmons et al 1998). The ACRPC staff expanded studies employing this strategy in 1998 with similar positive results (Antilla, et al, 1999). Subsequently an increasing number of cotton growers in Arizona have begun to test the embedded refuge concept in their own commercial farming operations. Four of such fields were selected by ACRPC staff to monitor effects of resident PBW populations. Pre arrangements were made with each grower for ACRPC inspectors to be present when fields were planted. Non-Bt rows were flagged for later evaluation. Two late season boll samples were collected, one in September and a second in October. At each collection date 500 bolls were harvested from Bt rows (50 from each of 10 randomly selected rows) and 150 bolls from non-Bt rows (50 from each of 3 randomly selected rows). All bolls were placed in ventilated incubation boxes for three weeks. Following incubation, exit holes, exited larvae and/or adults were counted and recorded.

### Rapid Response Team

The Arizona Cotton Research and Protection Council is working closely with the University of Arizona to track sustained effectiveness of Bt cotton. To this end, a Rapid Response team has been established and managed by the ACRPC staff. Its purpose is to provide a standardized format to investigate reported cases of inadequate performance of Bt cotton in

Arizona. Widely publicized, the Rapid Response Team draws on the monitoring efforts of hundreds of growers and pest control advisors statewide to assess Bt efficacy on a continuing basis. The Rapid Response protocol is as follows:

1. A grower or field pest control advisor (PCA) observes three or more pink colored larvae (3<sup>rd</sup> instar or later) or exit holes in approximately 50 bolls.
2. The grower or PCA calls the ACRPC main office to report observations.
3. An ACRPC field supervisor arranges to meet the grower/PCA at the field to analyze the extent of the problem.
4. If evidence of unusual PBW survival is verified, a much larger sample (2000 bolls) is collected and forwarded to the University of Arizona (EARML) for analysis.
5. If EARML tests verify a resistance episode, remedial action can then be taken.

### Results and Discussion

#### Population Monitoring

The purpose of the annual spring pheromone trapping survey is to comparatively assess the relative degree of severity exerted by PBW populations on early season cotton. When compared year-to-year the survey provides important insight into overall population dynamics statewide. Figure 1 reports the summarized annual averages for the monitoring effort since 1992. The graph denotes significant declines in PBW populations following the widespread general use of Bt cotton by Arizona growers beginning in 1997.

#### Paired Field Studies

Since its commercial introduction in 1996, Bt cotton has enjoyed increasingly widespread use in Arizona. Because of the high risk of resistance development of pink bollworm populations to the Bt endotoxin, a monitoring program to establish baseline field efficacy data was established by Dr. Hollis Flint, USDA ARS Western Cotton Lab in 1996 and expanded by the ACRPC in 1998 following Dr. Flint's retirement. Table 1 summarizes this cumulative data set and compares PBW infestation levels for the Bt/non-Bt field pairings statewide. Of the more than 258,000 bolls incubated and examined for PBW life forms, the overall percent of infestation for Bt and non-Bt both has averaged 0.17% and 38.2% respectively. Clearly, to date, no field failures of the Bt technology have been observed.

#### Embedded Refuge Studies

For the past four years, studies originated by Simmons et al, and expanded by the ACRPC staff focused on utilizing a non-Bt single row embedded strategy to address the refuge issue in Bt cotton fields. Ratios have varied according to planter box configurations, normally ranging from 1 non-Bt/5Bt (16.7%) to 1 non-Bt/3Bt (25%). In all tests to date, late season pink bollworm pressure in the non-Bt rows has produced relatively high population numbers without resulting in demonstrable reductions in yield. Table 2 reports infestation level data for the four fields sampled in the study.

#### Rapid Response Team

A standardized approach enabling trained field personnel to rapidly investigate reported cases of inadequate performance of Bt cotton in Arizona represents a critical element of any established monitoring program. Despite numerous responses, the Team has yet to encounter any instances of Bt field failure.

In conclusion, a series of standardized multi-level Bt/non-Bt monitoring surveys provide a solid framework for quality assurance of a transgenic technology critical to the economic viability of the Arizona cotton industry.

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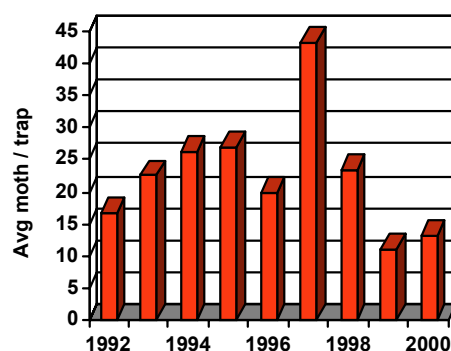


Figure 1. Status of over wintering PBW population sampled by spring emergence pheromone trapping.

Table 1. Multi year comparison of pink bollworm infestation levels recovered from cotton boll incubation boxes at the interface of adjacent non-Bt and Bt fields.

YEAR	Bt/NBt	Total Bolls	PBW	
			Recovered	Percent Infestation
1996 (n=6)	Bt Non-Bt	33350	14	0.04
		33850	11572	34%
1997 (n=7)	Bt Non-Bt	36650	139	0.4
		35100	16962	48%
1998 (n=33)	Bt Non-Bt	38000	30	0.078
		3200	1337	42%
1999 (n=35)	Bt Non-Bt	30300	79	0.26
		2900	817	28%
2000 (n=36)	Bt Non-Bt	37500	44	0.117
		8085	1134	14%

Table 2. Comparison of PBW life forms recovered from single row embedded non-Bt refuge bolls versus adjacent Bt row boll sample. Each replicate represents one field.

<b>Rep</b>	<b>Bt/NBt</b>	<b>Total Bolls</b>	<b>PBW Recovered</b>	<b>Percent Infestation</b>
1	Bt	1000	0	0
	Non-Bt	150	82	54.7%
2	Bt	1000	0	0
	Non-Bt	300	7	2.33%
3	Bt	1000	0	0
	Non-Bt	300	5	1.67%
4	Bt	1000	4	0.4
	Non-Bt	300	61	20.3%