

**BT RESISTANCE MONITORING OF TOBACCO
BUDWORM AND COTTON BOLLWORM
IN ALABAMA COTTON:
LET'S GET EVERYONE INVOLVED!
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Abstract

Greater than 400,000 and 350,000 acres of Bt cotton were planted in Alabama in 1996 and 1997, respectively. These Bt cotton plantings represent the largest percentage of cotton being planted with Bt cotton of any state. As a result, the concern over the development of Bt resistance occurring with the tobacco budworm (and to a lesser extent the cotton bollworm) has increased, especially in areas within Alabama which use Bt cotton primarily under the "96/4" strategy. A monitoring service for Bt resistance determination has been established by USDA/ARS, Stoneville, MS. In Alabama, a Bt Resistance Monitoring Kit was created and made available to all interested parties. Use of this kit facilitated delivery of "suspect worms" from Bt and non-Bt cotton to USDA/ARS for Bt resistance determination.

From July-Sept. 1997, 23 insect samples were sent to USDA/ARS from 8 counties (7 counties in Alabama, 1 county in Florida). Of the 23 samples sent, no surviving tobacco budworm were received having been removed from Bt cotton. Two samples contained tobacco budworm removed from non-Bt cotton. Most samples (12) received were cotton bollworm removed from Bt cotton whereas six samples contained cotton bollworm from non-Bt cotton. All bioassay results from the subsequent generations of larvae suggest no Bt resistance or tolerance by tobacco budworm or cotton bollworm. Plans for the 1998 cotton growing season also will be discussed.

Introduction

Transgenic cotton expressing the Cry1Ac gene from *Bacillus thuringiensis* (Bt) to primarily control the tobacco budworm, *Heliothis virescens* was introduced commercially in the US in 1996 on 1.8 million acres. Control of this pest with Bt cotton was considered excellent, and acreage planted in 1997 has increased. With this increased acreage of Bt cotton also comes increased concerns over the development of Bt resistance by tobacco budworm (and to a lesser extent the cotton bollworm, *Helicoverpa zea*). As a result of these increased concerns, the USDA/ARS facility in Stoneville, MS established a Bt screening service to monitor for potential Bt resistance development in both pest

species. Briefly, USDA/ARS receives larvae and rears them out to adulthood (identifying to species). These adults are then allowed to mate and lay eggs. Hatching larvae are then bioassayed at discriminating concentrations using a single Bt protein (that protein expressed in all Bt cotton) in either a spray chamber and/or diet overlay tests. These results are compared to results using a laboratory Bt susceptible larvae.

Greater than 400,000 and 350,000 acres of Bt cotton were planted in Alabama in 1996 and 1997, respectively. These Bt cotton plantings represent the largest percentage of cotton being planted with Bt cotton of any state. Although the USDA/ARS monitoring program was available in 1996, few, if any, samples were received from Alabama cotton. This probably was due to several reasons including 1) the service was brand new and 2) there was no organization involved with obtaining samples.

The data presented in this paper illustrates how a BT Resistance Monitoring Kit was developed and used in Alabama cotton in 1997, and what the results of the samples and Bt screening by USDA/ARS indicate.

Materials and Methods

Several prototypes of mailers were experimented with in July, 1997 to determine which type of container and mailer would be the most secure and "user friendly" while being as inexpensive as possible. Because of the concerns of keeping insect diet and collected insects cool, a biomailer package (Freeze Safe, Polyfoam Packers, Corp., Wheeling, IL 60090) was selected. This package contained an exterior cardboard box, internal styrofoam container, freezer pack, and a foam container which could hold 12 1oz. diet cups.

A Bt Resistance Monitoring Brochure was developed to inform participants about the need for Bt resistance monitoring, and how to use the Resistance Monitoring Kit: This brochure contained information regarding collection of heliothines from Bt and non-Bt cotton, storage, and shipping of the larvae, The brochure also contained a tear-off data collection sheet which would contain information such as collector's name and telephone number; grower's name, county, and field; cotton variety; location of larvae collected from the plant; and date. This data collection sheet was to be included with the shipment of insects to USDA/ARS.

One ounce plastic cups containing about 10 ml of a pinto bean-based artificial diet was used to provide food for collected larvae during shipping.

A preaddressed, prepaid Federal Express Airbill was included in the package for shipping to USDA/ARS Stoneville, MS.

Bt Resistance Monitoring Kits were shipped to anyone interested in participating in the program. The presence of this service was made known throughout the state primarily by extension entomologists and industry representatives. Participants usually contacted either W. Moar (Auburn), R. Smith (Auburn), or R. Weeks (Headland). Participants were primarily consultants and university/extension personnel, although industry and growers also were involved. Shipping was initiated in early July primarily in southern Alabama, and continued until early September.

Results

A total of 23 insect samples were received by USDA/ARS in Stoneville, MS from Alabama. All 23 samples came from counties in the central to southern part of the state: Autauga, Bullock, Coffee, Escambia, (Florida), Henry, Houston, Mobile, and Monroe. Autauga county had the most samples (7) which was primarily due to the proximity of Prattville Experimental Field to Auburn University. Of the 23 samples: received, no samples contained tobacco budworm collected from Bt cotton; two samples contained tobacco budworm collected from non-Bt cotton; one sample contained tobacco budworm from an unknown host; twelve samples contained cotton bollworm collected from Bt cotton and six samples contained cotton bollworm collected from non-Bt cotton.

Only 10 out of 23 samples sent to USDA/ARS resulted in adult emergence and egg production. All insects sent to USDA/ARS collected from Bt cotton and reared to adulthood were cotton bollworm. This result was not surprising because of the increased tolerance of this insect (compared to tobacco budworm) to the particular Bt protein expressed in Bt cotton. All progeny from these insects (as well as those cotton bollworm collected from non-Bt cotton) were susceptible to the discriminating concentration of Bt. No tobacco budworm were collected from Bt cotton, although numerous tobacco budworm were collected from non-Bt cotton. All progeny from these insects were also susceptible to the discriminating concentration of Bt.

Discussion

Participation in Bt resistance monitoring in Alabama in 1997 was outstanding considering that 1997 was the initial year for this program. Participants surveyed stated that the Bt Resistance Monitoring Kit was extremely "user friendly". Many participants were glad this service was available for both resistance monitoring as well as for species identification.

Results of the 1997 Bt resistance monitoring showed no Bt resistance development by tobacco budworm or cotton bollworm. This is especially important because numerous

samples were collected from regions within Alabama that contained a high percentage of Bt cotton fields that used the "96/4" strategy for two years (1996-1997). Additionally, these results should serve as baseline preliminary data in which to base future tobacco budworm and cotton bollworm Bt susceptibility.

Although the results from 1997 showed no resistance development, samples were collected only from certain cotton growing regions of the state, only from certain fields, and usually not throughout the growing season. Additionally, no fields were thoroughly searched for surviving larvae. Because resistant insects occur initially at extremely low levels, the current Bt resistance monitoring program probably will not be able to document many of the potential resistant insects in the state. Clearly, additional samples from more locations throughout the growing season are needed.

Only 10 out of 23 samples sent to USDA/ARS resulted in adult emergence and egg production. This low percentage of survival is a concern, especially when the occurrence of potential Bt resistant tobacco budworm and cotton bollworm should initially be present at extremely low numbers. Several of the possible problems associated with this low number include 1) collected larvae were not old enough (4-5 days old) and therefore died from Bt toxicity in transit 2) low sample size did not allow for adequate numbers of fertile females.

Plans for 1998 are to continue the resistance monitoring essentially as was conducted in 1997. Several modifications will be made such as encouraging an increased sample size of 40 insects if possible and requesting at least 4-5 day old larvae or older. The number of collections made throughout the state is expected to substantially increase in Alabama in 1998. This will be primarily because 1) Northern Alabama should have more Bt cotton acreage available July-September and 2) increased advertising of this program should make more people aware of this service. Efforts also will be made to concentrate samples in regions within Alabama that will have grown Bt cotton using the "96/4" strategy for three consecutive years.

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