

# 2009

## NCC PINK BOLLWORM TECHNICAL ACTION COMMITTEE Minutes

October 27<sup>th</sup>, 2009 Fiesta Inn Resort Tempe, AZ

### Table of Contents

Call to Order and Summary of Objectives.....	1
Announcements, Operation and 2008 Minutes.....	2
Summary of Progress and Program Overview.....	2
PBW Geographic Slides - Dr. Staten pgs. 4-7	
24C / Sec. 18 Terminology / Minimum Standards Discussion.....	7
Bt Cotton Sterile Insect Use / USDA Recommendation .....	10
Sterile Insect Use in Bt Cotton - Dr. Staten pgs. 12-13	
DS Red Review & Discussion .....	13
USDA APHIS PPQ CPHST - Dr. Zink pgs. 14-23	
Moth Movement.....	25
TX PBW Program - Larry Smith pgs. 25-36	
AZ PBW Program - Leighton Liesner pgs. 38-39	
Initial Distribution Moth Production .....	39
2010 Sterile Release Rates - Dr. Staten pg. 40	
Temperature Standards for Seed Handling .....	40
Motion to Adjourn .....	40
PBW Mating Disruption Report .....	42
[Provided at a later date to attach to Minutes] - Dr. Michelle Walters pgs. 42-44	
Resolution Summary / Recommendations.....	45

PBW TECHNICAL ADVISORY COMMITTEE members:

Present  
Charles Allen  
Larry Antilla  
Ted Boratynski

Absent  
Steve Birdsall

Craig Brown  
Joe Ellington  
Jim Ed Miller  
Tom Miller  
Steven Naranjo  
Don Parker  
Jim Rudig  
Bob Staten  
Bruce Tabashnik  
Robert Hull

## **Call to Order and Summary of Objectives**

Chairman Staten noted that there are four areas of major importance. One is a composite of issues surrounding the use of Bt cotton, how we use sterile insects as refugia, and how we define terms, in order to maintain the Special Local Needs labels (24c and Section 18 labels) for use of sterile insects as a replacement for spatial refugia in Bt cotton to prevent insecticide resistance. We must use caution when making definitions in order to continue to maximize use of Bt cotton, particularly as we go through eradication and in what we are now calling "confirmation eradication stages", so that we have no run-away populations, and no population at all if possible, and certainly no resistant population. Our goal is when we are through with Pink Bollworm Eradication in a very large area, the labels can be reconstructed in one set up. There will be a lot of time and they are intertwined.

We will then discuss the impact of migration, and very briefly about how we expect to distribute next years' income and sterile insects. Please remember that we will be operating with a reduced sterile insect production budget for next year.

We will also discuss DS Red, the transgenic marker strain of insects. Dr. Zinc will present APHIS' data. There will be a quick program summary so we can concentrate on these items. Tomorrow, each area will report their progress to the PBW Action Committee. Dr. Staten requested a roll call to assure there is a quorum. Tish Tamulis, ACRPC took roll call (reflected above) and advised there is a quorum.

Bob Staten yielded to Dennis Palmer. Chairman Palmer thanked everyone for attending. He expressed gratitude, especially to our Mexican counterparts. Dennis emphasized the need to come to resolutions in our efforts to continue with the 24c, as well as some of the information concerning the confirmation of the Pink Bollworm Program. It seems that as we get closer to end of it, there are more and more questions, so the Committee has a lot of work ahead of them. On behalf of cotton farmers from Thatcher, AZ and the region, he thanked everyone for their hard work and advised that as a farmer he is able to see the end result when he picks clean cotton with no bug damage.

Dennis advised that as we move forward, we will need consensus of many issues including migration, DS Red, and the impact this program is going to have as we come to a conclusion. He then yielded the floor to Don Parker.

## **Announcements, Operation and 2008 Minutes**

Don advised that there will be a Pink Bollworm Action Committee Executive Session at 6:00 p.m. He introduced Lance Murchison, National Cotton Council, Communications Department. He will be filming some of the Pink Bollworm meeting. He is capturing many aspects of cotton production in this part of the world. Don yielded the floor to Chairman Staten.

Dr. Staten advised that there is an International Working Committee Meeting in Mazatlan, Mexico starting November 10<sup>th</sup>. Individuals interested in attending can speak to Ted Boratynski, Steve Birdsall or Jim Rudig for details.

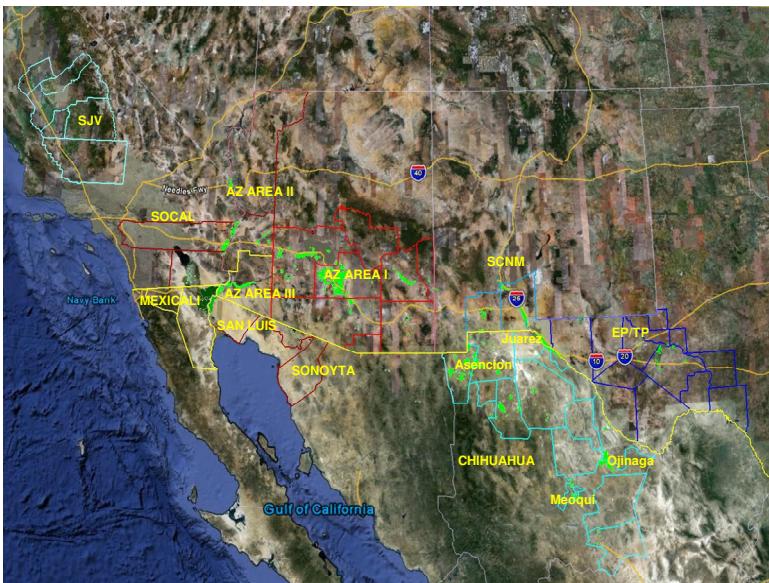
Dr. Staten advised Tish Tamulis, ACRPC replaced Jim Rudig, CDFA in taking the minutes and has copies of all slide presentations and minutes from last year, as well as the March NCC PBW Technical Action Committee minutes. She has a CD for every member of each Committee, and a few extra. If you are interested in receiving a copy of the minutes, please let her know. It is also available on the web (azcotton.org). If you have any questions or comments concerning the minutes, feel free to call Dr. Staten or Don Parker.

### **Summary of Progress and Program Overview**

Dr. Staten thanked everyone, particularly his colleges in Mexico, for getting him information ahead of time. He will give a very brief overview of the program, and hopefully it will be suitable enough so that the members of the Technical Advisory Committee, who aren't involved on a daily basis, will have a clear enough understanding on where we stand. Dr. Staten proceeded to give a power point presentation. He placed all of this area on one Google Earth map so that we can understand the magnitude of the area we are now working in.

The State of Chihuahua is broken into four areas; El Paso is broken into three. South central New Mexico is one unit because it is a very good area and is very easy. They are doing incredible. Arizona is broken into three areas. We also have Southern California, and then Sonora and Mexicali. This pretty well represents one block in terms of populations.

The San Joaquin is unique and has been operating for a long time. It is not in the documentation of the Pink Bollworm Eradication Program. It is very much an eradication program, but it has its own structure. You will be hearing from Jim Rudig tomorrow. The Phase I area is pretty much a block within its own sphere.



This simply tells you where we were by the end of the season, before there was any evidence of any pink bollworm movement. The only positive captures in the State of Chihuahua until August or September, were four captures in the Juarez area right below El Paso, along the river. They were right next to a port of entry (not a truck port of entry). It seemed to be a very unique small cluster. In the El Paso, TX area almost the same week, during the same trap cycle, they have two adjacent to the next port of entry towards El Paso.

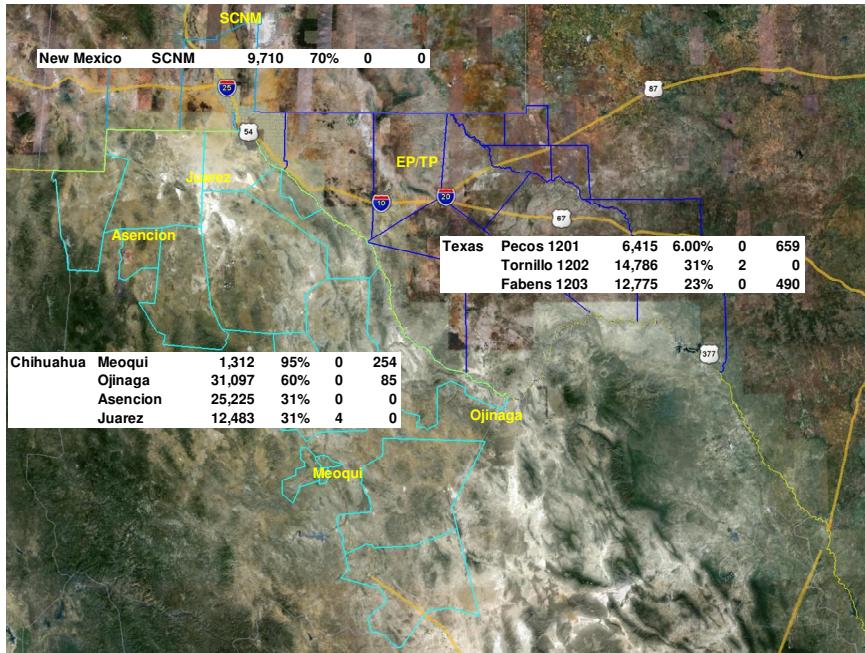
We were doing and are doing incredibly well. Larry Smith, TX contacted Dr. Staten concerning a capture in the Pecos area. It was very clear and very clean. They have had a lot of moth captures in a non-programmatic area during that general time frame. It started northeastern and made a pattern downward. It was over in a relatively short period of time, which is understandable. It is identical in general structure to the movement that also hit the Meoqui and Ojinaga areas in Chihuahua. We all know we have pink bollworm movement at the end of the season. This was very clear.

Last week, Dr. Staten was advised by Edward Herrera that there were a series of finds in the El Paso Valley that we did not expect. As of Thursday night, there were approximately 490 insects in about twenty fields. This does change the amount of freedom we have with sterile insects concerning our limited budget. It does impact our thinking. That area, at least in the El Paso Valley, needs to be kept under a full release, but we will discuss those things later.

It is very important to know these numbers, as it becomes apparent that what we are getting is largely externally driven. We have taken a lot of samples. Some have gone to Bruce Tabashnik. We need to get a shipping address for Dr. Tabashnik to Edward Herrera's personnel. Some samples will be used for an experimental project to see if we are going to get gossypol acting as a marker for an insect that has gone through on pink bollworm. We are just getting started, so we will not yet have a report. Currently, we have gathered the samples.

New Mexico is fine and has remained pink bollworm free. It is rather isolated and has a buffer including the city of El Paso from that area below it. The entire Ascension area has maintained zero. Ascension has

grown 25,000 acres of cotton this year, and approximately 70,000 ac were grown last year. Ojinaga and Meoqui had followed suit, until a migratory sequence occurred.



Arizona is very interesting. The central and eastern portion was essentially below detection levels for pink bollworm in non-Bt. In Area I, all but one of the twenty-two were in Bt cotton at the beginning of the season. There was one in a Bt field early in the season. All of Arizona now has been heavily influenced by what we have left.

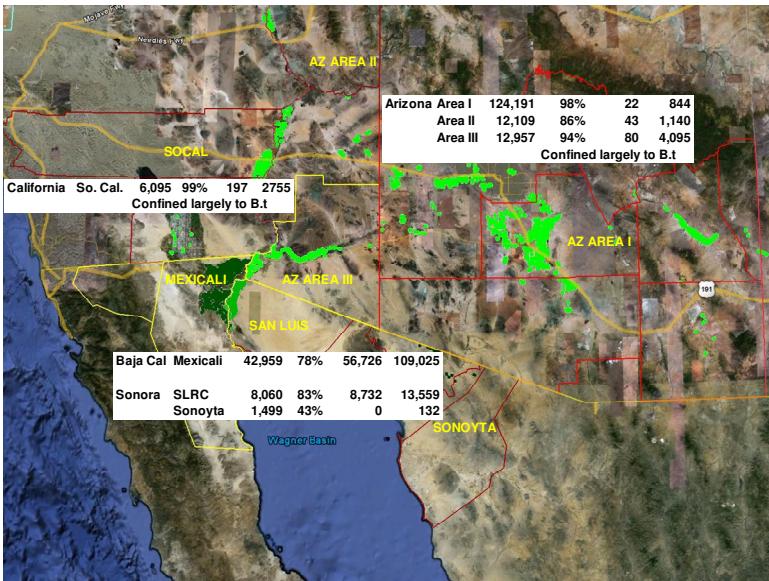
There is a very strong effort on the part of Mexico. This is their first full year on the program, and their first year with sterile insects. They had very good recovery, but their population levels are at first year levels. We are seeing the impact of that population. The numbers are very good, given how much is moving. This just illustrates how far and how much is moving, even though they are at eighty to ninety percent suppressed from the previous year.

Bruce Tabashnik inquired, and Dr. Staten answered a column represents total acres, percent of Bt, and the numbers in traps both before and after the migration appears.

California was similar. They were 99%+ Bt. Their biggest concern is okra (over 500 acres), which is a poor host, but we must be concerned with it. The okra is located mostly in the Coachella Valley, above the Imperial Valley, and in a few spots in Imperial.

A majority of the 197 were very early in Spring, followed by a very long period of virtually no activity. It is a dicey question to try and answer when things start moving.

Mexicali cuts their water off on August 15<sup>th</sup>, so there are a lot of plants hardening up even before that. Things become very unstable as far as moth movement at that time, but we are looking good.



You can see San Luis and Rio Colorado. Baja California (Mexicali Valley) is 78% Bt and 42,000 acres. They had a pheromone suppression program last year. The number seems high, but in comparison to the other respective areas, it's probably right on target.

Everything is moving, so what's late and what's early is probably a little bit more dicey. The vast majority of moths were probably in the last month and a half of the entire six-month trapping season.

San Luis and Rio Colorado are contiguous with it. It is running at 83% Bt and its numbers are very well split also along those lines. In non-Bt cotton in San Luis, there was a very long period with no recovery of any native insects. We did recover larval populations in a few locations in Mexicali. One will show up in their surveys. The others were used in terms of management decisions. They were not high-level populations.

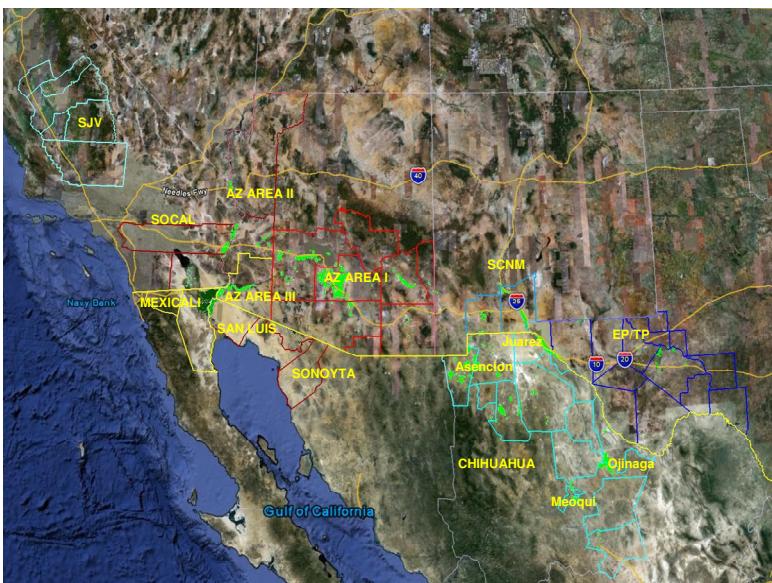
The Sonoita area received the same influence very late in the season. They were essentially at zero all year. All of these areas will give you a more detailed report tomorrow, and you will be able to receive the more fine tuned nuances of data.

Area III is the Yuma area in Arizona. It is 94% Bt and had about eighty moths moving early in the season. It is all part of the continuum. We will talk about migration and how we should be thinking about the future towards the end of this. This should give you enough of an overview.



Steve Naranjo inquired on the rules on percentages of Bt cotton in Mexico. Dr. Staten advised that they are operating under the 5-20 rule. They may run at five percent non-treated refugia, and twenty percent refugia. They are required to have spatial refugia. In essence, it would be a double safety factor in some place (like Mexicali) where they are running high percentages of refugia compared to everything but El Paso, and are getting sterile insects over the top of it.

Upon inquiry as to how many genes are in the Bt cotton in Mexico, Dr. Staten deferred to Ted Boratynski, who translated for Hector, and answered there is one gene.



## **24C / Sec. 18 Terminology / Minimum Standards Discussion**

Dr. Staten yielded the floor to Don Parker. Don advised that a lot of time was spent last year working on the Minimum Standards document. A week ago, members were sent a revised version. There were two issues we have run into this year concerning 24c and Section 18. California has a Section 18 that allows the potential for 100% planting of Bt. Arizona has a 24c which allows for the planting of 100% Bt. Don confirmed and clarified that Texas is amending the 24c in order to make sure all counties in west Texas are now covered.

Don reported that conference calls were conducted with Program Managers in regards to the 24c and Section 18, attempting to get uniform language. The 24c is state issued and approved by the EPA. The Section 18 is federally issued because the State of California decided that it would not regulate transgenic plants. Additionally, several of the programs have tried to encourage maximization of Bt as part of the eradication program. In active eradication programs, it is an important component in bringing populations under control.

In regards to post eradication, Program Managers have been trying to get a common language in forming a definition. The most recent wording identified a program either being active or under post-eradication and did not allow for a transition period. A need was identified to have some sort of safety net of transgenic Bt cotton to help prevent reinfestation.

California's Section 18 comes up next year. At that point, California will have a new Section 18 application. USDA applied for the last one and has already agreed that Susan will work on the new one as well. That is the background on the proposed revision for the Minimum Standards document.

The Minimum Standards document was sent to members on October 15<sup>th</sup>. There had been some discussion since then and this document on the screen is slightly modified. Don Parker put the document on the screen in "format view" so that changes were highlighted in red.

Don advised that we had proposed in this revision to put a new time frame in where we have active eradication, and then have confirmation of eradication, followed by post eradication. There allows for a transition period to have some safety nets in place. The program is still monitoring and has some safety nets to make sure we don't miss something. One of those safety nets would of course be to encourage maximizing the use of Bt. As we start out, it talks about the three phases. The definitions of eradication are laid out with some slight modifications of what we did last year.

An area may be considered for confirmation of eradication status after a year in which no evidence of a reproducing population is detected. That evidence of a reproducing population we went over many times last year until we finally got that part ironed out. Now it is just calling it confirmation in order to give us a four-year period which will allow us to have a safety net confirmed before we make a transition.

At that point, post eradication status, is where the document changes a little bit from where you originally reviewed it, there was some discussion of some risk assessments from other sources. What is being proposed here is that post eradication status is obtained when an area has completed four consecutive years

with no evidence of reproductive native populations. Don asked if there were any questions. No one had questions.

We will move on down where you can see we replaced *post eradication* with *confirmation* in place. This gives us four years of how we are going to operate as a minimum standard. Remember, this is a minimum standard. This does not mean that a Program Manager may not decide to intensify his trapping density, or something else. This is the minimum standard that the Committee is saying has to take place. After year four, you have confirmation of eradication, and the last sentence was stricken because it made reference to our previous post eradication reference page.

One other item that is recommended is the insertion of an area called *Program Population Management Tools*, which identifies the tools that are being used in the program. Active eradication programs have four basic components. The tracking is covered elsewhere. You have pheromone major disruption, encouraging maximum of two gene transgenic Bt, the release of sterile moths, chemical applications will be utilized only when necessary to achieve native population controls. That is a component that is in the program too.

As programs transition from active eradication to confirmation of eradication, and subsequently post eradication, programs shall continue monitoring for native populations while encouraging the maximum use of two-gene transgenic Bt cottons to provide a safety net that will minimize the chance of large reinfestation establishment. Mitigation measures for confirmed native captures are described in this document under Minimum Standards when pink bollworms are detected.

Last year we already laid out what we wanted to do if we captured something. This is just saying here are the tools that we have in place at these different stages. Don asked if there were any questions.

Bruce Tabashnik has a question regarding ***Year 2 where it states “assume” no native captures, and asked if we could remove the word assume because it is the only year where it appears.*** Discussion ensued between Don Parker, Bob Staten and Bruce Tabashnik. Bruce felt that in looking at the document, he finds it difficult to see whenever we have a detection, we do say how to immediately respond to the detection, but is not clear on where we revert back to as far as Year 1, Year 2, etc. There needs to be ***further clarification and a diagram or flow chart would more easily reflect the chain of occurrences.*** Steve Naranjo concurred.

Don asked if there were any further questions. Bob Hull asked if there is a single find, does the entire zone go a step back in eradication. Discussion concerning the “nine-square mile” rule ensued. Dr. Staten advised that we have set up a nine square mile block radius and if you find an insect, you would put that nine square mile block into a treatment scenario. That would be the portion of the entire state in essence to “square one”. It may well be that management in that area says no because there is too much contiguous area, and may choose a bigger area. We need to have something that sets a minimum standard. This will also allow EPA to be assured there will be no risk of resistance developing. Secondly, this allows us to manage the amount of trapping a program has to do, where you can take into consideration the amount of resources they have to spend in subsequent years. It should be kept as minimal as we can while still maintaining effectiveness, and still having latitude. Don Parker asked if there was any further discussion concerning that point.

Larry Antilla advised that you will notice in the document too that any additional finds during that period of time, will expand the grid area. It is not static. It establishes the initial criteria, and anything beyond that can expand the area.

Dr. Staten commented that it is the user, the client of the eradication program, that has the most to lose and the greatest reason to actually be conservative about this, so he does not have a big concern about it. However, someone who is totally unfamiliar with our environment, may well require some articulation.

Don reiterated that as the Technical Advisory Committee, we need to focus on the minimal that would ultimately work. It doesn't mean that a program may not decide to put a larger area in there. They still have flexibility of their own. This is just what we are establishing as a technical group, what we recommend would functionally work. If you think of it as this is a trap capture, you've got a moth capture. Nine square miles are being penalized for a moth. Now if there is another moth captured in proximity, you have another nine-square miles, and so on. Would you want to put a whole state back to ground zero based on one moth? We went through that a lot last year. More evidence is going to offer that.

Charles Allen commented that the subsequent trapping to enlarge the area is what makes it work. Bob Hull advised that under the minimum standards document under ***number seven, it is not written clearly concerning the nine square mile compared to the entire area. Dr. Tabashnik clarified that the section continues, but it could be further clarified.***

Don advised that it is very important to get this correct in this meeting. Next week, the Action Committee Chairman, if they adopt our recommendations, will go to Washington D.C. with it. Dennis Palmer and Clyde Sharp will be going there next week, and we need to have it completed in this meeting.

Earl Anders asked if there was any flight range data to support the nine-square mile range. Dr. Staten answered that there is a lot of greater migration capabilities. The greatest threat of this insect is during the season when it's relatively stable. There is no definitive boundary you can use. In this case, you will always have a monitoring program going around it. It is the kind of question that can not be answered. No one knows the range of a trap either. A nine-square-mile block is a very conservative configuration when you look at the long-term success rate of the San Joaquin, who are basically operating on a square-mile basis. We do not have definitive data, but we have a program that has operated in keeping and reducing populations out of the San Joaquin, using perhaps less stringent guidelines.

Larry Antilla commented there was an interesting point brought up in the Boll Weevil Action Committee meeting. Someone said it was kind of like buying insurance. You can buy catastrophic insurance and not pay quite as much, or you can pay a lot and have very close coverage. Remember, we have approximated eradication from areas that were thoroughly infested in these states using the protocols and guidelines we are using. Going back to a situation where we are looking at a nine-square-mile area will help us react to it before it gets out of control, and without breaking the bank with our producer's contingency funds.

Bruce offered giving the nine-square-mile reference a name. Discussion ensued including *refining the reference in a flow chart*.

Jim Rudig reported that nine-square-miles are a minimum standard in California.

If detections indicated that he needed to go beyond that, he did. If detections indicated there was a pattern and there might be some gaps within that area, we include the gap. We do the same thing with our one-square-mile. It gives you a lot of flexibility to look at past data, and react accordingly.

Don Parker advised that we have come to agreements concerning the changes that we will make to the Minimum Standards document. *Dr. Staten asked for assistance from Steve Naranjo, Bruce Tabashnik and Bob Hull concerning the clarification changes that need to be made and requested they meet with him after the meeting.* They agreed.

**M/S/P Don Parker moved to adopt the Minimum Standards document with revisions to clarify as discussed here. Charles Allen seconded the motion and the motion passed unopposed.**

### **Bt Cotton Sterile Insect Use / USDA Recommendation**

A few years ago, we interacted with EPA concerning sterile insects and were allowed to continue the program for that summer. Subsequently, we went before a Science Panel, and were told it was the best presented Science Panel they ever had go before them. We did an incredibly good job of not only supporting the position, but also garnering unanimous support.

Last year, Don Parker, Larry Antilla and Dr. Staten went to Tucson to see Bruce Tabashnik. The meeting concerned the relaxation of the ten per acre per day standard, which are nine above the lowest modeling number showed to be successful. If you cannot detect pink bollworm, what refugia standard do you need, and especially if you will then respond to any detection in a very vigorous and forthright manner. The Action Plan does outline this.

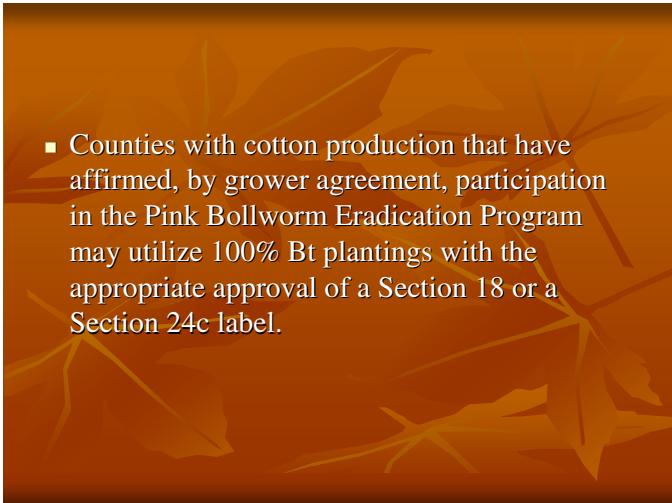
The 24c was allowed simply stating that the refugia requirements worked as recommended refugia standards. We spent a lot of time discussing areas where there is no evidence of reproduction, or we have already contained pink bollworm, and areas such as New Mexico, where it would be an extreme expense to release sterile insects for no known population. All of these areas need a standardized label.

We have gone to the effort to standardize the 24c's and get them across the border. Dr. Staten advised he solicited input from Bill Grefenstette, USDA. Coupled with this Action Plan, this is a suitable method of determining how we release sterile insects on Bt cotton (not non-Bt cotton). Non-bt cotton will always be released at far greater rates than what we are discussing.

Bill Grefenstette made his presentation. Bill advised that counties with cotton production that have affirmed, by grower agreement, (usually in the form of a grower referendum) participation in the Pink Bollworm Eradication Program, may utilize 100% Bt plantings with the appropriate approval of a Section 18 or a Section 24c label.

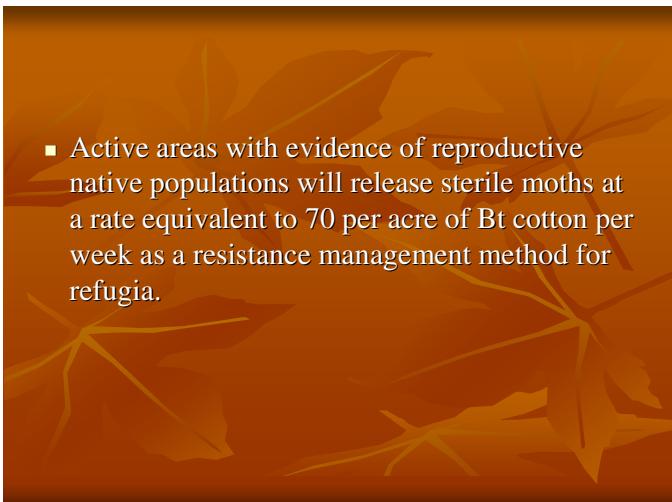


## Sterile Insect Use in Bt Cotton

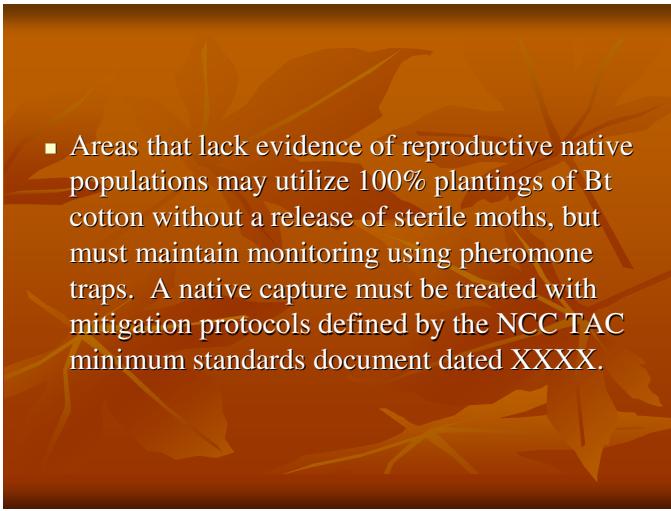


- Counties with cotton production that have affirmed, by grower agreement, participation in the Pink Bollworm Eradication Program may utilize 100% Bt plantings with the appropriate approval of a Section 18 or a Section 24c label.

Active areas with evidence of reproductive native populations will release ten sterile pink bollworm moths per acre of Bt cotton per day at a rate equivalent to 70 per acre of Bt cotton per week as a resistance management method for refugia.



- Active areas with evidence of reproductive native populations will release sterile moths at a rate equivalent to 70 per acre of Bt cotton per week as a resistance management method for refugia.



- Areas that lack evidence of reproductive native populations may utilize 100% plantings of Bt cotton without a release of sterile moths, but must maintain monitoring using pheromone traps. A native capture must be treated with mitigation protocols defined by the NCC TAC minimum standards document dated XXXX.

Finally, areas that lack evidence of reproductive native populations may utilize 100% plantings of Bt cotton without a release of sterile moths, but must maintain monitoring using pheromone traps. A native capture must be treated with mitigation protocols defined by the NCC TAC Minimum Standards Document dated tomorrow if it is approved.

***The USDA is recommending (ppt slides above) to this Technical Committee as the position we would take regarding the use of sterile moths on Bt cotton going forward.***

Dr. Staten asked the Technical Action Committee if this is a suitable system of stepping downward and using sterile insects for resistance management. In this case, we do need to understand that it is a cumulative rate of ten per acre per day. An area may only be able to fly a plane twice or three times a week.

Perhaps we can word it as "... of reproductive native populations will release *a mean* of ten sterile pink bollworm moths per acre of Bt cotton per..." ***Bruce Tabashnik said that he preferred the term "70 per week". Dr. Staten agreed. Dr. Staten asked if the USDA would consent to that change. Bill Grefenstette advised that as long people don't camp on the 70 and may be thinking of a daily type release rate and then they start thinking too high.*** Dr. Staten concurred.

Bob Hull expressed concern that as a grower if we go forward with planting perhaps a lower percentage of Bt cotton in some of these areas, there are too many occasions where we become careless about cleaning the planter good enough when working Bt and non-Bt fields.

Dr. Staten commented that there is always the fear of that situation even the very beginning year of the eradication program in most areas. It is usually demonstrated very quickly with moth captures. In the areas where we are releasing at ten per acre per day, you are really in essence in an eradication program and there is going to be movement around there. This deals with resistance management.

Dr. Staten asked Dr. Tabashnik what the number of the potential frequency is now. Bruce stated it is very low. He advised that he cannot find any resistant individuals for any resistant alleles. The resistant

frequency is very low, essentially below detection. For the molecular method, in the cases we know about, the frequency is probably less than one in one thousand, which means that the frequency of resistant individuals would be less than one in one million. Dr. Staten advised from that perspective, he believes a trapping grid would tell us if we had one of those situations going on in your area.

Dr. Staten requested the wording be changed to “*...release of mean of ten sterile insect moths per acre per day or a cumulative release of seventy sterile moths per acre of Bt cotton per week....*” Jim Rudig advised that in California they use the term of “*will receive a release rate*”. *The USDA will craft that into this document.* USDA does not need Technical Advisory Committee permission to set forth a recommendation, but the Committee should give a resolution of support.

**M/S/P Bob Hull moved to adopt a resolution of support for the USDA recommendations for sterile insect release as refugia, with the understanding that “*will receive a release rate*” will be crafted into the document (as noted above). Jim Rudig seconded the motion and the motion passed unopposed.**

There was a brief break at 2:20 p.m. The meeting reconvened at 2:43 p.m.

## **DS Red Review & Discussion**

Dr. Staten advised Richard Zink, Lab Director of Ft. Collins and CPHST in Phoenix, AZ will be giving a presentation on DS Red. Michelle Walters was acknowledged for her efforts in putting together day degree modeling and calculations for pink bollworm in Texas.

Dr. Zink will report on his group for all of the field trials for release of transgenic DS Red. Oxitec is the proprietary owner of the technology. Dr. Zink will go through the experiments that were run this year and answer any questions for the Technical Committee in reference to where we think it needs to be within program status and is it ready for use.

Dr. Zink introduced himself. Dr. Zink reported that this was very much a team effort. He has been in his position for about a year and a half. Dr. Zink has worked on parts of the analysis which will be presented today. Members were distributed other documents regarding previous field studies, a manuscript that is in development, and a few other reports.

Pink Bollworm Technical  
Advisory Committee

USDA-APHIS-PPQ-CPHST  
Phoenix, AZ  
October 2009

## Overview

- Background Information
- Objectives of 2009 field test
- Methods and Results
  - Mass Rearing
  - Field Release
- Recapture Rates
- Conclusions

## Background Information

- Rationale for using a genetic marker
  - Heritable marker
    - In larval, pupal, and adult life stages
    - F1 Sterility
    - Low radiation dose for sterility
  - Increased identification screening efficacy
- 2007 Test Results
  - No differences observed for recapture rates, mating performance, and dispersal distance between the two strains APHIS and OX1138BB
- 2008 Test Results
  - Demonstrated high accuracy in use of Ds Red marker to identify recaptured sterile moths
  - Ability to obtain large scale permit

Dr. Zink reported that this will improve moths that are caught concerning the florescent marker. There will also be a test using PCR, which we do not have at this time. Observations were fairly good at indicating that the two moths performed reasonably similarly. In the 2008 trial, some real concerns about the competitiveness of the two strains, DS Red and the APHIS strain, were addressed. We were able to obtain permits to do large-scale trials, which was a large accomplishment on our part.

The objectives for 2009 were to answer some of the questions that surfaced in 2008, specifically just how competitive these moths are in the field. We were to design a study that was simple, and to have as many replications as possible. We set it up in three phases, where we are actually repeating the work many times in order to maximize the number of observations over the summer. Then we look at this comparison based on recapture rates.

# Objectives of 2009 Field Test

- Provide answers to questions raised from 2008 Yuma test
  - Recapture rates of OX1138 strain were lower than APHIS strain in nearby area
  - Raised doubts about strain performance among some stakeholders
- Obtain comparative performance data for APHIS and OX1138 strain
  - When both strains are reared, collected, shipped, and released under the same conditions
  - Measured by recapture rates of two strains in pheromone baited traps

## Methods—Mass Rearing



They were reared under fairly similar conditions. The APHIS moth was reared in the APHIS moth facility, and the transgenic moth in our quarantine facility at the laboratory. From there, they were collected and packaged under the same conditions, taken to the field, and released under similar conditions.

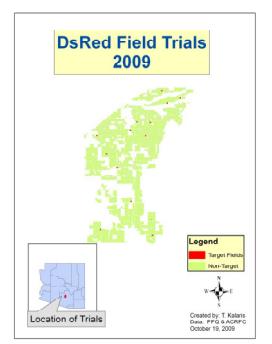
Upon inquiry by Dr. Staten, John Claus advised that the APHIS moths were actually raised in the APHIS rearing facility, however, they were raised in the CPHST labs there. Dr. Zink advised that this would give us the most similar conditions since we needed to keep two strains separated, and does not believe we had the facilities within our CPHST lab to do that, so this was the next best approach.

## Methods—Field Release



## Methods—Field Release

- Pinal County
- Release rate of 150 moths/acre/day
- Total of 2,775,124 moths APHIS
- Total of 2,504,876 moths OX1138
- Each plot 20 acres
- Each site was >2km from Non-Bt
- Total coverage of test, 305 acres
- Releases done 6 days/week
- Three releases were conducted
  - June 1-20
  - July 6-17
  - August 17 & 18
- Number of sites
  - 14—1<sup>st</sup> & 2<sup>nd</sup> releases
  - 10—3<sup>rd</sup> release



The field releases were fairly conventional. There were fourteen fields (about 20 acres per field) in Pinal County with a release rate was approximately 150 moths/acre/day, six days per week. We averaged 2km from any non-Bt cotton. Total acreage was 305 acres.

Dr. Tabashnik asked what the concern is regarding the distance from non-Bt. Dr. Staten advised that non-Bt field fields receive very heavy release rates. We wanted an absolute buffer, and used the DS Red and APHIS releases in lieu of our refugia releases during those periods. It was designed to avoid the influence of outside released moths. Dr. Zink advised it could have interfered with our tracking without the buffer. The recapture rate is the only tool that we have for assessing the competitiveness.

Dr. Zink stated that the trial was done in three phases. Tom Miller verified that both strains were eradicated. Dr. Zink concurred. Dr. Staten advised the difference in the locations is a matter of which rooms. The DS Red was reared adjacent to the Pink Bollworm Rearing Facility, by the same people in separate rooms in order to simulate the same handling. Dr. Miller asked and Dr. Zink answered that the moths are reared on the same source of diet.

Ernie Miller, USDA asked and John Claus answered that the APHIS and DS Red were actually enclosed in the same chamber for the first two phases. During the third phase, they were enclosed in the same chamber, but separate compartments, and eradicated next to each other.

## Methods—Field Release



- Delta traps
  - Baited with 2 mg gossypolure
  - Placed around edge of fields
  - 1 trap per 5 acres
  - Collected 3 times per week



- Identification
  - Visual screening for Calico Red dye
  - OX1138 moths identified using DS Red fluorescence
  - Double blind PCR testing by Oxitec

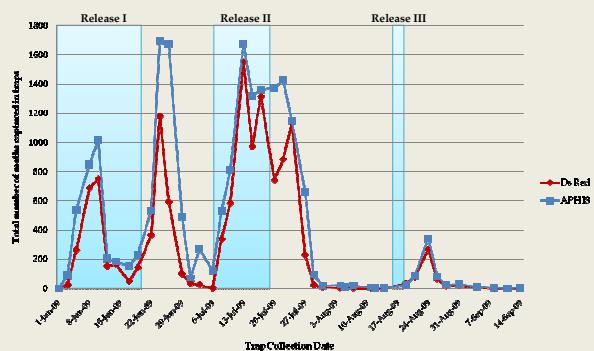
Dr. Zink advised that conventional traps were used in the field. There were four traps per field collected three times per week. A visual identification was done for calico red. Then the DS Red was screened under a fluorescent compound dissecting microscope.

In addition, we did another study called a double blind PCR. We sent traps we had already read to Oxitec in England. They did a visual identification and came up with their own numbers for DS Red or any wild moths. They ran PCR to confirm. We compared our results. It gave us interesting information that became critical in analyzing some of our data in this trial. In particular, we found that the ability to visually identify the DS Red moth (fluorescence under the scope) significantly dropped off after the traps had been held for 65 or more days. Unfortunately, this didn't become an issue in our trial because some of the traps from the first phase of the trial did remain in the laboratory at room temperature for 65 days or longer, which compromised some of our supporting data for the first phase of the trial.

The DS Red moth has had some genes introduced into it. Those encode for fluorescence, and there are also some other genes. The molecular test allows us to test the DNA for the presence of those sequences, which provides absolute confirmation that it is a DS Red moth. Of course, if this moth was being used in the program, this would be the ultimate test to determine whether or not it is a wild moth. The fluorescence may be difficult to read, and calico red can be a problem after time. The presence of the molecular marker makes identification absolute.

Upon inquiry by Steve Naranjo, Ann Kramer explained that there were a few specimens they were unable to obtain a PCR reading because the material had deteriorated. There was an error rate in reading of approximately three-percent. In speaking with Greg Simmons concerning the trials last year and the year before, this is a consistent observation. Clearly after sixty days, the error rate is enormous at seventeen-percent. This is relevant because of how the traps were held in storage. It is important to look carefully at the data.

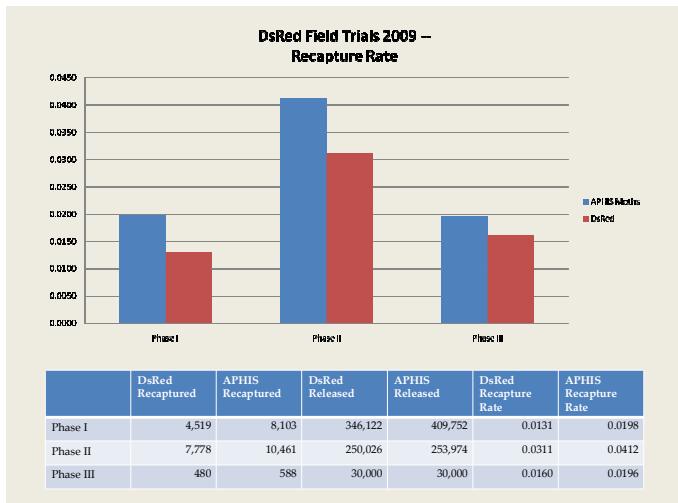
## Moth Recaptures



Dr. Zink indicated there are three principle phases (replications) to the trial and an overview looking at total moths captured in traps and the days traps were brought in from the field. Blue areas indicate the time span of were release. The first set of we had eighteen, the second twelve, and the third was a two day release. We are not putting a lot of confidence in the observations for phase one, as a number of traps did not get read for the DS Red fluorescence within an appropriate period of time. We are looking more closely at phase two and phase three.

We do have some concerns. Perhaps we could get some input from the Committee on why we see this peak occurring after we had done releases, we had a peak, our numbers are dropping off, and there is an increase in collections after we were done releasing. It is a peculiar anomaly. Ted Boratynski suggested possibly temperature. Dr. Zink also advised there was a change in plant canopy, and reaching full canopy at that time. Clyde Sharp asked if there were any insecticides used in the field. Dr. Zink said he did not believe so.

Dr. Zink advised they are recapturing the APHIS moth at a higher level than the DS Red moth. It is consistent throughout the study and the analysis.

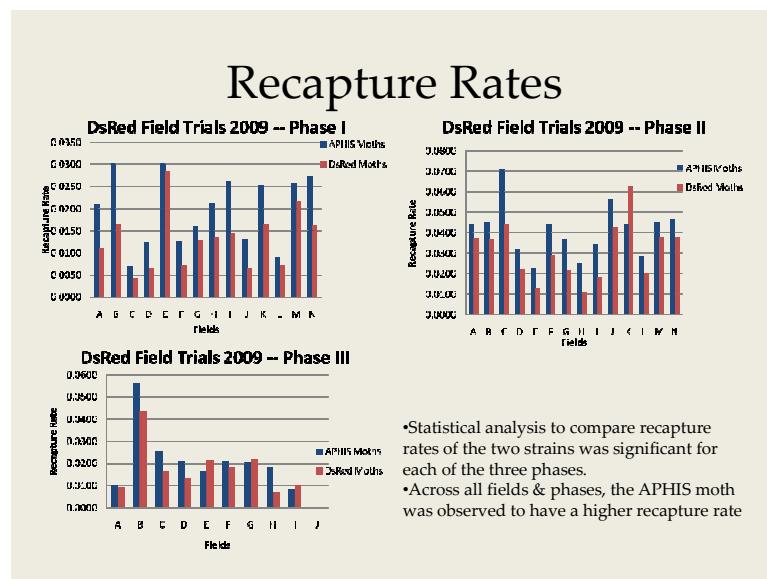


Jim Rudig suggested looking at what your anticipated sterile density was. Perhaps your insects were competing with your trap, and as those original populations died off, your trap data may become more efficient. Dr. Zink advised that it is quite possible as well.

We compared recapture rates of the two strains for the three phases. The APHIS moth is being recaptured at a higher rate. The numbers are adjusted for the differences in releases of DS Red verses APHIS moths, as they were not always a fifty-fifty ratio. In fact, there are differences between where the ratios were the greatest in phase one, where we typically had significantly more APHIS moths being released.

In phase two, the ratio was closer. The comparison of DS Red release at approximately 250,000 and the APHIS moth released at a total of 254,000. The difference between DS Red and APHIS are not significant concerning the ratio. It was statistically an equal number of moths being released, with the same pattern.

In phase three, we had a fifty-fifty ratio of moths being released. The numbers are lower, but were exactly the same with a total of 3,000 of each strain released.



This just shows you the recapture rates for each one of the phases for the whole trapping period.

The statistical analysis to compare recapture rates for the two strains was significant for each of the three phases. All phases, the APHIS moth was observed to have a higher recapture rate. It is fairly consistent. Another way of looking at it, for every 100,000 moths released over a field, typically you are going to recapture about 373 more APHIS moths based on this recapture rate.

Clyde asked if the increase in recapture rate could be attributed to fly ins from surrounding fields where APHIS moths are released. Dr. Zink stated that it is certainly a possibility although we did have a two-kilometer buffer. Dr. Zink asked Leighton Liesner to comment.

Leighton advised that the two-kilometer buffer was utilized based on earlier research as the influence range. He also looked the distance closest to the edge of the experiment area, as opposed to the program release area, and actually buffered some of that as well. Their focus was to reduce the likelihood of influence regarding program releases. During the third test, the buffer was increased to five kilometers. There are similar results between phase two and phase three. Preliminary analysis of data on a field-by-field basis does not indicate any interference.

Bob Hull questioned the importance of the recapture rate. Dr. Zink advised it is an observation we need to determine what effects it might have on the program from an operational standpoint. Bob Hull questioned the value and importance of conducting these trials.

Bruce Tabashnik advised that we discussed this two years ago. Why not count spermatophore in wild females and see if the males are getting the job done. It would be much more informative. Dr. Staten advised in this stage of the program, we do not have them. There was a lot of work using inactivated females and comparing data to trap data, and suppression data in cage experiments early on. He speculated that Greg Simmons may not have been able to get enough to show a significant difference. It takes a Herculean to collect wild females from the few. Trap data is no more or no less than an index of how well the insect is performing in general, and is probably acting as a surrogate for mating data, although he does not know of a statistical study that would show that. It is the index we have used to run programs for the last thirty or forty years. Field cage work is where it was one of several tools, including mating, used to assess competition. It is a pretty good index of the vigor of the two moths as they were put in the field at the same time through the same airplane opening. After that, we will have to make judgment calls.

## Recapture Rates

	Moths Released	Recapture Rate	Moths Recaptured
DsRed Moths	100,000	0.01626	1,626
APHIS Moths	100,000	0.01999	1,999
Additional APHIS Moths in Traps			373

Bruce Tabashnik advised there is a slightly lower DS Red recapture rate in 2009. In 2007, the recapture rate was higher. What about 2008. Dr. Zink advised that he believed it was lower for DS Red. Dr. Tabashnik asked how much lower. Dr. Zink was not sure. Ann Kramer, Oxitec answered that in 2008, they were not dropped together and were not recaptured together. The trial was designed in a large-scale normal programmatic fashion. One area was DS Red. A different area was treated with APHIS. There was no

side-by-side comparison. The mating table data is included in the manuscript data for the write up in the 2007 files. Dr. Zink advised that some of the rearing data and longevity, the two moths were quite similar.

Dr. Zink advised this is only a preliminary report. We will put together a formal report with considerably more explanation and discussion about the data. Dr. Zink asked if there were any more questions or comments. There were none.

## Results—Mass Rearing Data

Trait	OX1138BB (Ds Red)	APHIS
Pupae yield/cell/week	1938.9 ( $\pm$ 47.2)	2033.4 ( $\pm$ 58.3)
Pupa size	15.229mg ( $\pm$ .054)	14.950mg ( $\pm$ .121)
% Egg Hatch	91.3% ( $\pm$ .437)	94.1% ( $\pm$ .357)
Egg production/cage/day	25,910.9 ( $\pm$ 149.3)	25787.8 ( $\pm$ 206.2)
Infested Eggs/day	164,294 ( $\pm$ 2581)	168,133 ( $\pm$ 2292)
% Eclosion	99.0	98.8
% Longevity in lab (14day)	90.5 ( $\pm$ .857)	83.0 ( $\pm$ .1.5)

Mass Rearing Data showing mean ( $\pm$  standard error mean)

In conclusion, we observed approximately a twenty-percent difference in the recapture. The Committee can decide what this means from an operational standpoint. The trial went well this summer, and we have the data. We certainly are ready to look at any additional phases that the Committee feels are necessary.

Bruce Tabashnik asked if there were any wild males during the test. Maria advised that they sent a couple of unmarked pink bollworm insects to England for PCR analysis. They do not know the results.

Dr. Staten asked whether or not the genitalia were dissected, and was there a strip test run. Maria advised that there was not. Dr. Staten verified there were no confirmed natives within that area. PCR technology could confirm absolutely whether or not they were pink bollworm, as well as if they were DS Red.

## Conclusions

- Preliminary analysis indicates that the Ds Red moth has a recapture rate 20 to 25 % lower than the APHIS moth
- The operational implications of this factor are to be determined

Ann Kramer commented that they saw the data a week ago, and have not had a real chance to look through it. Seventy-nine natives were seen. We were sent some moths in vials, but not that many. This is one of the big questions we have with the data.

Clyde Sharp advised that if you do the side-by-side next year, you should have two or three fields that you release no APHIS on, and that would give you an idea how many fly-ins you are getting, which would help to make the data more accurate.

Upon inquiry, Dr. Zink advised that he would like to see a comprehensive final report that expresses the trial very clearly. As far as future work, he would look to the industry to see where they want to take it next. He is very open to whatever the industry wants. There probably needs to be some more work done on longevity and cage work.

Dr. Zink wants to complete the report on this study and write up last years study. Upon completion, he would look to the Committee and the industry concerning where they want to go with the technology if it is adopted. If not, direction on any additional studies that needs to be done in the coming year.

## Acknowledgements

### ACRPC

- Larry Antilla
- Leighton Liesner
- Travis L. Perkins
- Joahna Solis
- Robert Staten
- Lee Trekas
- Oxitec**
- Neil Morrison
- Adam Walker

### APHIS

- John Claus
- Tom Kalaris
- Anna Lowe
- Marcella McDonald
- Ernie Miller
- Greg Simmons
- Maria Sims
- Mickey Sledge
- Guolei Tang
- Cloti Tate
- Mike Vincent

Dr. Zink acknowledged individuals for their hard work including John Claus, Maria Sims, Tom Polaris and Oxitec. Dr. Zink will be working with them on the final report. Dr. Zink yielded the floor to Dr. Staten.

Dr. Staten asked for any comments from the Technical Advisory Committee, and leave whether or not we push forward as far as the Action Committee is concerned, for tomorrow.

Bruce Tabashnik asked if we should address whether or not we should release DS Red in the future. Dr. Staten felt there were several options. There are a number of business questions, which he will not ask this Committee to address, regarding whether or not it is permissible, what it cost, does it cost too much in terms of technology fees, etc. The one question that has not been addressed is the cost of rearing.

There are some differences that appear very minor to someone who is used to looking at small-scale experiments. The mass rearing trial was a fairly large-scale trial and those numbers can be projected in terms of how many APHIS and non-APHIS moths could be reared in a year. Dr. Ernie Miller advised that

he did that. It is important to realize that small differences may make a difference in terms of how much we can expect in a year.

Dr. Ernie Miller stated he has slides on the cost, which he based on the mass-rearing trial. The slide presented on the mass rearing data results are considerably different. Dr. Staten verified that in the original mass rearing trial, it would be a cost the slides do not necessarily indicate. Ernie agreed. Ernie projected approximately \$132,000 more for DS Red versus the APHIS strain.

Dr. Staten advised the Committee's role right now, if they liked in this technology and thought it was without risk as far as efficacy, could entertain a motion to replace the APHIS strain with the DS Red, realizing that it may be an irreversible act. Another option is to table it until we see all of the reports, and have a meeting of just the Technical Advisory Committee, possibly by phone. We could go through it in a more detailed fashion and ask more questions. Another option is to leave it strictly unresolved.

Jim Rudig asked if CPHST was prepared to make a recommendation. Dr. Zink advised that it is an industry decision. Steve Naranjo commented that the decision is based on a risk benefit, weighing that against what the benefit of the technology is going to be in the field in terms of protecting native versus release moths. Charles Allen asked if there was analysis in the past comparing the pheromone production of females. There was not. Larry Antilla agreed with Steve Naranjo and advised that the need for a marker drove this whole approach to begin with. Certainly, the very least we should do is wait for a more detailed report, and reconvene with a Committee to look at that data. We should also think of other issues too, for example, if the DS Red is slightly lower in potential, are there possibilities for out costing, or for some kind of rearing work to be done that would make it competitive. There has been a lot of time, effort and concern put into this and we shouldn't do away with this without giving full attention to all of the details. Dr. Staten advised that this is a very robust test in terms of the numbers of observations. Tom Kilaris has been a good resource. There were a very large number of data bits in the whole analysis. It is very robust. The differences are real. A twenty-percent difference is probably twenty-percent plus or minus a few percentage points. That is real. Whether we were to take it or not, we should not want it in the program without continued work on it. Dr. Staten advised his opinion is to table it until we get the entire report. We can have individual sessions going over data with the people involved. The group can answer any questions about whether it's going to go into the program. We should resolve some of the other questions on the agenda tomorrow so that we keep moving forward with the technology. The USDA should continue to work on this technology regardless of which direction it goes, as it is needed by agriculture in general and would be foolish for this to slow down at this point.

If there is no desire for a motion to push this technology forward, the Chairman will ***table it and call for the full reports, and a second meeting of the panel.*** Jim Rudig requested a time line be placed on that. Discussion ensued. Dr. Zink responded that he could provide the report by the end of November. Dr. Staten urged Dr. Zink to spend some time discussing questions with Steve Naranjo, and asked if he would prefer to have an additional month to provide the report. ***Dr. Zink clarified that he would provide a full report on the trial this past summer by the end of November.*** In order to do the risk analysis, he would have to draw on some other people and would need to make those connections and find out their availability. That would be an additional concern.

**Dr. Staten requested that Dr. Zink get together with him or Don Parker with the risk analysis information and full report, and they will work with the Committee.** Dr. Zink asked to discuss the risk analysis further with Dr. Staten, in order to direct Raleigh headquarters to assist with the analysis. **Dr. Staten asked Steve Naranjo to volunteer, and Steve agreed. Bruce Tabashnik noted that on the benefit side concerning the manuscript in 2008 there was a trial done with not only recapture rate measured, but control efficacy of the DS Red strain was measured, and felt that information should be included in the analysis.**

Ted Boratynski stated that a decision has to be made on what the Committee recommends. Secondly, if it is decided that the technology is worth using in the program, when is the earliest it could be in the field? Dr. Staten advised that there would be a number of things we would have to know first.

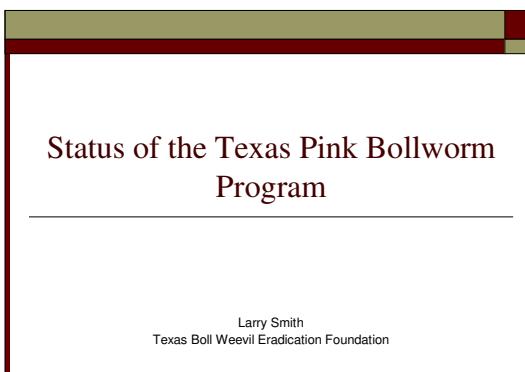
We have to know the minimum number Ernie Miller needs to start a colony, and how much lead time he needs, as well as whether or not the existing strain colony is that large, which is not a simple answer. We have to consider these things when considering the entire operation. **Dr. Staten suggested that Ernie give us an assessment of how many he would need and when, and what the ramp up capabilities are. Ernie has actually done this exercise several times, so if he could compile that data and send it to us. We will give him a week deadline. We will explore trying to put together a conference call in either December or January.**

There was a five-minute recess.

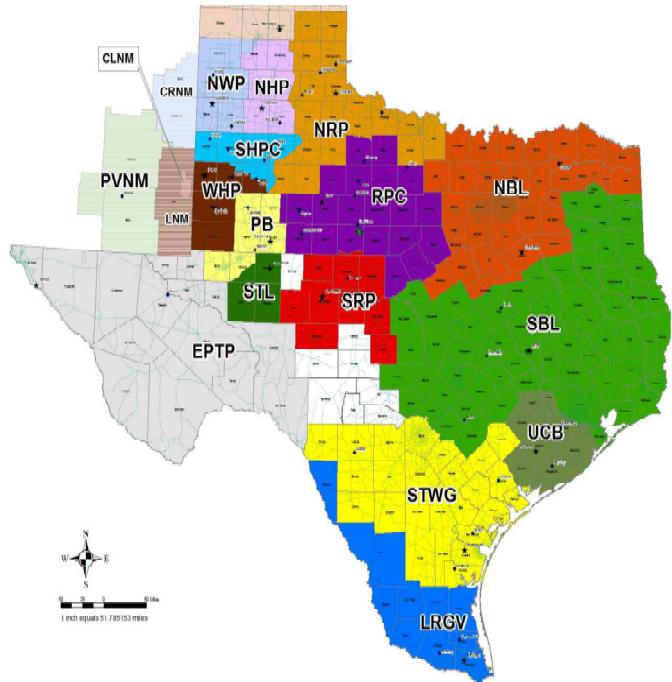
## Moth Movement

Dr. Staten advised that we need to talk about Moth Movement, and asked if Larry Smith, Larry Antilla and Edward Herrera have slides that document the moth movement. There is some information from Mexico, but would like to start with those slides.

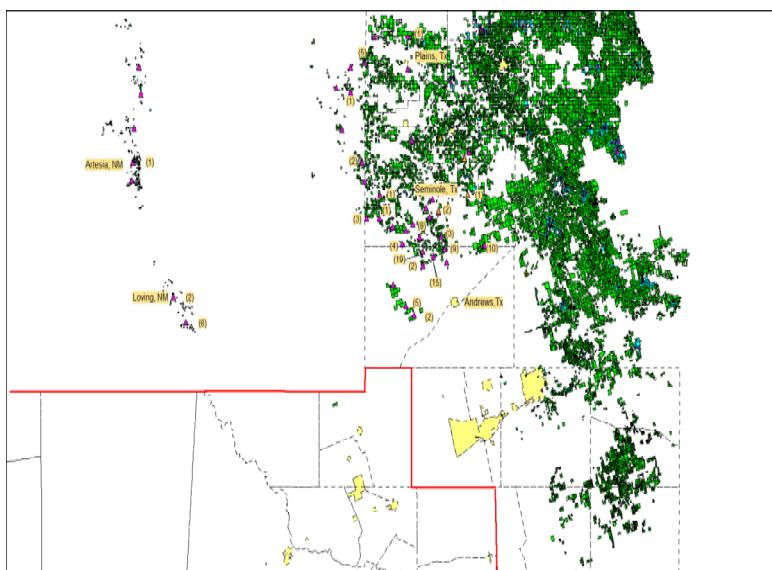
Larry Smith, Texas introduced himself. Time permits him to also discuss some of the issues Texas is currently facing, primarily in the last three weeks.



Here is our cotton production areas in west Texas. Our Pink Bollworm Program is in El Paso. We have cotton in the Pecos area, along the Texas border with Mexico and quite a bit in the High Plains and St. Lawrence area.



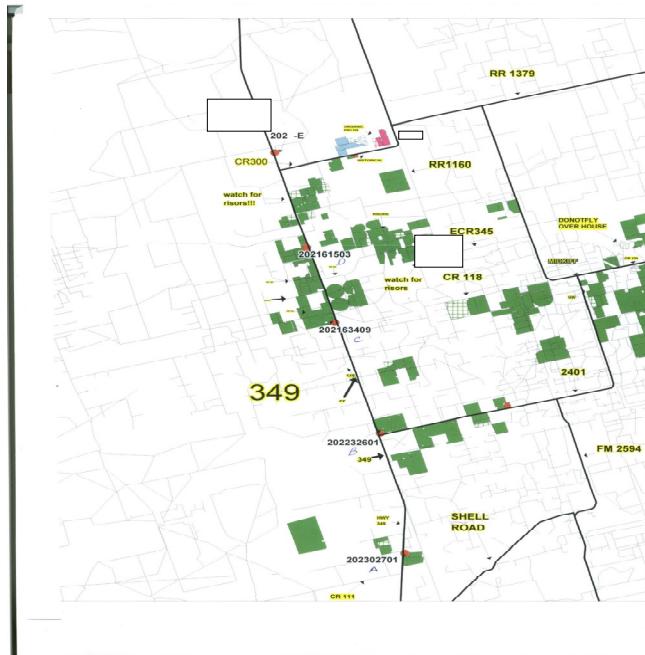
This reflects all of the cotton acres that we have to the north and east of the Pecos area and the eastern side of New Mexico. Dr. Staten inquired and Larry Smith answered there is approximately three million acres.



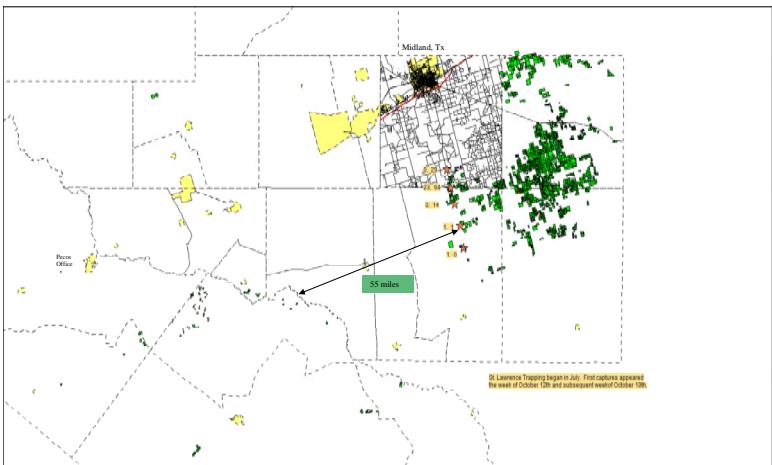
## St. Lawrence Zone

2009 Pink Bollworm Trapping Program

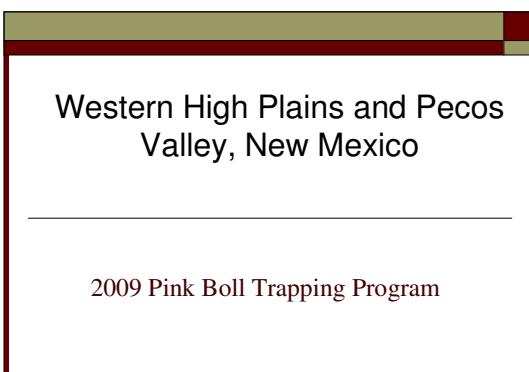
There are five traps along the west side of the St. Lawrence area approximately four to five miles apart. They started out with fifteen traps for that entire area early in the season. As they started capturing, they expanded the number of traps. Larry advised this was a USDA sponsored program and thanked them.



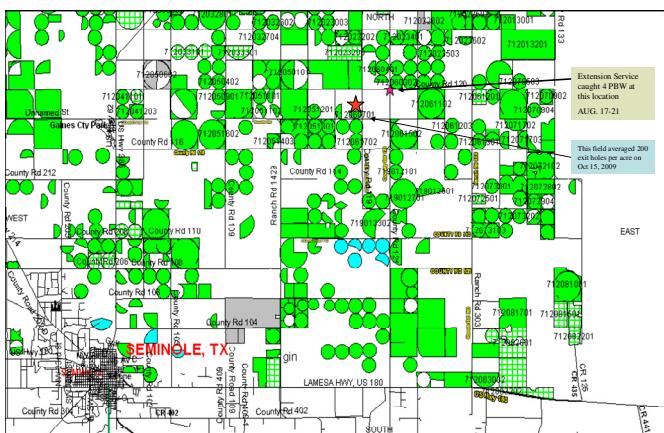
This is Midland Texas and these are the fields in the El Paso Trans Pecos area, approximately fifty-five miles from this zone to the edge of the cotton. The first week they captured was the week of October 12<sup>th</sup>. The second line is the October 19<sup>th</sup> trap captures.

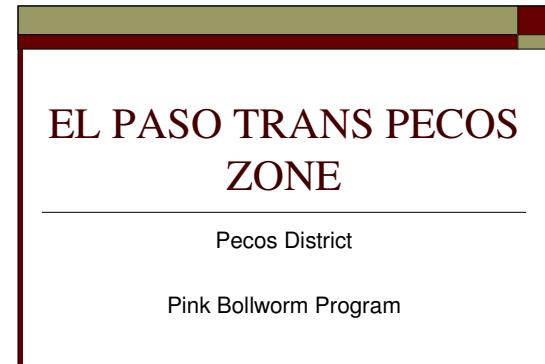
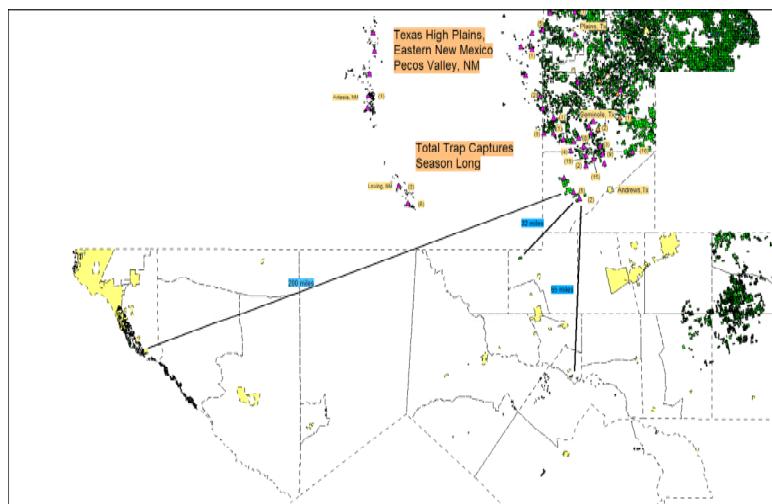
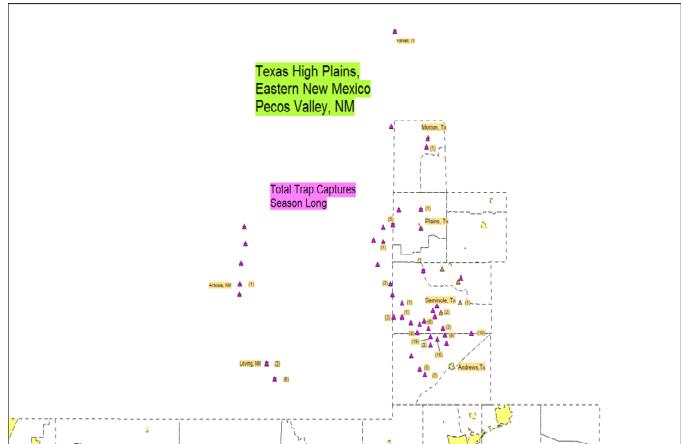


We started the trapping program in July. We did not capture any pinkies until the 12<sup>th</sup> of October.



This is Seminole, Texas. The extension service caught four pink bollworm on August 17<sup>th</sup>. Mr. Smith referenced particular fields and provided details as illustrated in the following slides.





## El Paso Trans Pecos 2009 Acreage

Unit 1201 Pecos District- Non-Bt 340

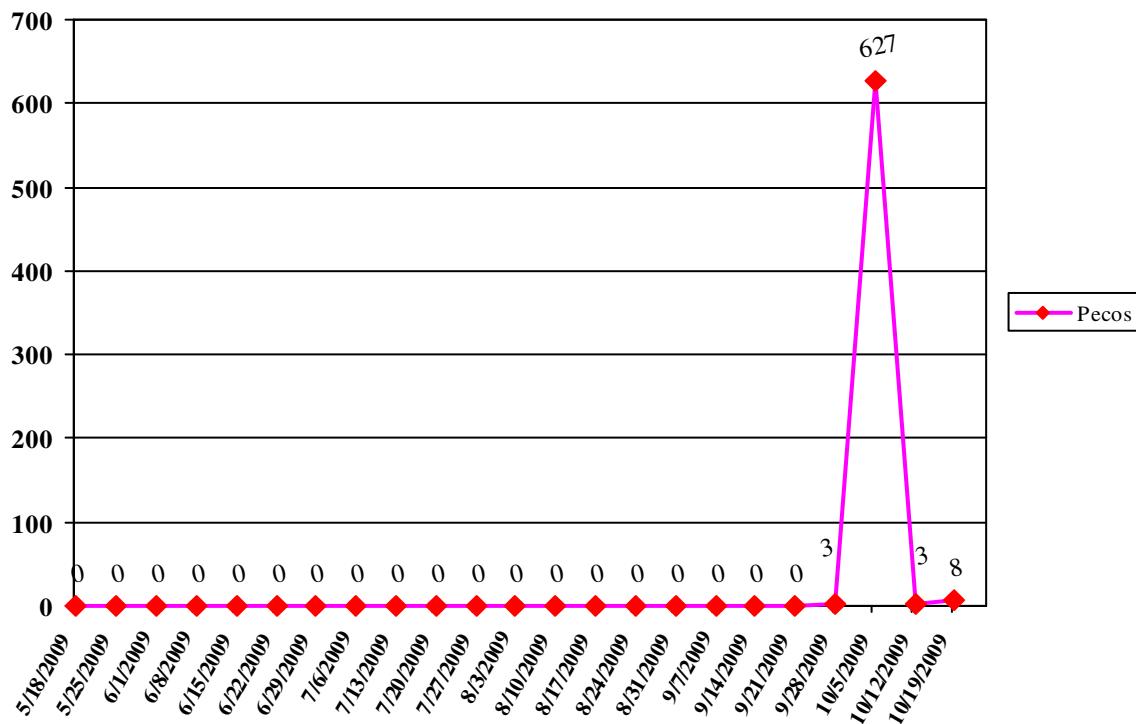
acres + Bt 6,075.7 = 6,415.7 acres

(Organic 320 acres in Van Horn, Refugia 5 acres in Saragosa, 5 acres in Pecos, 10 acres in Ft Stockton Prison)

Unit 1202 Lower Valley- Non-Bt 10,198.8 acres +  
Bt 4,587.6 = 14,786.4 acres (Upland Non-Bt 1,016.1  
acres in Acala with the rest Non-Bt in Pima)

Unit 1203 Upper Valley/Fabens north) - Non-Bt 9,860.6  
acres + Bt 2,914.5 = 12,775.1 acres (Organic 106.8 acres  
in Upper Valley, Non-Bt in Pima)

## Pecos District Pink Bollworm Trap Captures by Week



## 2009 El Paso-Trans Pecos

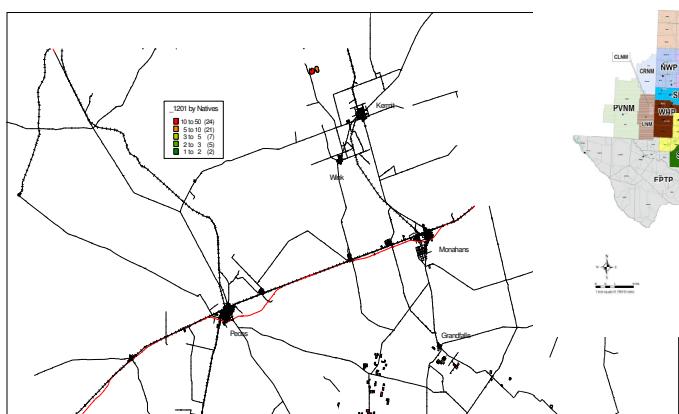
### Pink Bollworms per Trap Inspection by Week

	Pecos / 1201	Upper Valley / 1203	Lower Valley / 1202
Week Beginning			
9/21/2009	0 / 327	0 / 925	0 / 1095
9/28/2009	3 / 330	0 / 928	0 / 1094
10/5/2009	627 / 330	0 / 926	0 / 1093
10/12/2009	2 / 332	0 / 929	0 / 1094
10/19/2009	8 / 330	573 / 929	0 / 1094

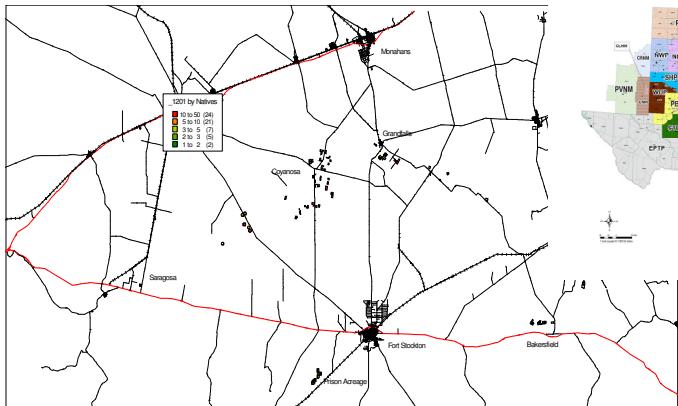
## Pecos District Historical Data

- Last recorded PBW larval find in Pecos District was on July 7, 2007, sixteen miles northwest of Fort Stockton TX in pima cotton acreage.
- Last recorded native PBW trap capture prior to 2009 migration was on August 7, 2007 in Coyanosa, TX next to Bt cotton acreage.

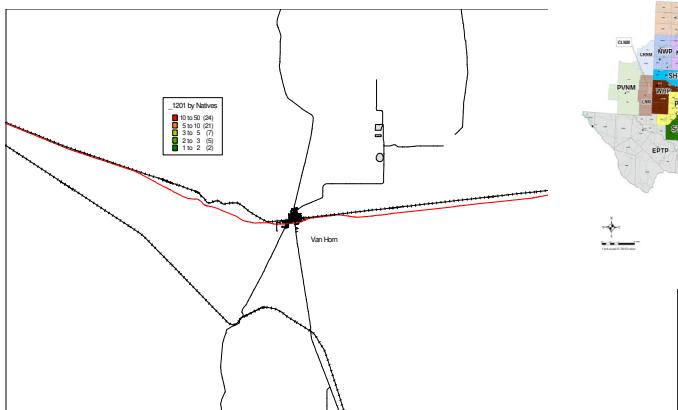
## Northeastern Pecos District



## Central Pecos District



## Western Pecos District



## 2009 Pecos District Sterile Releases

- July 7, 2009  
Released 5,140,485 steriles over Van Horn, Pecos, Saragosa and Fort Stockton cotton acreage.
- October 6, 2009  
Released 7,028,360 steriles over Van Horn, Pecos, Saragosa, Coyanosa and Grandfalls cotton acreage.
- October 9, 2009  
Released 7,107,474 steriles over Van Horn, Pecos, Saragosa, Coyanosa and Fort Stockton cotton acreage.
- October 10, 2009  
Released 6,821,699 steriles over Van Horn, Pecos, Saragosa, Coyanosa and Fort Stockton cotton acreage.

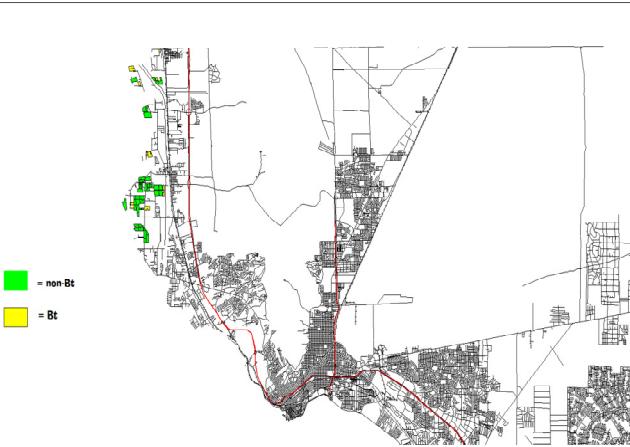
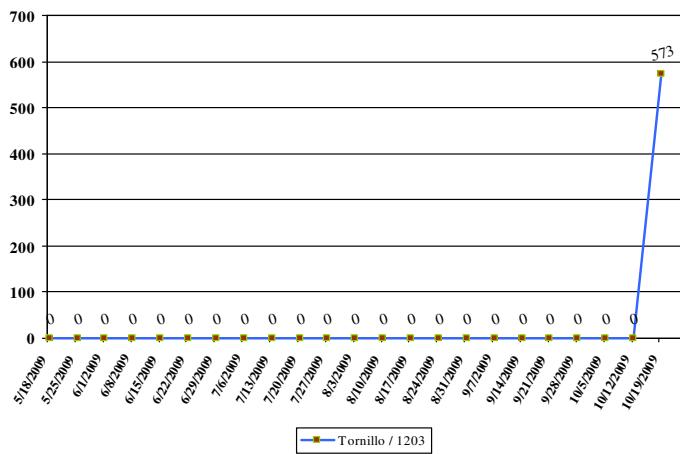
## EL PASO TRANS PECOS ZONE

Tornillo District

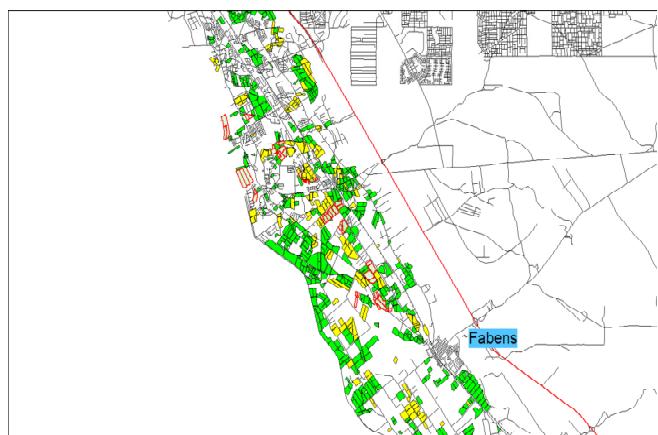
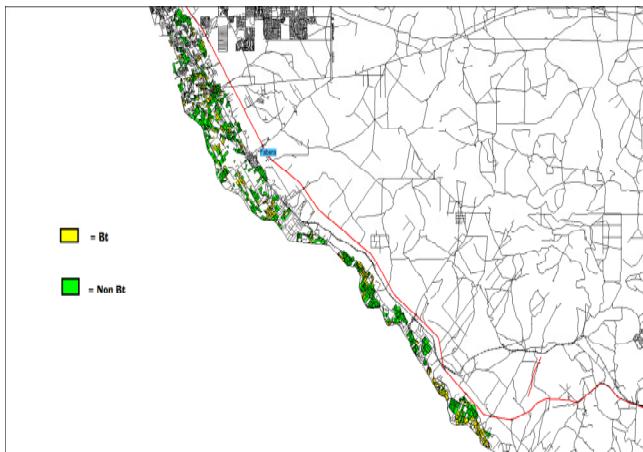
Pink Bollworm Program

In the Tornillo district, we had zeros all season, and then went to 573 in one week.

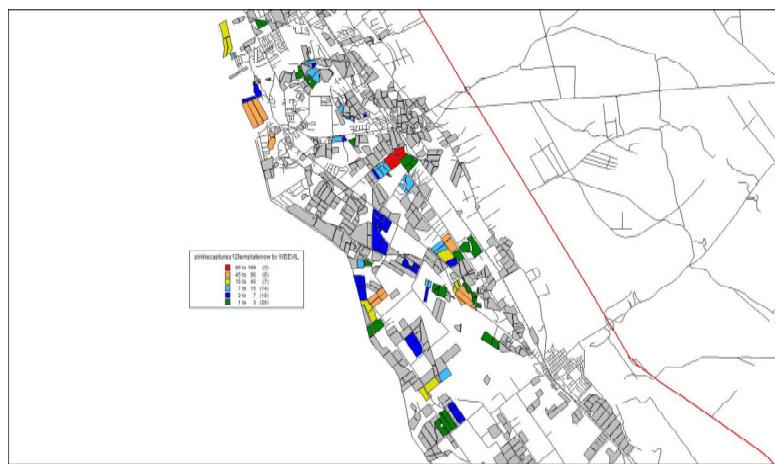
Tornillo District Pink Bollworm Trap Captures by Week



This is the upper Valley, north and west of El Paso. The grain is the non-Bt.



2009 Pink Bollworm Trap Captures (Week of October 19, 2009)



## Pink Bollworm Captures by Field -- Work Unit 1203

Sterile moths were dropped for 142 days with an average of 9,683,169 per day.

Total sterile moths dropped for the 2009 Season is 1,375,010,090

1203871501	169	1203870504	8	1203872507	2
1203871020	163	1203871703	8	1203872621	2
1203872602	73	1203851401	7	1203873605	2
1203873607	70	1203870921	7	1203910103	2
1203873601	69	1203860110	6	1203910404	2
1203870709	50	1203872716	6	1203911404	2
1203861203	48	1203872811	6	1203911405	2
1203873305	47	1203873518	6	1203911410	2
1203873303	44	1203860112	5	1203843110	1
1203910401	44	1203870903	5	1203870515	1
1203873505	22	1203871603	5	1203870611	1
1203872603	21	1203871702	5	1203870908	1
1203911102	17	1203910306	5	1203871521	1
1203853501	16	1203870503	4	1203872511	1
1203911111	16	1203872201	4	1203872813	1
1203871604	12	1203872203	4	1203873508	1
1203873507	12	1203910305	4	1203873513	1
1203870501	11	1203872202	3	1203873603	1
1203872604	10	1203872612	3	1203873606	1
1203872812	10	1203872712	3	1203873612	1
1203911118	10	1203873304	3	1203873615	1
1203870815	9	1203873307	3	1203873619	1
1203871504	9	1203911403	3	1203910403	1
1203871517	9	1203870603	2		
1203853613	8	1203871515	2		

## Recapture rates on Fields with Pink Bollworm Captures

FieldFull	Natives	Sterile Moths
1203871501	169	6527
1203871020	163	4712
1203870709	50	5000
1203861203	48	18212
1203873607	70	10095
1203873601	69	2122
1203872602	73	10257
1203853501	16	13109
1203873505	22	1765
1203873507	12	4510
1203853613	8	2653
1203870815	9	4249
1203851401	7	2974
1203870504	8	6028
1203870903	5	2733
1203870921	7	4163
1203870503	4	3513
1203860110	6	6007
1203872507	2	5112
1203870603	2	4649
1203870515	1	7379
1203873518	6	5143
1203843110	1	3838
1203870611	1	3685
1203073416	0	17587



Charles Allen inquired, and Edward Herrera confirmed that they were catching steriles when they were catching natives, after they stopped releasing for the season. The sterile recaptures are down. The last day of release was the 12<sup>th</sup>. Larry Smith advised that he did have reliable trapping reading information up until the spike reading. Clyde Sharp asked if that movement could have been attributed to any storms moving through. Larry advised that on the 28<sup>th</sup> they had a cold front. They triggered Pecos about two weeks earlier than he triggered St. Lawrence.

Dr. Staten advised that in looking at the data for this anomaly, they seem to be two very distinct events. The movement that came into that Pecos area is a separate event from whatever occurred in the El Paso area. He has no idea what occurred in the El Paso area.

Dr. Staten had discussed this with Don Parker and Dennis Palmer the night before. Don Parker suggested getting assistance from a climatologist modeler, which we will probably pursue. He would look at vector wind systems through out that whole area, which may prove to shed light on what might have happened. The closest possible source is Carlsbad. You didn't see a whole lot of activity there. That whole artesian New Mexico area is a unique area where you can have one little three acre plot somewhere that produces a lot of pink bollworm when nothing else seems to be moving. That may be a possibility as well. There may be something else distinctly behind this, but we simply don't know at this point. The most important El Paso data, when doing heat unit models, progeny from these moths will not, under normal circumstances, make forth in-star by January 1<sup>st</sup>. The biggest fear with the El Paso Valley is offspring be located in a pocket of moths that we did not detect. The data in their trapping does not indicate that, and the extensive search that was made in the fields for reproduction does not indicate that. It leaves you wondering. Next year, that area will need 17 million moths, and we will have to consider some pheromone systems.

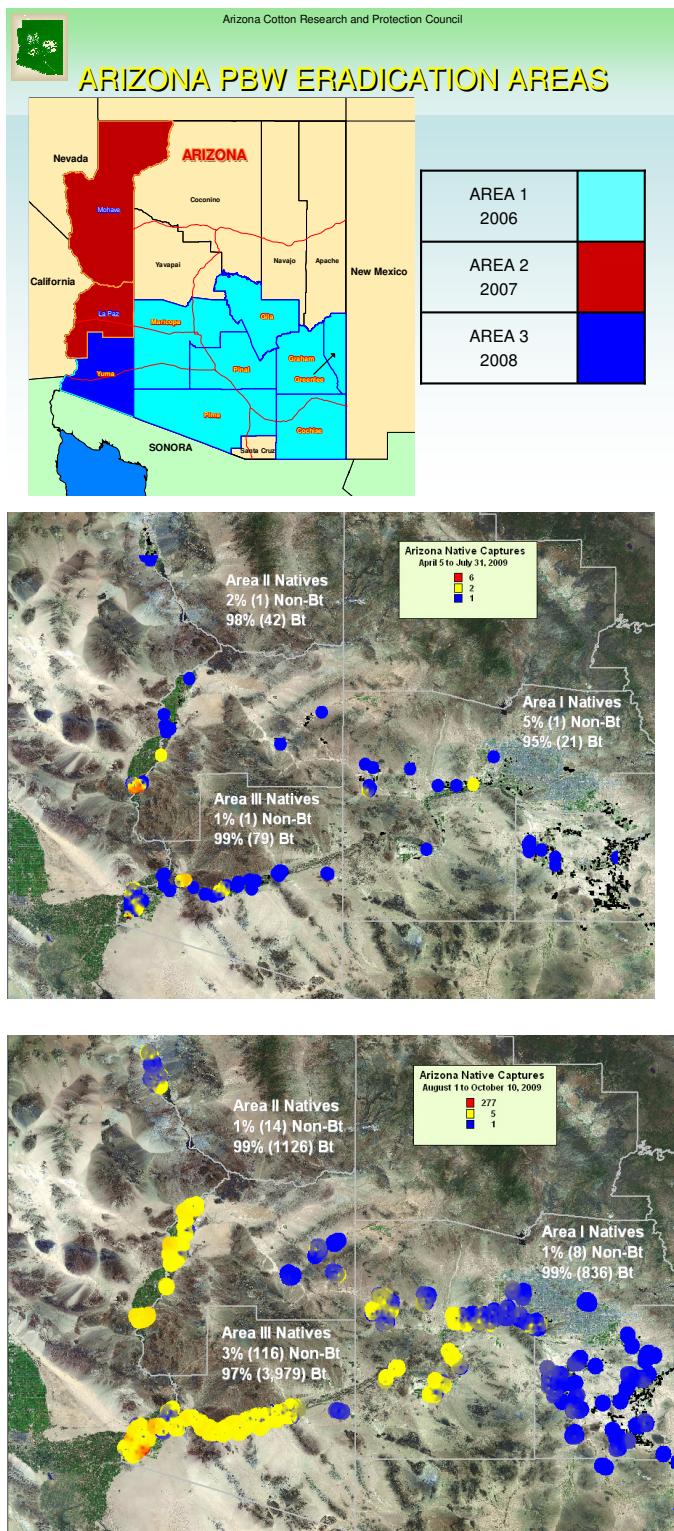
Per Ernie Miller's inquiry, Dr. Staten explained that there are more than one million acres up there. He is not sure how good of a grid you would be able to have. It would certainly be a wise investment to do some of the trap strings like they are done in Arizona and Chihuahua, from those other areas towards this area in order to be able to detect migration. Certainly, some sort of an indicator system up there would be useful. It would be useful long-term for planning when this program goes post-Phase III. That whole area is going to take a lot of strategic thought and planning before you talk about it. Sooner or later, you will want to go after pink bollworm in that area. Jim Ed Miller concurred, stating that we need to kill the bugs from where they came from. Dr. Staten is guessing this may be the same situation as San Joaquin and they have been dealing with it for forty years. It looks very similar. We have to clean up areas I, II and III with the resources at hand, and then ultimately, we will have to appeal to our colleges in Mexico to go after the Torreon and Durango area.

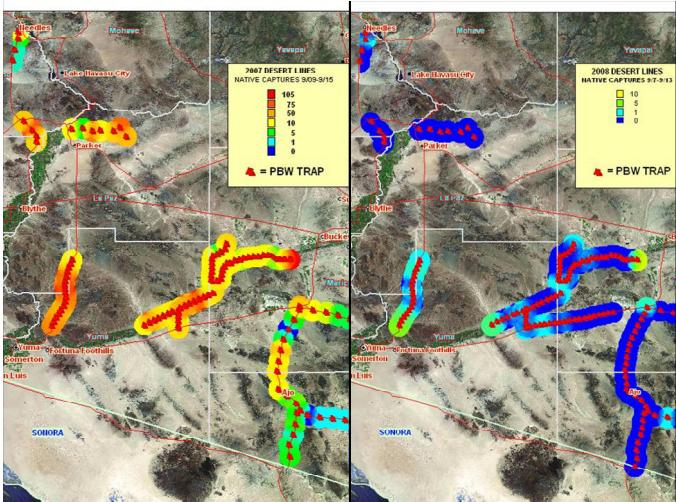
Larry Smith advised that there is normally a lot of Bt cotton planted in that area. Texas coming into the Spring was very dry. Possibly, a lot of these producers considered the insurance and planted more non-Bt planted than usual, which may have allowed some of these things to happen. We cannot be certain and we don't have a lot of research up there. In talking to John Westbrook, we can find out more about wind patterns.

Bruce Tabashnik asked how much Bt cotton is planted in that area. David Kearns estimated that during a typical year you can see 75-80 percent, maybe even a little higher. This year it was a little lower.

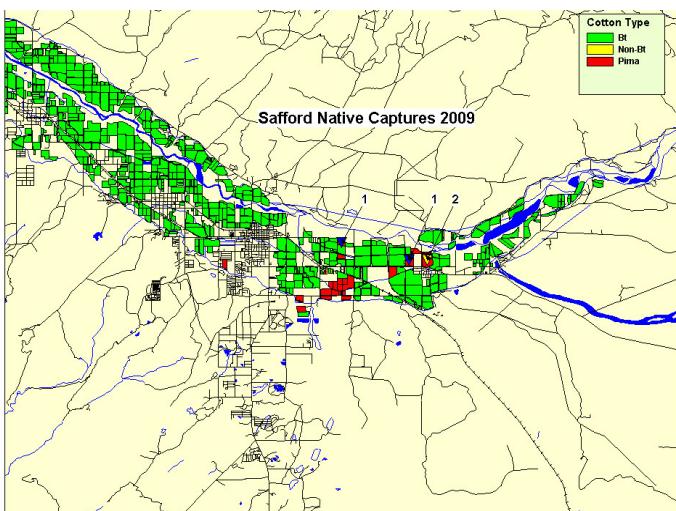
Dr. Staten asked Larry or Jim to discuss the movement in the Colorado River Basin. Larry Antilla reported that we have had some very good success in reduced numbers this year. There were good numbers in Yuma too, above the ninety-percentile reduction. Leighton put together some really good thematic maps illustrating early season versus late season movement. Larry noted that all of the non-Bt was treated with pheromone rope.

Leighton reported that Arizona is broken into three areas. Central and Eastern Arizona completed their fourth year this season. Next year will be our forth year in Area 2, and 2011 will be Yuma's forth year.





We are still seeing a large distribution of movement. The numbers are steadily going down as progress is made in the region. There are a lot of moths in program traps during migration.

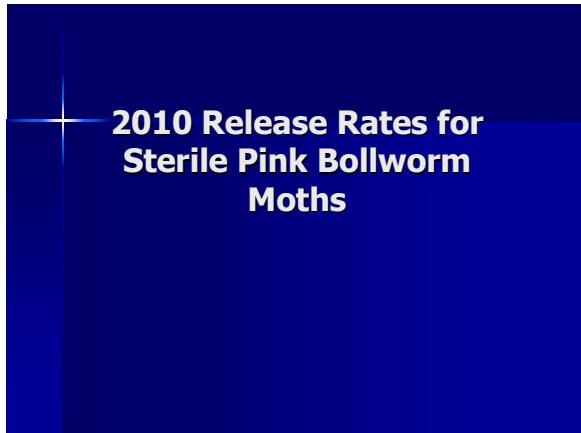


Leighton discussed the captures in the Safford area during migration. There were four insects captured and no evidence of reproduction was found. Larry reported they perform season-long boll sampling in all non-Bt fields. We perform boll cracking, and then forward 500 to 1,000 boll samples to Bruce Tabashnik for boll box incubation. So far, no larvae have been found.

Upon inquiry by Dr. Staten, he reported that the samples for Safford have not been completed yet. Upon inquiry by Charles Allen, Leighton reported that all three fields that caught pink bollworm were Pima fields. Dr. Staten advised the Committee will have to spend considerable time thinking about where these programs may go in the future, particularly to the adjacent areas. They will have to do the same thing in Mexico. That will be done in planning sessions with Sanidad Vegetal, and possibly during the Mazatlan meeting. Perhaps we should put together a Work Group within the Committee if the Action Committee so desires.

## Initial Distribution Moth Production

We will now go over the tentative break down for distribution of sterile insects.



<b>Release Rates Per Week</b>	
■ Texas	30 M
■ Juarez	7.5 M
■ New Mexico	0 M
■ Arizona	43.5 M
■ Southern California	2 M
■ SJV	12 M
■ N.W. Mexico	45 M
■ TOTAL	140 Million

Dr. Staten reported this year there is 140 million moths per week available, which works out to 28 million less than last year in terms of the way the budget is set up. Dr. Staten had hoped for less pressure in the El Paso area in particular, which has taken one of the highest cuts of moths.

After his recent trip to El Paso, it is quite apparent that the area north of Fabens is going to have to continue at its existing release rate, which is about 17.5 million per week. In the Pecos area, you have very little non-Bt cotton that had a positive find. There is a large area of Bt cotton that is in that umbrella for release.

On Bt cotton, it will need a very low level of release and require flying time, but even at a high release rate on Bt cotton, there is a commitment of about 2.5 million. There is 20 million with those two areas. Dr. Staten feels very strongly that you will need at least 10 million starting with concentrated releases around the Port of Entry find in the Tornillo area. The entire situation will have to be watched very carefully.

Arizona has been reduced. This will largely have to come from Bt cotton areas, where we will take advantage of what we have just accomplished. The hope will be that the relative low percentages of non-Bt cotton will be stable, but we have a tremendous amount of flying to do in Arizona. Dr. Staten is hoping that we can find a way to make that number work for Arizona. We are talking about reducing by half, based on the fact that they have four total recaptures on or about the third of September. We set up a concentrated release with half of their insects. We can run that release that way next year unless we discover a population to the contrary.

This could change at any time based on captures. This is the tentative release. We have some debate in California on how we will handle okra, but it is basically at the same level that we were releasing last year. The graphs last year showed southern California and Arizona combined. The San Joaquin Valley has a unique problem which Jim Rudig will address later. There is a much higher level of native moth captures in the San Joaquin this year compared to last year. They are concentrated around a variety trial. Any release has to be concentrated. We ended the season with additional moths there. We will have to start the season with about the same moth release there as last year.

We left northwest Mexico as it was, unfortunately. Dr. Staten would like to have more there, but we just don't have them. The crux of the entire program is getting complete control and eradication in Mexicali and San Luis as the final stages of this push westward. If that fails, it would spread rapidly westward as opposed to the east.

Given these resources and all of the information, this is the best distribution available. Dr. Staten asked if anyone has any comments concerning a better distribution of insects or if there was any further discussion. There was not.

**M/S/P      Larry Antilla moved to accept Dr. Staten's Release Rates per Week slide as a starting point for sterile release distribution for next year. Bobby Hull seconded. The motion passed unopposed.**

### **Temperature Standards for Seed Handling**

Dr. Staten advised that there is an issue around how particularly small-plot planting seed is handled, and also seed that is being moved around the country and around the world for experimental purposes. Apparently, there needs to be a review of all of the standards. There is an issue of whether or not temperatures are being met, or are we substituting acid concentrations, and so on. *Dr. Staten requested Jim Rudig, Bill Grefenstette and Joe Ellington and to volunteer to work with him putting a draft together and research literature in order to review it. They agreed. Dr. Staten will also contact Jim Shoenholtz. Dr. Staten further instructed Dr. Ellingson to find information in the Las Cruces Ginning Lab.*

### **Motion to Adjourn**

**M/S/P Bobby Hull moved to adjourn. Jim Ed Miller seconded. The motion passed unopposed.**

# Pink Bollworm Mating Disruption: Sprayable Pheromone Development

Safford, Arizona

May 2009 – October 2009

Michelle Walters, Barry Barnes, and Nelson Foster, USDA-APHIS-PPQ, CPHST Phoenix Lab, 3645 E. Wier Avenue, Phoenix, AZ, Jack Jenkins, Pacific BioControl, 620 E Bird Lane, Litchfield, AZ, Tim Roland and Daryl Hill, USDA-APHIS-PPQ, AEO, 22675 N. Moorefield Rd. Bldg. # 6415, Edinburg, TX, Mike Whitlow and Larry Antilla, Arizona Cotton Research and Protection Council, 3721 E. Wier Avenue, Phoenix, AZ and Robert Staten, USDA retired, 414 E. Barbarita Ave, Gilbert, AZ

## Introduction

Since its arrival in the Southwestern United States, the pink bollworm, *Pectinophora gossypiella* (Saunders), has proved to be a devastating cotton pest, one of the main crops of the region. Through the area-wide and programmatic use of sterile insect release, genetically modified cotton, good cultural practices of growers, and mating disruption via the dispersal of sex pheromones, PBW populations have been suppressed and eradicated. The current standard mating disruption product, PB-Robe L, is effective and long-lived but requires hand application. The eradication program needed a mating disruptant that could be mechanically applied by air or ground, is economically priced and would be effective for at least 30 days. Effectiveness in this case means trap catches in the treated fields remain under the economic threshold (catch an average of less than one moth per trap per night)

## Methods and Materials

We used PBW-GEL (supplied by Pacific Biocontrol, 9.2% AI in an acrylic formulation), the most promising formulation found in previous research. Ground application research, with a specialized, research ground vehicle, was conducted in 2007 in Wellton, AZ. Wellton was not then under eradication and had high levels of wild PBW against which to test the pheromone treatments. The GEL formulation provided 30+ days of mating disruption under very high population pressure, was heat stable and cleanup was acceptable.

In 2008, aerial application research was conducted in Virden, NM, just east of Safford, AZ, where we had sufficient acreage of untreated, uninfested fields and therefore no need of pesticide treatments that would interfere with the research. Sterile moths were released to provide a PBW population against which to test the mating disruptant. We used GEL modified with 1% NALCO-TROL (drift control additive supplied by Nalco Co., Naperville, IL, 30% polyvinyl polymer) to thicken it, with the expectation of larger droplets. We had tested the additive in 2007 to make sure that it did not interfere with the attractiveness or longevity of the pheromone. We conducted additional laboratory tests in 2008 to compare relative viscosities of GEL with and without several levels of NALCO-TROL. We found that with our equipment and all things being equal, 1% NALCO-TROL made the GEL about 3 times as viscous. We made only minor modifications to standard aircraft spray equipment, an Ag Truck, at 120 mph applying 109 grams of formulation/acre (10 grams active ingredient/acre). The first treatment was mid-season when the crop canopy was closing, making ground application difficult - a time when aerial application is very desirable.

In 2009, a pump was found that allowed a standard tractor rig to apply the GEL by ground. Further modifications to an aircraft were made to stabilize application rates. Subsequently, ground and aerial application research was conducted in Safford, AZ (Graham County), in 2009. Similar to the research done in Virden, NM in 2008, the fields used in Safford were untreated Non-Bt cotton and uninfested with wild PBW. GEL was applied to 7 Pima cotton fields. Four additional fields (two Bt and two treated with PB-Rope L) were not treated with GEL, and were used as checks. The fields included in the research ranged in size from 27-50 acres and received 2,700 sterile PBW moths per week per acre, in 3 aerial releases/week, to provide a PBW population against which to test the mating disruptant.

The first gel application of the 2009 season was done at the pin square stage (3-7 leaf cotton), 3 fields aerially where the GEL formulation was modified with 1% NALCO-TROL, and 4 by ground rig with straight GEL . All seven fields were treated in a day's time. A second application was performed over a two week period (delays due to irrigation schedules) in early mid-season, entirely by ground methods – the aircraft was not available. The third and final application of the season was performed in a two day span, entirely by the aircraft, owing to the height and condition of the cotton and the availability of the aircraft. All aerial applications were made by a Cessna (USDA APHIS, AEO) with minor modifications, while the ground applications were performed by a standard tractor (ACRPC).

Resembling previous years, moth populations were monitored with Delta traps (in field, 4 per field). Traps were placed 30 rows from the edge of the field and 30 paces into the field. Each trap was baited with a standard septa lure (changed biweekly), and checked two times a week.

## Results

In 2009, after the first application at pin square, the material on both the ground and air treated fields lasted at least 26 days but increased to  $\geq 1$  moth/trap/night by day 29. Note that at least 90% of the aerially applied formulation fell on open ground, vs. a cotton plant, because of the size of pin square cotton. After the second application, applied by tractor to much larger plants, the formulation remained effective for an average of 38 days. The third application, applied by aircraft with most of the formulation deposited on foliage, was effective for 42 days until the traps were pulled so the cotton could be picked. Releases of sterile moths remained constant throughout the test. Recapture rates in untreated fields declined, due to less activity from male moths, in the second and third application test stages (this phenomenon is consistent and observed annually across the eradication area).

### GEL Application, Safford, AZ - 2009

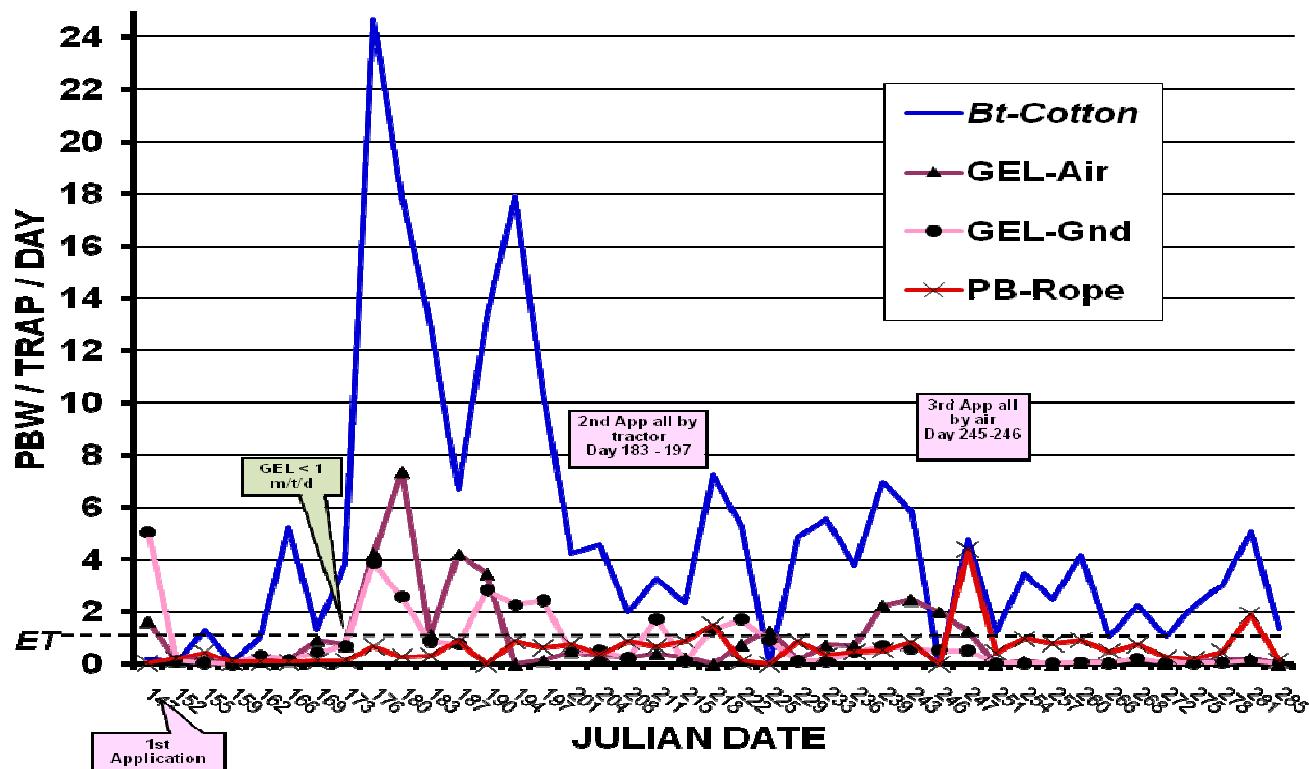


Figure 1: The average number of pink bollworm catches, by treatment and over time

## **Conclusions**

GEL is easy to apply aerially and by ground, without major modification to standard equipment, on pin-square stage cotton or post-bloom cotton. GEL is harmless to the cotton crop and does not cause observable phytotoxicity – the treated leaves appear normal and, most important, GEL does not cause early senescence nor leaf drop. GEL stands up well to typical levels of sun, rain and wind; very heavy rainfall (1" downpour within 1.25 hours following the first application in 2008) may shorten mating disruption longevity. In the 2008 tests, GEL lasted almost 20 days on first aerial application (probably due to rain immediately after application) and 46+ days for the second application. In 2009, the Gel was effective 26 days at pin square, 38 days mid-season and more than 42 days late season. Our experienced aerial application staff considered clean-up easy relative to standard pesticides. We are very encouraged by the performance of this formulation.

## **Plans for 2010**

Pacific Biocontrol and Shin-Etsu are pursuing registration for GEL. The PBW eradication program expects to use GEL during and following eradication.

Mike Whitlow, ACRPC, plans to adapt the ground rig to connect directly to the GEL container. This will further simplify application and cleanup.

USDA APHIS PPQ CPHST and AEO plan to finalize aerial procedures to make them more compatible with existing commercial aerial application equipment.

## **Resolution Summary / Recommendations**

*Dr. Staten asked for assistance from Steve Naranjo, Bruce Tabashnik and Bob Hull concerning the clarification changes that need to be made to the Minimum Standards document and requested they meet with him after the meeting.....* 11

**M/S/P Don Parker moved to adopt the Minimum Standards document with revisions to clarify as discussed here. Charles Allen seconded the motion and the motion passed unopposed.....** 11

*The USDA is recommending to this Technical Committee as the position we would take regarding the use of sterile moths on Bt cotton going forward.....* 13

Dr. Staten requested the wording be changed to “...*release of mean of ten sterile insect moths per acre per day or a cumulative release of seventy sterile moths per acre of Bt cotton per week....*” Jim Rudig advised that in California they use the term of “*will receive a release rate*”. *The USDA will craft that into this*

**document.** USDA does not need Technical Advisory Committee permission to set forth a recommendation, but the Committee should give a resolution of support.

**M/S/P Bob Hull moved to adopt a resolution of support for the USDA recommendations for sterile insect release as refugia, with the understanding that “will receive a release rate” will be crafted into the document (as noted above). Jim Rudig seconded the motion and the motion passed unopposed.....** 14

“....If there is no desire for a motion to push this technology forward, the Chairman will **table it and call for the full reports, and a second meeting of the panel.** Jim Rudig requested a time line be placed on that. Discussion ensued. Dr. Zink responded that he could provide the report by the end of November.....  
**Dr. Zink clarified that he would provide a full report on the trial this past summer by the end of November....”**.....24

“....**Dr. Staten requested that Dr. Zink get together with him or Don Parker with the risk analysis information and full report, and they will work with the Committee.....Dr. Staten asked Steve Naranjo to volunteer, and Steve agreed. Bruce Tabashnik noted that on the benefit side concerning the manuscript in 2008 there was a trial done with not only recapture rate measured, but control efficacy of the DS Red strain was measured, and felt that information should be included in the analysis....”**.....25

**“Dr. Staten suggested that Ernie give us an assessment of how many he would need and when, and what the ramp up capabilities are. Ernie has actually done this exercise several times, so if he could compile that data and send it to us. We will give him a week deadline. We will explore trying to put together a conference call in either December or January.”**.....25

**M/S/P Larry Antilla moved to accept Dr. Staten’s Release Rates per Week slide as a starting point for sterile release distribution for next year. Bobby Hull seconded. The motion passed unopposed.....** 41

**Dr. Staten requested Jim Rudig, Bill Grefenstette and Joe Ellington and to volunteer to work with him putting a draft together and research literature in order to review it. They agreed. Dr. Staten will also contact Jim Shoenholtz. Dr. Staten further instructed Dr. Ellingson to find information in the Las Cruces Ginning Lab.....** 41

Bobby Hull moved to adjourn. Jim Ed Miller seconded. The motion passed unopposed. .... 41