



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

OFFICE OF  
PREVENTION, PESTICIDES, AND  
TOXIC SUBSTANCES

January 11, 2007

**MEMORANDUM**

**SUBJECT:** Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting Held October 24 - 25, 2006 on the Evaluation of the Resistance Risks from Using 100% Bollgard and Bollgard II Cotton as Part of a Pink Bollworm Eradication Program in the State of Arizona.

**TO:** James J. Jones, Director  
Office of Pesticide Programs

**FROM:** Myrta R. Christian, Designated Federal Official  
FIFRA Scientific Advisory Panel  
Office of Science Coordination and Policy

**THRU:** Steven Knott, Executive Secretary  
FIFRA Scientific Advisory Panel  
Office of Science Coordination and Policy

Clifford J. Gabriel, Ph.D., Director  
Office of Science Coordination and Policy

Attached, please find the meeting minutes of the FIFRA Scientific Advisory Panel open meeting held in Arlington, Virginia on October 24 - 25, 2006. This report addresses a set of scientific issues being considered by the Environmental Protection Agency pertaining to the Evaluation of the Resistance Risks from Using 100% Bollgard and Bollgard II Cotton as Part of a Pink Bollworm Eradication Program in the State of Arizona.

Attachment

**CC:**

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OPP Docket

**FIFRA Scientific Advisory Panel Members**

Steven Heeringa, Ph.D. (FIFRA SAP Chair)  
John R. Bucher, Ph.D.  
Stuart Handwerger, Ph.D.  
Gary Isom, Ph.D.  
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**FQPA Science Review Board Members**

Andrew Paul Gutierrez, Ph.D.  
Gary P. Fitt, Ph.D.  
Ian V. MacRae, Ph.D.  
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Steven L. Peck, Ph.D.  
Linda J. Young, Ph.D.

**SAP Minutes No. 2007-01**

**A Set of Scientific Issues Being Considered by the  
Environmental Protection Agency Regarding:**

**EVALUATION OF THE RESISTANCE RISKS  
FROM USING 100% BOLLGARD AND  
BOLLGARD II COTTON AS PART OF A PINK  
BOLLWORM ERADICATION PROGRAM IN THE  
STATE OF ARIZONA**

**OCTOBER 24 - 25, 2006  
FIFRA Scientific Advisory Panel Meeting,  
held at the Holiday Inn Rosslyn at Key Bridge,  
Arlington, Virginia**

## NOTICE

These meeting minutes have been written as part of the activities of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP). The meeting minutes represent the views and recommendations of the FIFRA SAP, not the United States Environmental Protection Agency (Agency). The content of the meeting minutes does not represent information approved or disseminated by the Agency. The meeting minutes have not been reviewed for approval by the Agency and, hence, the contents of these meeting minutes do not necessarily represent the views and policies of the Agency, nor of other agencies in the Executive Branch of the Federal government, nor does mention of trade names or commercial products constitute a recommendation for use.

The FIFRA SAP is a Federal advisory committee operating in accordance with the Federal Advisory Committee Act and established under the provisions of FIFRA as amended by the Food Quality Protection Act (FQPA) of 1996. The FIFRA SAP provides advice, information, and recommendations to the Agency Administrator on pesticides and pesticide-related issues regarding the impact of regulatory actions on health and the environment. The Panel serves as the primary scientific peer review mechanism of the EPA, Office of Pesticide Programs (OPP), and is structured to provide balanced expert assessment of pesticide and pesticide-related matters facing the Agency. Food Quality Protection Act Science Review Board members serve the FIFRA SAP on an *ad hoc* basis to assist in reviews conducted by the FIFRA SAP. Further information about FIFRA SAP reports and activities can be obtained from its website at <http://www.epa.gov/scipoly/sap/> or the OPP Docket at (703) 305-5805. Interested persons are invited to contact Myrta R. Christian, SAP Designated Federal Official, via e-mail at [christian.myrta@epa.gov](mailto:christian.myrta@epa.gov).

In preparing the meeting minutes, the Panel carefully considered all information provided and presented by the Agency presenters, as well as information presented by public commenters. This document addresses the information provided and presented by the Agency within the structure of the charge.

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**OCTOBER 24 - 25, 2006  
FIFRA Scientific Advisory Panel Meeting,  
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**Steven G. Heeringa, Ph.D.  
FIFRA SAP Chair  
FIFRA Scientific Advisory Panel  
Date: January 11, 2007**

**Myrta R. Christian, M.S  
Designated Federal Official  
FIFRA Scientific Advisory Panel  
Date: January 11, 2007**

**Federal Insecticide, Fungicide, and Rodenticide Act  
Scientific Advisory Panel Meeting  
October 24 - 25, 2006**

**EVALUATION OF THE RESISTANCE RISKS FROM USING 100% BOLLGARD AND  
BOLLGARD II COTTON AS PART OF A PINK BOLLWORM ERADICATION  
PROGRAM IN THE STATE OF ARIZONA**

**PARTICIPANTS**

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**Designated Federal Official**

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## INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP) has completed its review of the Evaluation of the Resistance Risks from Using 100% Bollgard and Bollgard II Cotton as Part of a Pink Bollworm Eradication Program in the State of Arizona. Advance notice of the meeting was published in the *Federal Register* on August 2, 2006. The review was conducted in an open Panel meeting held in Arlington, Virginia, from October 24 – 25, 2006. Dr. Steven G. Heeringa chaired the meeting. Myrta R. Christian served as the Designated Federal Official.

The FIFRA SAP met to consider and review the Evaluation of the Resistance Risks from Using 100% Bollgard and Bollgard II Cotton as Part of a Pink Bollworm Eradication Program in the State of Arizona. The State of Arizona issued two Special Local Need Registrations under FIFRA section 24(c) in March 2006 permitting the use of 100% Bollgard® and Bollgard II® cotton varieties along with sterile pink bollworm (PBW) moths (i.e., the sterile insect technique or SIT), pheromones, and limited use of chemical insecticides in a sanctioned PBW eradication program. As a provision of these registrations, the State of Arizona agreed to provide the Agency with data to support the continued use of the FIFRA 24(c) registrations. These data focused on the uncertainties associated with the expected effectiveness of the PBW eradication program using the SIT, 100% Bollgard and Bollgard II cotton, pheromones, and limited insecticide use. The Agency was concerned with the potential for increased resistance of PBW to *Bacillus thuringiensis* (*Bt*) proteins that may be posed by the use of 100% Bollgard and Bollgard II cotton (i.e., with no structured non-*Bt* cotton refuge, very high selection intensity) and whether the SIT can be used effectively to replace the role of refuges in managing PBW resistance to *Bt* should it occur during the four-year eradication program. The State of Arizona, in consultation with USDA and University experts, devised a plan that replaces the biological function served by non-*Bt* cotton refuges with mass-reared sterile PBW. Any resistant PBW individual that develops will mate with a sterile moth and the resistant gene will be eliminated. The Panel was asked to comment on the Agency's review of the effectiveness of this plan using field level systematic monitoring and mapping data in conjunction with modeling simulations.

The agenda for this SAP meeting included an introduction of the Pink Bollworm Eradication Program in the United States and Mexico provided by Dr. Osama A. El-Lissy (USDA). An overview of the state of Arizona FIFRA Section 24(c) registrations in the PBW eradication program was provided by Mr. Alan Reynolds (Biopesticides and Pollution Prevention Division (BPPD), OPP), and a review of the likelihood of *Bt* resistance developing

during Arizona's PBW eradication program was presented by Dr. Sharlene Matten (BPPD, OPP).

Dr. Janet Andersen (Director, BPPD, OPP) offered opening remarks at the meeting.

In preparing these meeting minutes, the Panel carefully considered all information provided and presented by the Agency presenters, as well as information presented by public commenters. This document addresses the information provided and presented at the meeting, especially the response to the Agency's charge.

## **PUBLIC COMMENTERS**

### **Oral statements were presented as follows:**

Bruce Tabashnik, Ph.D., on his own behalf

Timothy J. Dennehy, Ph.D., on his own behalf

Mark Sisterson, Ph.D., on his own behalf

Mr. Clyde Sharp on behalf of Arizona Cotton Growers

Mr. Paul Ollerton on behalf of Arizona Cotton Growers

Larry Antilla, Ph.D., on his own behalf

Robert Staten, Ph.D., on his own behalf

Mr. James Rudig on behalf of State of California Department of Food and Agriculture's Pink Bollworm Program

Frank Carter, Ph.D., on behalf of National Cotton Council

Mr. Bill Lovelady on behalf of National Cotton Council Pink Bollworm Action Committee

David W. Bartels, Ph.D., on behalf of USDA APHIS PPQ Center for Plant Health Science & Technology

## **SUMMARY OF PANEL DISCUSSION AND RECOMMENDATIONS**

### **Estimations of Pink Bollworm Populations Using Pheromone Trapping and Spatial Analysis**

The Panel was impressed with the quality and comprehensive nature of the supplied data package and public comments supporting the Arizona eradication program for PBW using the sterile insect technology (SIT) in combination with 100% Bollgard and/or Bollgard II cotton, pheromones, and limited insecticide use. They felt it is critical that annual mapping of patterns derived from both pheromone trap and boll sampling are continued through the life of the program but that the intensity of sampling, trap placement, and boll sampling hot spots be adapted to reflect expected declines in the native PBW populations. The Panel noted that the accuracy and precision of estimates derived from the two sampling methods differs and is an important consideration given the need to provide early warning of the development of PBW resistance to *Bt* cotton. In addition, the panel noted it is important that direct monitoring of resistance using molecular tools continues through the life of the program as it is the best

measure of population level changes in the resistance allele frequency; boll counts and trap data provide early warnings of hotspots. However, a number of potential concerns were identified in both monitoring methods.

Boll sampling, while probably more accurate and precise in estimating levels of infestation, is time consuming and not practical for real-time, within-field estimates of PBW populations, especially at low densities. Pheromone traps will provide a useful relative measure of released and native PBW males (released sterile males are distinguished by color from dyes in their food) in the population but may be influenced by a number of characteristics of the traps, the insects and the environment. The efficacy of pheromone traps in non-*Bt* fields is especially problematic. The pheromone confusion technique also employed in these fields to disrupt PBW mating competes with that from the traps and causes an under-estimate of the moth population density and compromises the utility of data in the spatial analyses.

Despite these factors, the two sampling methods complement each other to the extent that both should continue throughout the period of the project. The panel recommends ongoing evaluation of both sampling methods during the period of the program to assure adequate sampling precision as native PBW populations decline. The Panel specifically recommends that the trap catch data from *Bt* and non-*Bt* fields be analyzed separately to facilitate explanation of population change predictions derived from spatial analyses locally and regionally. Further, the assumptions underlying the population estimates from pheromone traps should be continually assessed through the program period and potential relationships between trap catch and boll sampling through boll filling and maturation should be examined. Further specific recommendations can be found in the *Pheromone Trapping and Boll Sampling* sections of the response to charge #1.

While spatial analyses of PBW populations should definitely be continued throughout the program period, they currently do not provide satisfactory results and add little to the interpretation of population estimates. However, the extensive datasets (~3500 sample sites) provide an opportunity to employ a number of analyses to produce meaningful predictions. For example, the data used to create the interpolative predictions made with kriging should be detrended and assessed for isotropy. The use of potential PBW movement to set the range of the spatial autocorrelation may be better replaced by estimates of the range of autocorrelation after removing any trend in the data. The existing dataset may be large enough to provide subsets with which to cross-validate the predictive models. Maps of the prediction errors should also be constructed. There was little concern over the use of centroids instead of geographic coordinates as the management units for the program are whole fields. Coordinates may provide better insight for modeling purposes but will have minimal impact on management decisions. The creation of regions in spatial analyses may require the modifiable areal unit problem to be assessed (i.e. the increase of correlation among variables as area increases). The interaction of time and space may provide insight into the changing dynamics of PBW populations; non-separable space-time models are therefore suggested. Future monitoring efforts will have to adapt to declining populations as the eradication program progresses; a number of specific recommendations for analytical methods were made, including the eventual adoption of Poisson-based models over normal-based models, SAS's GLIMMIX procedure, and indicator kriging.

Further specific recommendations can be found in the *Spatial Analysis* section of the response to charge #1.

The sterile insect technology (SIT) must be used in a way to not only assure eradication but to replace the effects of removal of spatial refuges for suppressing the potential development of resistant genotypes. Current release rates seem adequate to achieve this. As PBW populations decrease, monitoring methods may need to be adapted to ensure adequate detection limits. It is recommended that the current number of traps not be decreased during the program period. Further specific recommendations can be found in the *Specific Comments on the Use of SIT* section of the response to charge #1.

The potential for local and long-distance migration/dispersal was discussed in the public comments; it has not been directly incorporated into interpretations of population dynamics or in the modeling effort. While there is evidence of regular long distance movement resulting in potential immigration into the program area, this will likely affect eradication more than the development of resistance. A number of methods for discriminating between local and long distance movement exist, including population genetic markers and tools such as the HYSPLIT model. It was also suggested to make the current model more reflective of the spatial pattern of fields in the program area. Further specific recommendations can be found in the *Sources of Uncertainty in the Estimates of PBW Population Levels* section of the response to charge #1.

### **Simulation Modeling**

Models are best used as research guides and to pose and test hypotheses that cannot be addressed by direct experimentation. The question of certainty posed to the Panel cannot be addressed by simulation models because they do not have predictive value in a statistical sense. The simulation model can only provide a ‘worst case’ scenario of resistance development in this system.

Although it was felt the modeling effort was well done, several weaknesses were identified: mating was assumed to occur only once, movement between more than three fields was not included (i.e. the distribution function should have considered the possibility of movement in the tails), and it assumed sterile PBW were as competitive as native individuals. The simulation could be modified to more realistically represent the spatial patterns of fields in Arizona, field sizes, and topology. However, the Panel thought that none of these considerations would affect the main conclusion that resistance is unlikely to develop during the period of the project. Increased realism, to more accurately reflect the biology of the situation, can be introduced into the model through the adoption of physiologically based models. In general, however, the predictions of the simulation modeling concerning the development of resistance agreed with previously published work and provide supporting evidence that resistance is unlikely to develop in the four years of the Arizona Area Wide Project.

The iterative examination and modification of models with biological data is an important part of the development of simulation, analytical and statistical models. It is recommended that this effort continue for the duration of the program. It is unlikely, however, that additional simulations will overturn the current predictions but they could be useful in exploring resistance

problems within this system, especially the implications to resistance development of additional pesticide applications triggered by low over-flooding ratios of sterile to wild insects, and by the presence of larvae in boll samples.

The model may not be the best reflection of “worst case” scenarios; sensitivity studies of the model changed only one factor at a time. A factorial design could be used to examine the interaction of important combinations of factors that allow for richer combinations of parameter values. Variation in the stochastic model’s output should be examined carefully to determine the number of simulation runs necessary to capture that variation and to identify parameters contributing the greatest variation to the results.

Given that Cry1Ac and Cry2Ab2 are pyramided in Bollgard II, a product currently planted at less than 5% of *Bt* acreage in Arizona, the potential for resistance to Cry2Ab2 to develop during the program period was deemed unlikely. It should be noted that simulation models of the stacked products are unlikely to provide relevant information over and above the expectation from population genetics theory. Furthermore, PBW does not display cross-resistance between these two toxins and laboratory trials indicate Cry1Ac resistant individuals are susceptible to Cry2Ab2. Consequently, the initial probability of resistance is the numerical product of their individual initial probabilities of resistance. Simulation models conducted by other authors indicate that even low levels of gene-frequency dilution cause rapid declines in the frequency of resistant alleles. Discussions of cross-resistance in other meetings dealing with this program have also indicated it is unlikely to be a concern.

**Adequacy of Field Data, Spatial Analysis and Simulation Modeling to Provide Reasonable Certainty that PBW Resistance to Cry1Ac and Cry2Ab2 is Unlikely Through the Period of the Arizona Area Wide Program (Four Years)**

The Panel felt that the Arizona Area Wide Program is well designed and managed and is an exceptional model of an area wide IPM program. It incorporates significant monitoring efforts, high rates of grower adoption and support, and has adequate triggers for additional suppression tactics if needed. One of the most impressive aspects of the program is the inclusion of a remedial plan for responding to the development of resistance should it occur.

The protocols for testing for known *cadherin* alleles ( $r1$ ,  $r2$ , and  $r3$ ) and any subsequent novel resistance alleles are excellent. The added use of polymerase chain reaction (PCR) techniques to detect resistance genes in PBW caught in pheromone traps and in larvae reared from sampled bolls complements the thorough approach to resistance monitoring in this program.

The lack of resistance genes from the large number of field samples tested to date suggests gene frequency in the population is low. However, the selection of resistance in the laboratory from a field collected source indicates that continued field monitoring of trapped PBW adults is vital to trigger the remedial action plan if necessary.

The spatial modeling does not currently have the ability to accurately assess the spatial extent of resistance within the PBW population. The extensive dataset and the suggestions made in this report should be used to seek the predictive ability required by the program.

The simulation model predictions, the historical lack of resistance in the field, and the lack of recorded genetic change all indicate a low likelihood that resistance will develop over the period of the program. The use of released sterile PBW adults to remove potential resistant individuals from the population and the remedial action plan to prevent the establishment of any developing resistance further decrease the likelihood of resistance developing during the program period.

Considering the data presented, the Panel concluded there would be a very low likelihood of resistance to Cry1Ac or Cry2Ab2 developing over the 4-year program period.

## **PANEL DISCUSSION AND RECOMMENDATIONS**

The specific issues to be addressed by the Panel are keyed to the Agency's background documents, references, and charge questions.

### **Agency Charge**

#### **1) Estimations of Pink Bollworm Populations Using Pheromone Trapping and Spatial Analysis**

Geospatial maps of the *Bt* and non-*Bt* cotton fields were provided by the Arizona Cotton Research and Protection Council (Appendix 4 - EPA Technical Review of Materials Supporting Arizona's 24(c) Applications [AZ05009 and AZ050010]). There were 4,626 total fields (approx. 156,000 acres) in the eradication zone, of which 334 were non-*Bt* fields (6.92%) and 4,292 *Bt* fields (93.08%) (Table 2 - EPA Technical Review of Materials Supporting Arizona's 24(c) Applications [AZ05009 and AZ050010]). Each field is numbered. All fields were targeted for sterile moth releases, and insecticide treatments if needed. Non-*Bt* fields also received pheromone for mating disruption. There were a total of 4,541 pheromone traps placed in all fields with 3,541 pheromone traps placed in *Bt* fields and 1,000 pheromone traps placed in non-*Bt* fields. The protocol for the sterile moth releases is found on p. 11 of EPA's technical assessment (EPA Technical Review of Materials Supporting Arizona's 24(c) Applications [AZ05009 and AZ050010]). The actual sterile moth release rates through August 25, 2006 are found in Table 4 (EPA Technical Review of Materials Supporting Arizona's 24(c) Applications [AZ05009 and AZ050010]). The number of traps per field ranged from 0 to 14. The scheme for using the trapping and map data is shown in Figure 5 (EPA Technical Review of Materials Supporting Arizona's 24(c) Applications [AZ05009 and AZ050010]).

To present the trapping data as a predicted surface of PBW numbers, kriging was used to calculate a predicted value for areas between the known values of each field. Kriging is a regression technique used in geostatistics to estimate the optimal interpolation of these points across the spatial domain. This method handles spatial autocorrelation and is not sensitive to uneven sampling in specific areas, such as the distribution of cotton fields in the eradication program. Ordinary kriging using a spherical model was applied to trap counts for each week

(see Volume 2, Table 1 of the submission, MRID# 469048-02 for the data) to develop a predictive surface model encompassing the cotton fields. Kriging constructs a weighted moving average that estimates the value of a spatially distributed variable from adjacent values while considering their interdependence. Kriging results in a smoothing effect in which high original values are underestimated and low original values are overestimated. It is a best linear unbiased predictor because it minimizes the variance of the prediction errors.

The kriged maps of native and sterile PBW populations in Arizona's eradication program from June 25 through July 22, 2006 are found in Figures 6A-H (EPA Technical Review of Materials Supporting Arizona's 24(c) Applications [AZ05009 and AZ050010]). This analysis indicates that the sterile PBW adult populations are more abundant, consistent and more widely distributed than the native population. The native populations are limited to 1-5 moths per trap with 3-5 areas as "hot spots" (PBW captures > 25) during the four-week sampling period. The sterile PBW populations are more abundant with captures > 50 in many areas. Early results from the eradication program indicate that the sterile releases have been quite successful in reducing native PBW populations.

**a) The Panel is asked to comment on the accuracy and precision of the estimates of native and sterile pink bollworm population levels in the eradication zone in Arizona in 2006 using the described pheromone trapping and boll sampling methods, and spatial analysis (kriging method). Factors that may affect the native and sterile pink bollworm populations estimates include, but are not limited to:**

- 1. Number and location of *Bt* and non-*Bt* fields**
- 2. Size of fields (e.g., 15 ha)**
- 3. Use of pheromone traps, only males sampled, to estimate overall population size (and therefore population suppression estimates)**
- 4. Number and placement of traps within a field**
- 5. Number of traps in *Bt* vs. non-*Bt* fields**
- 6. Use of the centroid of the field to estimate trap location within a field vs. exact location of the field using GPS coordinates for the spatial analysis (kriging method), e.g., to address "hot spots" within a field or set of small fields**
- 7. Sterile release rates (*Bt* vs. non-*Bt*)**
- 8. Frequency of sterile releases**
- 9. Predictions about the ratios of sterile: native moths**
- 10. Estimations of overwintering larvae per field**
- 11. Boll sampling data (not available for these analyses, too early in the season)**

**Please identify major sources of uncertainty in the estimates of PBW population levels and comment on whether the estimates tend to overstate or understate actual levels of native and sterile PBW.**

- b) Given the discussion in a) above, what suggestions does the Panel have to strengthen the accuracy and precision of the native and sterile pink bollworm population estimates?**

## **Panel Response**

### **General Comments**

Overall the Panel was extremely impressed by the quality and comprehensive nature of the data package provided and by the ambitious nature of the PBW eradication program that is being coordinated and managed by the Arizona Cotton Research and Protection Council and University of Arizona researchers.

Questions on the accuracy and precision of population estimates derived from pheromone traps and boll sampling are important, since these are the two key techniques that will be used to assess the trend in population dynamics through the suppression and eradication campaign and to provide early warning of resistance hot spots, should they occur. It is thus critical that annual mapping of patterns derived from these two indicators are continued through the life of the program and that the intensity of sampling not decrease but be adapted as the native population declines. The two techniques being used for population assessment vary in accuracy and in the suite of complicating factors that require careful analysis to maximize value from the data and provide decision support for the program.

### ***Pheromone Trapping***

The use of an extensive network of pheromone traps specific for PBW males provides, at best, a relative estimate of population density, since catches of males will be influenced by many factors other than population size. These factors include characteristics of the trap itself (placement, height, pheromone quantity, trap shape and establishment of a plume), of the insect (mobility, age), the load of trapped adults and of the environment (competition from other pheromone sources nearby, weather).

Given these caveats, the pheromone traps can provide a useful indication of the ratio of sterile to native moths, but interpreting this ratio requires information on the competitiveness of sterile moths relative to natives (longevity, mobility, responsiveness to pheromone). Some information on the competitiveness of sterile PBW was provided, but no information on their responsiveness to pheromone. It has been demonstrated, however, that *Bt* resistant PBW adults are as responsive to pheromone as are susceptible ones (Carriere *et al* 2006).

In general terms the pheromone traps will be competing to attract males in a background of multiple alternative sources provided by the native females, the large number of sterile females that also presumably release pheromone and in the non-*Bt* cotton by the application of pheromone dispensers for mating disruption. The latter treatment severely disrupts the effectiveness of traps associated with non-*Bt* cotton resulting in apparently lower densities than in the *Bt* crops even though the population density is probably considerably higher than in *Bt*

cotton. This difference in trap efficiency suggests that the data from *Bt* and non-*Bt* crops be analyzed separately (see *Spatial Analysis*).

Concerns about the interpretation of spatial data would be simplified if there were no non-*Bt* fields, but these are being cultivated for reasons that are unlikely to change. One key issue raised was in relation to the layout and physical separation of traps and their potential to interfere with one another through overlapping pheromone plumes. Such an effect could potentially result in negative autocorrelation in the data. With the current density of traps in *Bt* (1 trap per 43.5 acres) and non-*Bt* fields (1 trap per 11.5 acres), it seems highly unlikely that between trap interference is occurring, but information on the attractive zone of traps should be provided. Trap density (1/40 acres and 1/10 acres in *Bt* and non-*Bt* respectively) appears more than adequate for monitoring purposes.

As the population is further suppressed, individual traps might become more effective because there will be fewer competing sources of pheromone, but the limits of detection of the network will also be tested. This raises the issue of what rules to apply to decide that eradication has been effected (Barclay and Hargrove 2005). It is likely that differing operational and statistical treatments will need to be applied at different stages through the 4-year program.

Despite the fact that pheromone traps only sample males and despite all the caveats and limitations about how to interpret catches, pheromone traps really are the only practical method to provide extensive monitoring of population change over the expanse of cotton fields involved in the program in Arizona.

### ***Boll Sampling***

Boll sampling either through cracking of bolls or the use of boll boxes provides a more direct measure of population density and integrates the effects of a number of other factors which impinge on adult fecundity, egg mortality and larval establishment. However, this is a time consuming and labor intensive technique that cannot be applied in the same extensive manner as pheromone trapping and from which results are inevitably delayed relative to the almost real time nature of trap catches. Nonetheless there were suggestions that the efficacy of boll sampling could be further enhanced by reducing the number of bolls per field and increasing the number of fields sampled. If the maximum number of bolls that can be adequately sampled and handled is fixed, then there will be a trade-off between extensive and intensive use of boll sampling.

There may well be opportunities to use adaptive sampling techniques (Thomson and Seber 1996) to improve identification of hot spots. This could be done with more intensive pheromone trapping or immediate checking of larval infestations in bolls to confirm indications that a hot spot may be developing.

### ***Spatial Analysis***

The nature of the trapping data makes prediction of the PBW population challenging. Although more sterile moths are released in the non-*Bt* fields and the native population would be

anticipated to be higher in these fields, the trap counts are actually lower. This is undoubtedly due to the use of mating disruption activities in the non-*Bt* fields. The trap counts from those fields may still be good relative measures of the number of viable male moths in that area, but the proportion of the population captured is undoubtedly much less than the proportion in the *Bt* fields. This needs to be carefully considered in subsequent analyses.

The number of sample sites in the program is extensive with approximately 3500 sites contributing to the interpolated surfaces. In fact, increasing the number of contributing sites may improve the precision but not significantly improve the accuracy of the predicted values. Further, the number of sites may provide an opportunity to select a subset of data to evaluate the predictions of the spatial model.

Statistical regression methodology can be used to improve the confidence in the pheromone trap count data. In particular, it may be possible to identify a relationship between the boll sampling data, which seem to be universally assumed to be a more accurate and precise estimate of infestation, and the pheromone trap data that may provide a better, more practical population estimate. This could only be done for the boll filling and maturation period of crop development.

An initial useful analysis would be to plot the numbers of sterile PBW against the numbers of native moths from the same trap. This should be done separately for *Bt* and non-*Bt* fields and for each sampling date. If the relationship appears linear, then Pearson's correlation coefficient might be a good way to quantify this relationship; otherwise, Spearman's rank correlation coefficient may be more appropriate. Finally, one could formally test whether the correlation coefficients are the same for *Bt* and non-*Bt* fields.

An underlying assumption in estimating populations from pheromone trapping data is equality of sex ratio. While data from Arizona indicate that PBW has a 1:1 sex ratio there are indications that the sex ratio of PBW populations in NE Africa may vary with time of year and food source of the female moths (Kostandy & Rashad 1997 & Naguib 2004). Sex ratio in the Arizona population should continue to be assessed.

Generally speaking, the kriging analysis on trapping data that has been conducted has not been appropriate. Given the quantity of data available over multiple locations, simply mapping the raw data and observing patterns over time may provide a good initial view of the available information. The differences in *Bt* and non-*Bt* fields should be accounted for in the model. For some of the dates, it appears that a trend might be present over the area though this may be an artifact of combining *Bt* and non-*Bt* fields. To produce a more informative map, it would be prudent to develop separate maps for *Bt* and non-*Bt* fields for each sample date.

Trends in the trapping data need to be removed before estimating the semivariogram, or else universal kriging should be used. It is possible that the difficulty in estimating the semivariogram was due to the presence of a trend and differences in *Bt* and non-*Bt* fields. There is no reason to believe that the extent to which moths move has a direct relationship to spatial autocorrelation. It would be better to estimate the range of the correlation after removing the trend than to force a range based on movement that was estimated in other unrelated studies.

Because the pheromone trap catch will also be biased with wind direction, directionality of the data may also be an issue. The assumption of isotropy should, therefore, be checked and anisotropic models used if the semivariogram changes significantly with direction. Cross-validation and other statistical methods should be used to evaluate the fit of the models. It should be noted that population predictions, and not estimates, are obtained with these procedures. Maps of the prediction errors should be constructed.

Although it is statistically preferable to have the GPS coordinates of each trap, use of the centroid for fields with multiple traps is not a major concern since the field is the unit of management and it is unlikely that hot spots will occur only in part of one field nor is there any indication that pest management would be targeted at areas below the size of a field. However, in modeling the trend, there will be greater value from considering each data point.

One challenge, especially if the suppression program is successful, is the appropriate modeling of low counts. One possibility is using Poisson regression, or perhaps the zero-inflated Poisson model, with correlated errors. Although routines exist in S-Plus and R for this purpose, SAS's GLIMMIX may be a particularly useful tool. This procedure allows for the use a generalized linear model with correlated errors. In addition, it has the capabilities for semi-parametric regression. This could be particularly useful in modeling trends in the data as it is unlikely that simple polynomial models will be able to accurately capture these over the region. As the PBW population decreases further, indicator kriging may prove to be a better choice. This approach would emphasize modeling the probability of finding a native moth for points across the region. However, the concerns mentioned earlier must still be considered.

It is important to consider the distance between traps. From a spatial analysis viewpoint, we cannot draw inferences on spatial correlation at distances shorter than that between the closest two traps. At the same time, if traps are too close together, they may have overlapping ranges of influence. In this latter case, one would anticipate a negative autocorrelation between neighboring traps with a positive autocorrelation being expressed as the distance increased. Most spatial statistics models assume a positive autocorrelation, making this a more difficult model to develop.

The analyses thus far have been based on the data for a given date. One could consider space-time models. If space and time in the data are separable, then the analysis would be a relatively simple extension. If space and time interact, then one could consider adding time and the interaction between time and the spatial variables in the model. Finally, one could consider non-separable space-time models (e.g., Gneiting 2002, Irwin *et al.* 2002).

A more thorough spatial analysis addressing the issues listed above might provide additional insight. Data from the non-*Bt* fields are surely causing an underestimate of the relative abundance of PBW in the area. Some of the issues mentioned above might tend to lead to over prediction and others to under prediction of the populations. It is not clear what the net effect would be.

When regions are created, one may encounter the modified areal unit problem (MAUP) (see, for example, Openshaw and Taylor 1979 or, for a critical review of statistical methods

associated with this problem, see Gotway and Young 2002). In general, it has been shown that, as the sizes of the areas increase, the correlation among variables also tend to increase, at least to a certain point. The reason for this lies to some extent in the smoothing that occurs in the aggregation process. At the very least, one should consider regions of differing sizes to gain some idea as to whether this is a problem. This recommendation is valid even if the regions have a biological basis (other biological definitions are possible). The MAUP also does not disappear if the sizes of the regions vary.

Finally, it is important to note that management requirements of the program, and the statistical approaches required for data analysis, may change as the population is suppressed. Normal-based models applicable early in the program may need to revert to Poisson-based models down to Indicator (zero or 1) data models as the data set becomes more dominated by zero values.

### ***Specific Comments on the Use of SIT***

It is critically important that the sterile PBW are used in a way that is likely to achieve the goal of eradication and also act as a replacement of refuges that are needed to provide protection against the potential evolution of *Bt* resistance. Release rate and frequency of release of sterile PBW into *Bt* and non-*Bt* crops (49.4 moths/ha and 247 moths/ha respectively) seem to be more than adequate and above previously suggested rates needed to achieve suppression. The capability for production and release of sterile PBW is impressive; however, there is an ongoing need to demonstrate the competitiveness and ecological effectiveness of the sterile PBW in the Arizona cotton environment.

Current reliance on red dye provided in the diet as a means to distinguish released sterile moths from the native population may be replaced during the course of the Program through new technology to simplify the distinction of moths in trap captures. A public commenter (R. Staten) reports that a method of genetically transforming PBW with a fluorescent protein is currently being evaluated. This would negate the need for the internal dye and improve the identification of sterile males.

As population suppression proceeds, it will be necessary to gain some indication of the limits of detection of the trapping network. Trapping efficiency will decline as the native population falls to very low levels. While the current trap network may seem extensive, there should be no reduction in the number of traps through the life of the program. If anything, there may need to be more traps to assist in determining that eradication has been achieved (as far as it is possible to say that). This may be problematic should trap catch be used as a criteria to define absence of the pest.

### ***Sources of Uncertainty in the Estimates of PBW Population Levels***

Interpretation of any real biological system will always be dependent on uncertainties in a number of parameters. Having to rely on relative estimates of population density means that uncertainties may be multiplied.

A major area of uncertainty that may impinge on the likelihood of eradication and on the dynamics of resistance relates to the frequency and extent of local and long-distance movements of adult PBW. Tabashnik et al. (1999) provide evidence of local movement that suggests males move distances of up to 400m, which is important for movement between *Bt* crops and non-*Bt* refuges but could also aid trapping design in this program.

There seems to be significant evidence for regular long distance movements at certain times of the year, driven in part by weather patterns, but this movement is not considered when seeking to interpret population dynamics or in the modeling effort. Especially lacking is an understanding of the tails of the movement distribution. Long-range dispersal may be important, although probably more related to eradication issues than insecticide resistance.

Possible approaches to help distinguish between local movement and immigration may come from population genetic approaches which utilize genetic markers to distinguish local from immigrant moths. Another post-hoc tool to assist in identifying sources of potential movement is the HYSPLIT model (<http://www.arl.noaa.gov/ready/hysplit4.html>), designed to model the movement of airborne particulates. HYSPLIT provides both forward and back tracking data from/to source or target location for a given date/time. While the model cannot confirm a source location for an immigration event, it can indicate if movement from a suspected source was possible on a given date.

A further suggestion was to utilize the existing spatially explicit model but make it more reflective of the Arizona spatial layout of fields and accommodate more realistic elements of movement. The inclusion of physiological and climatic parameters would add to the functionality and biological reality of the models for future application.

## **Agency Charge**

### **2) Simulation Modeling**

The Agency required that simulation modeling be used to compare the impact of PBW population suppression vs. resistance risk over the four-year period of the eradication program. The simulation model used was a revised version of the spatially-explicit, stochastic model discussed in Sisterson *et al.* (2004). The simulations examined population suppression (number of PBW per ha) and risk of resistance to *Bt* cotton (rate of increase of resistance allele frequency). This model assumes that resistance to the *Bt* toxin is controlled by a single, recessive gene. This model is based on PBW resistance to Cry1Ac. Modifications to the model include the release of sterile moths. A variety of scenarios were simulated using the best estimates of the parameter values as well as more optimistic and more pessimistic scenarios.

Preliminary modeling, even using more “worst case/pessimistic” parameter assumptions, predicts that the four-year eradication program in Arizona will suppress PBW without creating a problem with Cry1Ac resistance to *Bt* cotton. In 11 of 12 sets of assumptions examined, the simulated eradication program eliminated PBW from the 4096 fields modeled in two years or less without selecting for resistance. In the one exception, PBW was not

removed from the region when the model simulation assumed no release of sterile moths in *Bt* fields, 90% *Bt* cotton, and the resistance allele frequency ( $r$ ) = 0.01 in all five replications. In this case, the population density declined by 98% (460 final overwintering larvae per field from a starting density of 29,000 starting overwintering larvae per field) and  $r$  increased from 0.01 to 0.02 after four years, but was still far lower than the 0.50 value typically used as a criterion for the development of resistance. The model assumes that population suppression will occur if the mean PBW density in the region is equal to or less than 0.1 overwintering larvae per 15 ha (=0.0067 larvae per ha).

- a) **The Panel is asked to comment on the certainty of the preliminary outcomes of the modeling simulations using worst-case assumptions, in many cases, that pink bollworm populations will be suppressed and there will be no resistance to Cry1Ac during the four years of the eradication program simulations.**

Dr. Bruce Tabashnik (University of Arizona) plans to conduct additional simulations using field data collected in 2006 as model inputs in place of certain assumptions used in the 2005 simulations.

- b) **The Panel is asked to comment on whether there is any reason to expect these additional simulations of pink bollworm resistance over the four-year period of the eradication program will change the predicted outcome to indicate a greater risk of the development of resistance than seen in the 12 other simulations in which no resistance was seen.**

PBW resistance to the Cry2Ab2 toxin was not considered in either the simulation modeling or DNA screening analyses. Additional consideration of PBW resistance to the Cry2Ab2 toxin would only be important if the selection pressure dramatically increases in the next three years, i.e., much more Bollgard II is planted in the eradication zone. If some or all of Arizona's *Bt* cotton has two toxins, Cry1Ac + Cry2Ab, evolution of resistance would be much less likely than it is with only Cry1Ac. Modeling resistance to cotton that produces only Cry1Ac is the more pessimistic scenario. The modeling predictions (using only Cry1Ac resistance), therefore, are conservative, i.e., they tend to overestimate resistance risk. Based on simulation models examining the likelihood of insect resistance to pyramided toxins in *Bt* crops (e.g., Roush, 1998; Zhao *et al.*, 2005), even if Bollgard II acreage substantially increases, the likelihood of PBW resistance to both the Cry1Ac and Cry2Ab2 toxins would remain low during the four-year PBW eradication program in Arizona.

- c) **The Agency asks the Panel to comment on the likelihood of Cry2Ab2 resistance given that percentage of Bollgard II planted in Arizona has been <5% of the total *Bt* cotton acreage, and simulation modeling predicts that the likelihood of insect resistance to pyramided toxins in *Bt* cotton would be extremely low.**

### Panel Response

## ***Resistance to Cry1Ac***

The Panel is asked to comment whether resistance to Cry1Ac is likely given the “worst case” scenarios provided to the EPA by the simulation studies of PBW by Tabashnik and Sisterson (2004). The model is an individually-based, spatially-explicit stochastic simulation model that includes single-loci, two-allele population genetics, two age classes and their associated population dynamics, movement probabilities and distance, the addition of the SIT, spatial patterns, and management options including insecticidal sprays when the ratio of sterile to native moths becomes low.

Models typically reflect the question being asked, the experience and expertise of the modeler, and often their biases. Models are best used as research guides and to pose and test hypotheses that cannot be addressed by direct experimentation. The question posed to the Panel of certainty of the prediction cannot be addressed by simulation models because they do not have predictive value in a statistical sense. The results of a simulation model provide a ‘worst case’ scenario of resistance development in this system and have only a heuristic value.

The Panel noted several weaknesses of the model. These include; (1) mating was assumed to occur only once between a single pair of insects, (2) movement beyond three fields was not included, i.e., the distribution function should have included the possibility of movement in the tails, and (3) the assumption that sterile moths were as competitive as natural moths. This later assumption is suspect and may need to be reconsidered in the model. The Panel also felt that the simulation could be modified to be more realistic in representing the Arizona situation in terms of spatial patterns, field sizes and topology. With these caveats, the Panel felt that in general the modeling was well done, and that none of the problems identified would affect the main conclusion that resistance is very unlikely to develop. The parameters used in the model were likely to represent the biology of the situation, but it is important to keep in mind that this is a big picture model and does not contain within-field dynamics. Some Panel members suggested it would be more realistic to use physiologically-based models. Such models can provide additional information enabling the linkage of site-specific dynamics for the GPS referenced field data (e.g. Gutierrez and Ponsard 2006, Gutierrez et al. 2006). The Panel felt that the issue of more realism is important and other available approaches could be examined. In general, the predictions of the model on the declining resistance to the Cry1Ac toxin in *Bt* cotton during the four-year period of this eradication project agree with those of other models (Caprio 1994, Gutierrez and Ponsard 2006). The Panel felt that the model gave supporting evidence that resistance was unlikely to develop in the four years of Arizona Area Wide Project.

## ***Additional simulations - Risk of the Resistance Development***

The Panel is asked to comment on the expectation that additional simulation runs of the model provided by Dr. Bruce Tabashnik might reveal a greater risk of the resistance development in Bollgard Cotton. The Panel thought that working back and forth from models (including simulation, analytic and statistical) to biological data and back again is an important process, and that this effort to reexamine the model fits well with this process. The Panel thought that it was unlikely that the results of additional simulation runs would overturn the results noted above, i.e., that resistance was unlikely to develop over the course of the four-year program. However, the

additional simulations will be valuable in exploring resistance problems within these systems. In particular, it will be important to explore the implications to resistance development of additional conventional pesticide applications being triggered, not only by low over-flooding ratios of sterile to wild insects, but also by larvae being found in sampled bolls.

The Panel also questioned whether the parameter space of the model represented a “worst case” scenario, given that changes of only one factor at a time were evaluated in the sensitivity study of the model. The Panel pointed out that classical experimental factorial design methodology could be used to evaluate important combinations of parameters and statistics, and that using techniques such as linear models would be useful in analyzing the simulation model output, and would allow examining richer combinations of parameter values. It is also important to examine carefully the variation in the stochastic model’s output in order to determine the number of simulation runs necessary to capture that variation. This analysis allows one to understand which of the parameters contribute to the greatest sources of variation in model results.

Despite these problems, the Panel thought that the model provided an adequate evaluation of resistance and sufficient evidence to suggest that resistance is unlikely over the time period proposed for the program.

### ***Resistance to Cry2Ab2***

The Panel is asked to comment on the likelihood of Cry2Ab2 resistance given that the percentage of Bollgard II planted in Arizona has been <5% of the total *Bt* cotton acreage, and simulation modeling predicts that the likelihood of insect resistance to pyramided toxins in *Bt* cotton would be extremely low. The likelihood of resistance to Cry2Ab2 (Bollgard II) developing in *Bt* cotton appears to be unlikely given that this product is pyramided with Cry1Ac. Simulation models of the stacked products are unlikely to provide relevant information over and above the expectation from population genetics theory that suggests that if these two toxins are not cross-resistant, then the initial probability of resistance in stacked *Bt* cotton is the numerical product of their individual initial probabilities of resistance (see Zhou *et al.* 2006). Simulation results have demonstrated that even very low levels of dilution of the gene frequencies would cause rapid declines in the frequencies of these resistance alleles (e.g., Gutierrez *et al.* 2006). In greenhouse bioassays, Tabashnik *et al.* (2002), indicated that Cry1Ac resistant individuals die when fed Cry2Ab2 material. Reference was made to the June 2006 SAP meeting minutes where cross-resistance between Cry1Ac and Cry2Ab2 was discussed (USEPA 2006). This indicates that cross-resistance is not a problem and that resistance to the stacked product will be unlikely and that rates of resistance may actually decrease.

### **Agency Charge**

#### **3) Likelihood of Pink Bollworm Resistance in Future Years of the Eradication Program in Arizona**

**The Panel is asked to comment on the scientific validity of whether the preliminary field data, spatial analysis, and simulation modeling are adequate to provide reasonable**

**certainty that the likelihood of pink bollworm resistance to the Cry1Ac and Cry2Ab2 toxins will be extremely low during the four years of Arizona's eradication program.**

### **Panel Response**

#### **Development of resistance to Cry1Ac and Cry2Ab2**

The Panel is asked to comment on the scientific validity of whether the preliminary field data, spatial analysis, and simulation modeling are adequate to provide reasonable certainty that the likelihood of PBW resistance to the Cry1Ac and Cry2Ab2 toxins will be extremely low during the four years of Arizona's eradication program.

The amount of data provided is extraordinary and underscores how well the program was designed and managed, representing an exceptional model for area wide IPM. The program incorporates significant monitoring efforts, high rates of grower adoption and support, and triggers for combined suppression tactics. One of the most impressive aspects of the program is the program's inclusion of a remedial plan for responding to the development of resistance. Few other programs have been so proactive in their development and planning.

The Panel extended its compliments on the excellent protocols for resistance monitoring in field PBW populations to test for known *cadherin* alleles (*r1*, *r2* and *r3*) and novel ones that might exist using larvae reared from *Bt* bolls. In addition, the use of PCR analysis to detect the presence of resistance genes in adult males captured in pheromone traps and of larvae reared from bolls is lauded.

The failure to detect the resistance genes from the large number of field samples suggests that the frequency of the resistance allele(s) is below detectable levels. However, the selection of resistance in the laboratory from field collected inoculums is vexing, and suggests that monitoring of resistance in field populations should continue with greatest effort made using trapped PBW male adults to assure that if the resistance allele is detected, then in place intervention strategies may be employed.

The spatial modeling, as presented, does not have the ability to accurately assess the spatial extent of resistance within the population. The suggestions made by the Panel need to be adopted into the spatial modeling effort to provide the predictive ability required by the project.

The simulation model, historical lack of resistance developing in field populations and the lack of recorded genetic change also indicate a low likelihood of resistance developing over the program period. Further, the release of sterile PBW adults as a way to remove resistance genes from the native population is an effective method of preventing the development of resistance.

Finally, the program's incorporation of a comprehensive contingency plan (Remedial Action Plan) is laudable. Should any resistance be detected, the plan's responses are designed to prevent it from becoming established in the population.

Considering the data presented to the Panel, it is considered there is a very low likelihood of resistance to Cry1Ac or Cry2Ab2 developing over the 4-year program period.

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