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

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

Memorandum SEP 27 2018

SUBJECT: Transmittal of Meeting Minutes and Final Report for the Federal Insecticide, Fungicide and Rodenticide Act, Scientific Advisory Panel (FIFRA SAP) Meeting held July 17-19, 2018

TO: Richard Keigwin
Director
Office of Pesticide Programs

FROM: Tamue Gibson, M.S.
Designated Federal Official, FIFRA SAP Scientific Staff
Office of Science Coordination and Policy

THRU: Steven Knott, M.S.
Executive Secretary, FIFRA SAP
Office of Science Coordination and Policy

Stanley Barone, Jr., M.S., Ph.D.
Acting Director
Office of Science Coordination and Policy

Please find attached the meeting minutes for the FIFRA Scientific Advisory Panel open public meeting held in Arlington, Virginia on July 17-19, 2018. This report addresses a set of scientific issues being considered by the Environmental Protection Agency regarding resistance in lepidopteran pests to Bt PIPs in the United States.

Attachment

cc:
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OPP Docket

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Clifford P. Weisel, Ph.D.
Raymond S.H. Yang, Ph.D.

FQPA Science Review Board Members

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FIFRA Scientific Advisory Panel
Meeting Minutes and Final Report
No. 2018-06

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

Resistance of Lepidopteran Pests to *Bacillus thuringiensis* (Bt) Plant Incorporated Protectants (PIPs) in The United States

July 17-19, 2018
FIFRA Scientific Advisory Panel Meeting
Held at
Holiday Inn Rosslyn, At Key Bridge
Arlington, Virginia

NOTICE

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (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 U.S. Environmental Protection Agency (EPA or Agency) Administrator on pesticides and pesticide-related issues regarding the impact of regulatory actions on health and the environment. The SAP serves as a 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. FQPA Science Review Board members serve the FIFRA SAP on an *ad hoc* basis to assist in reviews conducted by the FIFRA SAP. The meeting minutes and final report are provided as part of the activities of the FIFRA SAP.

The FIFRA SAP carefully considered all information provided and presented by the Agency, as well as information presented by the public. The minutes represent the views and recommendations of the FIFRA SAP and do not necessarily represent the views and policies of the Agency, nor of other agencies in the Executive Branch of the Federal government. Mention of trade names or commercial products does not constitute an endorsement or recommendation for use.

The meeting minutes and final report do not create nor confer legal rights nor impose legally binding requirements on the EPA or any other party. The meeting minutes and final report of the July 17-20, 2018 FIFRA SAP meeting represent the SAP's consideration and review of scientific issues associated with "Resistance in Lepidopteran Pests to *Bacillus thuringiensis* (Bt) Plant Incorporated Protectants (PIPs) in The United States." Steven Knott, M.S., FIFRA SAP Executive Secretary, reviewed the minutes and final report. Robert E. Chapin, Ph.D., FIFRA SAP Chair, and Tamue Gibson, M.S., FIFRA SAP Designated Federal Official, certified the minutes and final report which is publicly available on the SAP website http://www.epa.gov/sap under the heading of "Meetings" and in the public e-docket, Docket No. EPA-HQ-OPP-2017-0617, accessible through the docket portal: http://www.regulations.gov. Further information about FIFRA SAP reports and activities can be obtained from its website at http://www.epa.gov/sap. Interested persons are invited to contact Tamue L. Gibson, M.S., SAP Designated Federal Official, via e-mail at gibson.tamue@epa.gov.

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Resistance of Lepidopteran Pests to *Bacillus thuringiensis* (Bt) Plant Incorporated Protectants in The United States

July 17-19, 2018
FIFRA Scientific Advisory Panel Meeting
Held at
Holiday Inn Rosslyn, At Key Bridge
Arlington, Virginia

Robert E. Chapin, Ph.D.
FIFRA SAP Chair
FIFRA Scientific Advisory Panel

Robert E Chapin

Date:

25 Sept 2018

Tamue L. Gibson, M.S.
Designated Federal Official
Office of Science Coordination and Policy

Tamue L. Gibson

Date: September 25, 2018

Federal Insecticide, Fungicide, and Rodenticide Act
Scientific Advisory Panel Meeting
July 17-19, 2018

Resistance in Lepidopteran Pests to *Bacillus thuringiensis* (Bt) Plant Incorporated Protectants (PIPs) in The United States

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LIST OF ACRONYMS AND ABBREVIATIONS

ACRONYMS	DESCRIPTION
BMP	Best Management Practice
Bt	*Bacillus thuringiensis*
CEW	Corn Earworm
DNA	Deoxyribonucleic Acid
EIL	Economic Injury Level
ET	Economic Threshold
FAW	Fall Armyworm
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
IPM	Integrated Pest Management
IRM	Insect Resistance Management
MAA	Mitigation Action Area
OPP	Office of Pesticide Programs
PIP	Plant Incorporated Protectant
RIB	Refuge-in-the-Bag
RNA	Ribonucleic acid
SAP	Scientific Advisory Panel
SWCB	Southwestern corn borer
US EPA or The Agency	Environmental Protection Agency
USDA	United States Department of Agriculture
UXI	Unexpected Injury
WBC	Western Bean Cutworm

INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel completed its review of the set of scientific issues being considered by the Environmental Protection Agency regarding Resistance in lepidopteran Pests to Bt PIPs in the United States. Advanced notice of the meeting was published in the Federal Register on March 5, 2018. The review was conducted in an open Panel meeting held in Arlington, Virginia, on July 17-19, 2018. The Agency position paper, charge questions, and related documents in support of the SAP meeting are posted in the public e-docket at http://www.regulations.gov (ID: EPA-HQ-OPP-2017-0617). Dr. Robert E. Chapin chaired the meeting. Tamue L. Gibson, M.S. served as the Designated Federal Official.

In preparing these meeting minutes and final report, the Panel carefully considered all information provided and presented by the Agency presenters, as well as information presented by public commenters. The meeting minutes and final report address the information provided and presented at the meeting, especially the Panel response to the Agency charge.

U.S. EPA presentations were provided during the FIFRA SAP meeting by the following (listed in order of presentation):

Welcome and Introduction – Anne Overstreet, Acting Deputy Director, Biopesticides and Pollution Prevention Division (BPPD), OPP, EPA

EPA's Identified Risk Factors for Resistance in Lepidopteran Pests of Bt and Proposed Options to Improve the Insect Resistant Management (IRM) Program– Jeannette Martinez, Ph.D., and Kara Welch, M.S., BPPD, OPP, EPA

PUBLIC COMMENTERS

Oral statements were presented by:

1) Don Glen, Director, Biotechnology and Crop Inputs, National Corn Growers Association, Hillsboro, Alabama

2) Graham Head, Ph.D., Global Scientific Affairs, Monsanto, Saint Louis, Missouri (Representing the Agricultural Biotechnology Stewardship Technical Committee)

3) Clinton D. Pilcher, Ph. D., Global Integrated Solutions Manager, Integrated Field Sciences, Corteva Agriscience™, Johnston, Iowa

4) Marlin E. Rice, Ph.D., Product Biology Technical Manager, Syngenta, Research Triangle Park, North Carolina

5) Graham Head, Ph. D., Global Scientific Affairs and Matthew Carroll, Ph.D., Regulatory, Corn IRM Technical Lead, Monsanto, Saint Louis, Missouri

6) Jim Steffel, Past President, National Alliance of Independent Crop Consultants (NAICC), Hamburg, Pennsylvania

Written statements were provided by:

1) Corteva Agriscience™, Agriculture Division of DowDupont

2) National Alliance of Independent Crop Consultants

3) National Corn Growers Association

4) National Cotton States Arthropod Pest Management Working Group

5) On behalf of the Agricultural Biotechnology Stewardship Technical Committee (ABSTC)
    a. Daniel L. Kendrick, Chair, ABSTC

6) On behalf of the Monsanto Company
    a. Matthew Carroll, Regulatory, Corn IRM Technical Lead

7) On behalf of the National Cotton Council
    a. Steve Hensley, Senior Scientist, Regulatory and Environmental Issues

8) On behalf of Syngenta Crop Protection, LLC:
    a. Marlin E. Rice, Ph.D.
    b. Isaac Oyediran, Ph.D.
    c. Paul Miles

9) On behalf of themselves:
    a. G. David Buntin, University of Georgia-Griffin Campus
    b. Patrick Porter, Texas A&M AgriLife Extension

EXECUTIVE SUMMARY

In 2014, the Agency initially became aware of lepidopteran resistance to Bt traits in corn and cotton in the continental U.S. from unconfirmed published reports by academic scientists. The reports of resistance were observed specifically for fall armyworm (*Spodoptera frugiperda*), corn earworm (*Helicoverpa zea*), and western bean cutworm (*Striacosta albicosta*). In 2016, industry registrants of Bt PIPs submitted annual monitoring data to the Agency which confirmed lepidopteran resistance for southwestern corn borer (*Diatraea grandiosella*).

Based on the information included with the monitoring data, the Agency concluded that there were major risk factors responsible for Bt resistance in these cases: 1) lack of available high dose traits; 2) use of single mode of action in Bt corn year-after-year; 3) use of corn seed blends in the southern U.S.; 4) poor refuge compliance for Bt corn in southern states; 5) continuous selection with the same traits expressed in Bt corn and Bt cotton in a given year; 6) shortcomings in current EPA recommended methodological approaches for monitoring for resistant field populations; and 7) challenges with identifying resistance with current diet bioassay methods.

The FIFRA SAP was charged with providing recommendations to the Agency in considering options to reduce resistance risks for lepidopteran pests, increase the longevity of currently functional Bt traits and future technologies, and improve the current insect resistance management program for lepidopteran pests of Bt corn and cotton.

The Panel addressed eight charge questions divided into resistance reports for lepidopteran pests of Bt PIPs (question 1); resistance monitoring for non-high dose pests (question 2); resistance risk of seed blend corn in the southern U.S. (question 3); Bt traits expressed in corn and cotton (question 4); resistance management for S. *albicosta* (question 5); mitigation of resistance (question 6); grower non-compliance with refuges in the southern U.S. (question 7); and new IRM framework for lepidopteran pests of Bt (question 8). The Panel provided the following overall summary of the major conclusions and recommendations detailed in the report.

Resistance Reports for Lepidopteran Pests of Bt PIPs Considerations – Major Conclusions and Recommendations

The Panel agreed that published reports of field resistance, taken collectively, were convincing for lepidopteran pests. Due to the extensive planting of Bt corn in the U.S.; the limited high-dose and/or pyramided traits available to target *H. zea*, the large range of suitable hosts for this pest, and the vast geographical range and migration patterns, it appears highly likely that *H. zea* will further evolve resistance to Bt crops on a broad scale in the continental U.S. For *S. frugiperda*, migrating individuals from Caribbean islands are the likely source of resistance in the U.S., rather than local selection. *Striacosta albicosta* may be described as at heightened risk of resistance, and resistance of *D. grandiosella* to Cry1F is reasonably well documented by industry.

There are strong environmental influences that could serve to slow the spread of resistance in these species, but are not yet well known. The Panel noted that it would be important to know the resistance mechanisms in these species and among populations for monitoring and mitigation efforts, and for future PIP development.

Resistance Monitoring for Non-High Dose Pests – Major Conclusions and Recommendations

The Panel acknowledged that pests such as *H. zea* are extremely challenging insects to manipulate in the laboratory (due to inherent challenges with inbreeding depression, fitness costs, high-dose requirements, apparent sudden shifts in susceptibility to PIPs, and others). Hence, bioassays of such pests carry inherent variability and are likely to continue to be recalcitrant in providing consistent patterns of susceptibility to PIPs in diet bioassays. The primary purpose of the bioassays should be to provide biological relevancy such that data can be used to assess unexpected injury (UXI) accurately in the field. For assessing genetic resistance, the Panel agreed that molecular detection of resistance alleles is a promising technology and has been used for many years as part of the Pink Bollworm, *Pectinophora gossypiella,* Eradication Program for the detection of resistance; however, methods for molecular detection of resistance in other lepidopteran pests have not been developed for large-scale use in the field, and therefore F2 screens should be used until such methods become available and are validated.

The Panel concluded that the current EPA definition of resistance is inadequate, because it does not set clear and appropriate thresholds of resistance needed to manage resistance evolution and phenotypic resistance once it evolves. A working definition should be clear so that appropriate actions can be taken in response to observational and monitoring data.

Recommendations

- For defining resistance, the Panel recommended that the Agency use three categories: (i) At Risk of Resistance, (ii) Heightened Risk of Resistance, and (iii) Observed Practical Resistance. The threshold between categories (i) and (ii) is crossed when resistance allele frequencies exceed 2% in any population, or at least one population shows a detectable increase in genotypic/phenotypic resistance. The threshold between categories (ii) and (iii) is crossed when UXI is observed and is shown to be caused by heritable resistance.
- For monitoring genetic resistance for category (ii) Heightened Risk of Resistance, the Panel recommended the Agency strengthen the existing program by increasing communication and transparency among current monitoring programs, and introducing currently available genetic (F2) screens to provide critical information for mitigating and managing resistance.
- For monitoring for category (iii) Observed Practical Resistance, the Panel recommended standardized diet bioassays to be used for key lepidopteran pests such as *Helicoverpa zea* and *Spodoptera frugiperda*.
- Bioassay conditions should be standardized across laboratories such that diet contents, Bt/PIP proteins, and other materials/methods allow for better direct comparisons and consistent assessment of susceptibility.
- The Panel recommended the Agency pursue the development of alternative technologies for enhanced genotypic resistance monitoring. Methods that incorporate molecular genetics and/or "omics" technologies could strengthen existing monitoring efforts.

Resistance Risk of Seed Blend Corn in the Southern U.S. – Major Conclusions and Recommendations

The Panel agreed with the Agency's conclusions that seed blends (refuge-in-the-bag, RIB) decrease the durability of PIPs to resistance evolution in ear-feeding lepidopteran pests. The potential for RIB to reduce durability was initially identified as a problem caused by plant-to-plant movement of larvae when using seed blends. This concern involves both stalk and ear-feeding pests. For ear-feeding pests, there is an additional problem of pollen exchange between Bt and non-Bt plants, and the mixing of Bt and non-Bt kernels within the same ear. Previous FIFRA Scientific Advisory Panels addressed the issue of seed blends primarily for corn pests, and concluded that a seed blend of 5% provided less durability than a structured refuge of 5%. The focus of this Panel was on lepidopterans (stalk borers) that feed on corn ears. Growing evidence suggests that the risk of decreased durability for ear-feeding lepidopterans in seed blend refuges is greater than for stalk and root-feeding pests.

The Panel also concluded that, as one of the long-term IRM tactics on corn seed development, the Agency's proposed approaches for mitigating Bt resistance in *H. zea* using the RIB products are scientifically and economically feasible and merit further detailed examination, although long-term investments are needed. The Panel shared possible alternative long- and short-term approaches for the seed industry to consider in reducing Bt resistance risk of seed blend corn in the southern U.S. states.

Recommendations

- The Panel recommended the prohibition of seed blends for Vip3A corn crops in the southern regions of the U.S. where ear-feeding lepidopteran pests overwinter and cotton is also grown.
- The Panel recommended the permissibility of seed blends where ear-feeding lepidopteran pests do not overwinter, but with a minimum of 10% non-Bt seed within the blends for functionally pyramid varieties. Seed blends should not be permitted for functionally single-toxin varieties.

For seed development (as a long-term approach), the Panel recommended:

- Utilizing corn germplasm with native insect resistance for non-Bt hybrid development, in addition as parental lines for Bt hybrid development.
- Expanding the transgene portfolio beyond Bt genes by including transgenes from other bacterial and fungal entomo-pathogens.

For seed production (as a short-term approach), the Panel recommended:

- Adopting the Plus Hybrids system to prevent cross pollination between Bt and non-Bt plants.
- Providing seed treatment with endophytic entomo-pathogens for lepidopteran pest.

Bt Traits Expressed in Corn and Cotton – Major Conclusions and Recommendations

Most Bt toxins expressed in corn are also expressed in cotton. The presence of the same Bt traits in corn and cotton exerts a continuous selection pressure on the multiple generations of *H.*

zea per year in the southern U.S. and presents a resistance concern. Resistance management for cross-resistance conditions across multiple pest generations per year has limited options to reduce selection pressure. The problem is further compounded with the limited availability of efficacious Bt toxins against *H. zea*. If such toxins are expressed in non-functional pyramids of corn and cotton, the durability of these traits could be reduced substantially.

Recommendations

The Panel Recommended the Following:

- The Agency to quickly assess and approve PIP traits for deployment against pests of corn and cotton.
- The Agency to prohibit the use of blended refuge against ear feeding pests of field corn in the southern zone.
- The Agency and the commercial seed industry to work to incentivize the planting of block refuges.
- Development of high yielding non-PIP field corn hybrids for use in refuges.
- The Agency to institute strengthened requirements for structured refuge in southern field corn.
- The prohibition of the use of field corn cultivars which produce Vip3A insecticidal proteins in the south.
- Modeling of the strategy of non-deployment of Vip3A in southern field corn for resistance durability and economic impact.
- Review of the Vip3A non-deployment strategy in the south as new PIPs become available.
- Continued use of the natural refuge strategy for cotton.
- Maintaining refuge requirements for field corn expressing Cry toxins at levels sufficient to forestall resistance development in stalk borers and secondary pests.
- The use of refuge strategies to protect Cry toxins in field corn should be modeled for best fit against pests in the crops in which they are deployed and to evaluate economic and IRM concerns.
- The Agency to allow, until better products are available, the use of "non-functional, pyramided PIPs" from corn and cotton due to their continued effectiveness against important primary pests.
- The Agency to support the use of effective, targeted insecticide treatments with modes of action that are independent from those of PIPs when pest populations exceed economic thresholds
- Development of a monitoring network to assay *H. zea* populations for resistance to PIPs in field corn, empowering state Extension, consultants and commodity groups to educate growers about changes in pests and management of them.
- Development of non-Bt field corn hybrids that are incompatible for pollination of surrounding Bt plants in blended refuge resistance management.
- The promotion of IPM strategies including post-harvest stalk and volunteer plant destruction to limit late season development of pests and PIP resistance.

Resistance Management for *S. albicosta* – Major Conclusions and Recommendations

Consensus of the Panel was that a separate Corn Belt-wide refuge approach for western bean cutworm (WBC), *Striacosta albicosta*, likely would be burdensome for producers and registrants and would interfere with resistance management for other, more widespread primary pests, particularly European corn borer and corn rootworm. The Panel recognized that WBC is a significant pest in localized areas, especially areas with sandy soils that are commonly found in Great Plains and Great Lakes areas. In these areas the Panel recommended locally-appropriate best management practices (BMP) should be developed that focus on plant incorporated protectants (PIP) traits and IPM. The Panel noted the importance of Vip3A because it is the only highly effective Bt protein for WBC. There were concerns by the Panel about resistance evolution to this trait, especially because of refuge-in-bag (RIB) refuge that could compromise assumptions of the high-dose/refuge IRM strategy. In this case, larvae could avoid high dose by moving among PIP and non-PIP plants and by feeding on a mosaic of PIP expressing tissues in corn ears. Due to these challenges several members of the Panel recommended EPA consider implementing a monitoring program for Vip3A resistance and an action/education plan for if/when Vip3A susceptibility shifts in problematic areas. The Panel also recognized that because of the recent range expansion, the pest status of WBC is dynamic. If WBC becomes a serious pest over a larger area, then the Panel recommends current guidance should be reevaluated. One member of the Panel noted that the problem of managing insect susceptibility is inherently dynamic in the sense that management actions now have implications for costs and benefits at later points. Thus, in addition to current benefits and costs, at least some attention should be paid to the probability that a problem will increase in scale.

Recommendations

- The Panel does not recommend a separate Corn Belt-wide refuge approach for western bean cutworm.
- The Panel recommends that in areas where western bean cutworm is an important pest, locally-appropriate best management practices should be developed that focus on PIP traits and IPM.
- Several Panel members recommended that for problematic areas EPA consider implementing a monitoring program for Vip3A resistance and an action/education plan for if/when Vip3A susceptibility shifts.
- The Panel also recommends that if western bean cutworm becomes a serious pest over a larger area, then current guidance should be reevaluated.

Mitigation of Resistance– Major Conclusions and Recommendations

Similar to conventional pesticides, Bt plant PIPs are at risk of resistance. Nevertheless, Bt PIPs are likely at greater risk than conventional pesticides targeting the same insects because they are expressed at relatively high and also variable levels throughout the life cycle of the plant compared to conventional pesticides, which typically have shorter periods of efficacy and are applied when pests are likely to cause yield loss. Constant exposure to Bt PIPs, often over multiple generations per season, exerts a significant selection pressure and facilitates pest resistance development.

The Panel recognized that adoption of IPM tactics to reduce the pressure of pest population in Bt-crops could contribute to mitigation planning by reducing the pressure of resistance selection. The Panel acknowledged that there are many factors that must be considered when determining Mitigation Action Areas (MAAs), particularly for the highly mobile noctuid moths. The Panel recognized the impact of cross-resistance to Bt traits should be considered when determining the MAA and limiting the spread of resistance genes in a specific region.

Recommendations

- The Panel recommended that it is more applicable or appropriate to proactively manage resistance of lepidopteran, such as *H. zea* and *S. frugiperda,* rather than mitigate specific outbreaks.
- The Panel recommended the creation of a proactive mitigation plan in a region-specific scale that needs to be designed within an IPM framework.
- The Panel recommended a program that employs clear and coordinated communication among all involved parties when implementing management tactics (proactive or reactive) in order to build trust, decrease complexity and uncertainty, and increase the likelihood that adopted IRM tactics work within an IPM framework.

Grower Non-Compliance with Refuges in the Southern U.S.– Major Conclusions and Recommendations

The EPA concluded that refuge compliance in the southern U.S. had further decreased over the past several years. The Panel concurred with the Agency that compliance in this region decreased and believes that owners of smaller farms in the southern U.S. were less likely to plant a refuge due to different labor and management constraints on smaller farms than larger farms, as well as the fact that larger growers might derive more direct (personal) benefit from planting a refuge so are more likely to do so. Additionally, operators of smaller farms might be less likely to believe their non-compliance contributes negatively to the common pool of susceptible insects due to their small size; and, it might be more difficult to justify economically the planting of a refuge on their farms. The Panel believes growers likely favor planting RIB products because it expedites the planting process and reduce labor inputs. Likewise, RIB products could be favored by industry because they guarantee that refuges for Bt corn are planted and growers remain compliant with refuge requirements. The Panel noted that RIBs are viewed as the solution to grower refuge non-compliance in southern states; though several lines of evidence support the conclusion that RIBs are less successful than IRM strategies for ear-feeding pests of Bt corn (e.g., *H. zea, S. frugiperda,* etc.) than block refuges, especially in areas where pests have multiple generations per year and overwinter. The Panel believed compliance with IRM requirements is in part a function of perceived probability of receiving a sanction, and the severity of any sanction from non-compliance (Hurley and Mitchell, 2014). Problematic in this regard is the reliance on a consortium of seed firms (ABSTC) to monitor regulatory compliance. The regulatory regime is indirect and the penalty for non-compliance is denial of seed sales which reduces revenue for the seed firms. The Panel found that data provided by the Agency through the ABSTC on denial of seed sales are unclear and difficult to interpret.

Recommendations

The Panel Recommended the Following:

- Tailoring marketing programs for smaller and larger farms. Smaller farms in total control a substantial amount of acreage and their compliance is necessary to realize beneficial outcomes. Additionally, as acreage of a farm increases, the benefits of delayed resistance become more direct which in turn could inform future IRM marketing initiatives.
- Listening sessions or focus groups led by Extension specialists to dialogue with growers about the need for establishing and maintaining refuges, and the constraints growers face to do so.
- The assessment of fees on Bt seeds, and discounts on refuge seed or subsidies for planting refuge. This approach would require quantitative evaluation of product quality and variety of Bt versus non-Bt traits in privately collected seed sales data on corn hybrids across the U.S. These data could be used in econometric modeling to compute how large the relative price discount or subsidy for refuge seed would have to be in order to achieve different refuge targets.
- Investigative measures within the U.S. crop insurance program, or other agricultural subsidies, to compel compliance. Recent efforts to incorporate cover crop incentives into crop insurance might be a model. In the case of refuges, that would require EPA coordinating with USDA, specifically the Risk Management Agency.
- Implementing a ‘refuge in time’ policy, e.g. withhold VIP3a from corn in south.
- Encouraging growers to adopt technology that reduces variable costs of planting refuge. This could include adoption of multi-hybrid corn planters, which would reduce growers’ variable costs of planting multiple corn varieties with different soil and sowing density optima, as well as greater use of “precision agriculture” services, for example in calculating efficient refuge configurations.
- The Agency require more detail on reporting of regulatory outcomes, especially denial of seed sales to non-compliant growers.

New IRM Framework for Lepidopteran Pests of Bt– Major Conclusions and Recommendations

The Panel was in consensus regarding the use of broad-based, multi-tactic, biologically sound IPM principles as the basis for stewardship and the foundation on which IRM strategies are built for both non-high dose and high dose pest of Bt corn and cotton. General consensus was reached for strategies that mitigate resistance development versus strategies that manage resistance after development. This was deemed especially important in non-high dose and highly mobile pests such as *H. zea*. A consensus of the panel supported the concept that the most effective strategies for slowing/suppressing the development of resistance among non-high-dose pests are PIP pyramids and refuge strategies.

Recommendations

- The Panel recommended strengthening the proactive insect resistance management approach in which IPM measures are applied “before, during and after” insect resistance occurs.

- The Panel recommended the use of interdisciplinary teams of natural and social scientists expert in agro-ecology, human behavior and social institutions related to agricultural production and common pool resources to inform a strategic IRM framework that identifies key leverage points to control lepidopteran pests in Bt crops.
- The Panel recommended convening key stakeholders to IRM for Bt crops to assemble real-time experiential and intellectual knowledge on the spatial and dynamic patterns of resistance and cost-effective tactics to foster sustainable IRM tactics in each crop production region.
- The Panel recommended strengthening comprehensive scouting and monitoring programs with shared public and private resources to detect Bt PIP resistance and communicate real time for proactive management. Assure that an independent third party is responsible for confirmation of insect resistance to remove potential conflicts of interests.
- The Panel recommended adopting an area-wide approach to implement the new IRM framework with monitoring and management actions tailored to the specific biophysical and socio-ecological conditions in each distinct area.
- The Panel recommended to secure appointments of trusted leaders (champions) in each area, e.g., Extension specialists, to facilitate grower cooperation to implement community-driven IRM programs. Access private, e.g., industry, and public, e.g., USDA, organizations to build an integrated network of responsibilities, incentives and compliance requirements and penalties.

DETAILED PANEL DELIBERATIONS AND RESPONSE TO CHARGE

Charge Question 1. – *Resistance Reports for Lepidopteran Pests of Bt PIPs*

Field resistance to three out of four lepidopteran species targeted by Bt PIPs has been reported by academic scientists in the continental U.S.: *H. zea* collected from sweet corn fields in eastern U.S., *S. frugiperda* collected in southeastern U.S., and *S. albicosta* populations from northern lake states in the U.S. and Canada.

> **a.** Please discuss these reports and address the degree of confidence that the reports represent cases of confirmed resistance to Bt PIPs.
>
> **b.** Please comment on the adequacy or limitations of the scientific assumptions driving conclusions that under current conditions resistance will broadly evolve in *H. zea*, *S. frugiperda*, and *S. albicosta* in the continental U.S., given the cited reports.

Panel Response 1:

a. The Panel found that the published reports by academic scientists, taken collectively, of field resistance were convincing for corn earworm (or cotton bollworm), *Helicoverpa zea* (*H. zea*) collected from the eastern and southern U.S. (Bt PIP toxins: Cry1Ab and Cry1A.105, Cry2Ab2), based on papers from Dively et al. 2016; Yang et al. 2017; and Reisig et al. 2018. However, there is no doubt that frequencies of alleles conferring tolerance to Cry proteins in *H. zea* are increasing.

Corn Earworm/Cotton Bollworm (*Helicoverpa zea*)

The publication by Yang et al. (2017), for example, compared the survivorship of F1 and F2 generations of field-derived *H. zea* cotton bollworms collected from the Mid-South region of the U.S. to four individual Bt proteins (Cry1Ac, Cry1F, Cry2Ab2, and Vip3a) with that of a susceptible counterpart using diet overlay to estimate susceptibility of field populations of cotton bollworm to Bt proteins. The Panel noted that not all populations were equally affected. In comparison to susceptible laboratory colonies, populations from the states of Louisiana and Mississippi exhibited lower susceptibility to the Cry1Ac protein, with resistance ratios of 35-50-fold. One Panel member with expertise in cotton agro-ecosystems indicated that agricultural consultants scouting cotton fields previously reported broad declining efficacy as well.

Fall Armyworm (*Spodoptera. frugiperda*)

The Panel highlighted the reported evidence of field-evolved resistance for fall armyworm (FAW) collected in the southeastern U.S. from the publication by Huang et al. (2014). The Panel noted the actual quantification of resistant individuals in the field and practical consequences of resistance were not specifically provided (with Huang et al., [2014] observing the decline of field efficacy suggesting resistance is highly pervasive, but not actually quantified), though it was observed that an F2 screen using leaf tissue from Herculex I (Cry1F) in Florida and Louisiana demonstrated that 47% of families harbored *r* alleles (a 10% allele frequency). Multiple diet bioassays and greenhouse tests confirmed resistance to Cry1F. Two families were found to have >270-fold RR to purified Cry1F and at least eight more with >50-fold RR. Likewise, FAW resistance to Cry1F was well documented in Puerto Rico (Storer et al. 2010, 2012), and Brazil (Farias et al. 2014).

Western Bean Cutworm (*Striacosta albicosta)*

Based on published data by Smith et al. (2017) and on observations of which the Panel was aware, populations in Ontario, Canada and the Great Lakes and Great Plains States in the U.S. may fall under the "heightened risk of resistance" category (Charge Question 2D). These species were marginally susceptible to Cry1F, and plausibly needed only a "minor "resistance mechanism, which may be relatively more common than high magnitude mechanisms.

Southwestern Corn Borer (*Diatraea grandiosella*)

The Panel believed resistance to Cry1F in this species appeared reasonably well documented by industry (Crespo, et al. 2017).

The Panel noted in the public comments posted at https://www.regulations.gov (Docket ID Number EPA-HQ-OPP-2017-0617) that there was broad acceptance from industry and academics based on the following conclusions.

Corteva Agriscience™ noted "*The EPA's whitepaper provides a thorough overview of the current status of pest susceptibility to Bt crops, in particular summarizing information indicating or confirming changes in susceptibility in populations of four pest species (S. albicosta, S. frugiperda, D. grandiosella and H. zea). Corteva Agriscience™ agrees that, from a practical standpoint, reductions in product performance against these pests have been documented, and in some cases a link to pest resistance as defined by EPA in registrations has been confirmed*."

The National Cotton States Arthropod Pest Management Working Group (NCSA PMWG, https://southernpests.org/), a collaboration of 35-45 entomologists from land-grant universities specializing in management of arthropod pests of cotton, corn, soybean, grain sorghum, and wheat in all cotton producing states from California to Virginia "*agrees with the reports for H. zea resistance to Cry proteins in sweet corn and has similar data from field corn and cotton in other regions of the U.S. H. zea resistance to Cry1Ac and Cry2Ab is not complete nor geographically universal. Supplemental control is often needed more frequently than in the past in many areas. Likewise, significant H. zea infestations have been observed in single-gene and dual-gene Bt corn since their respective commercialization. Finally, severe control problems with H. zea in pyramided cottons have been experienced recently across the U.S. Cotton Belt; with problems intensifying across the southern U.S. during 2017 requiring multiple foliar insecticide applications.*

We also agree with the findings of resistance for S. frugiperda. The group understands the complexity of the situation for a pest that does not always overwinter in the U.S. and relies on migration from southern latitudes. It is likely that resistance to Cry1F in this pest developed outside of the U.S. and potential resistance to other Bt PIPs will be difficult to manage."

G. David Buntin, Grain Crop Entomologist, University of Georgia stated "*The two pests relevant to this section in Georgia are fall armyworm and corn earworm (CEW). Fall armyworm: Field failures of Cry1F corn were reported in 2013. Several very late planted fields with unexpected damage were reported in southern Georgia. Corn earworm: 2 and 3 gene corn containing the Cry2Ab toxin (Gen. VT Triple and Double PRO, SmartStax) provided about 80-90% reduction in kernel damage by CEW from 2009 through 2014. Reduced efficacy has occurred since 2015 but these products still provide up to 40-50% reduction of kernel damage. Despite the reduced efficacy of these Bt toxins in corn, CEW/Bollworm damage to 2 gene cotton has not been widespread in Georgia*."

Marlin E. Rice, Isaac Oyediran, and Paul Miles, Syngenta Crop Protection, noted that with respect to S. albicosta there were, "*No apparent resistance problems in several U.S. states; only Ontario*."

b. Panel members agreed that based on the evidence presented in the current published reports, resistance is supported by field observations that characterize reductions in Bt PIP efficacy for CEW and FAW were not geographically isolated and is likely that populations of both species will continue to evolve.

Further, the Panel agreed that several factors such as extensive planting of Bt corn in the U.S., the limited high-dose and/or pyramided traits available to target CEW, the large range of suitable hosts for this pest, and the vast geographical range and migration patterns, appears highly likely that CEW will further evolve resistance to Bt crops on a broad scale in the continental U.S.

The Panel believes specifically for FAW, selection is not likely the source of resistance in the U.S., but rather migrating individuals (species) from Caribbean islands (where Cry1F resistance is apparently persisting due to "complete resistance" and lack of fitness costs) to the U.S. mainland. Some level of cross-resistance exists between Cry1F and Cry1A.105, but data less clear between Cry1F and Cry2Ab2. The Panel also notes that there is no apparent cross-resistance between Cry1F and Vip3A. Prediction that FAW will broadly evolve resistance to Bt crops in the continental U.S. is difficult to determine because of limited overwintering in the continental U.S. and uncertain extent of dual/cross-resistance to Cry1A.105, Cry2Ab2, and/or Vip3A (as well as associated fitness costs) in field populations.

The evolution of resistance of western bean cutworm, has some uncertainties, not that it has occurred, but where did it occur and how often. Based on conversations with a scientist from the USDA, it appears Cry1F resistance is widespread in Nebraska, Michigan, Ontario and New York, everywhere FAW has been surveyed so far. Non-high dose events as predicted by most modeling, will likely continue to be problematic, due to resistance development, as long as such events are commercialized. This begs the question whether Vip3A is high dose for corn earworm, fall armyworm and western bean cutworm.

In summary, the Panel agrees there are strong environmental influences that could serve to slow the spread of resistance in these species, but these influences are not yet well known. The Panel suggested it is be important to know the resistance mechanisms in these species and among populations for mitigation efforts and for future Bt PIP development. The Panel discussed that it is unclear whether the general positive effects of pyramiding still exist among these populations that have become resistant to one or more of the toxins. If Vip3A is not high dose for a pest, and is used against multiple generations, the Panel would expect resistance to develop relatively quickly.

Charge Question 2. – *Resistance Monitoring for Non-High Dose Pests*

The success of a resistance monitoring program relies on effective insect collection methodologies, accurate and quick resistance determination assays, uniform damage thresholds for unexpected injury fields, and a practical definition of resistance. The current sampling approach, by which populations are collected from different locations year-to-year, does not track the susceptibility of individual populations over time. Diet bioassays presently in use require rearing of insects, which may bias against or eliminate resistant genotypes. These assays delay reporting of resistance occurrences and are too variable for non-high dose pests, such as *H. zea*.

A. Insect Sampling

> **a.** Consider effective insect sampling options for resistance monitoring of non-high dose pests such as *H. zea* that would result in timely resistance detection. Please compare and contrast EPA's current approach to a focused sampling of sentinel populations and a targeted sampling of populations obtained from UXI fields.

Panel Response 2A:

a. The Panel acknowledged the Agency is considering two insect sampling options for the timely detection of resistance in non-high dose pests. One option is the adoption of sentinel plots in corn and cotton growing areas for resistance monitoring purposes instead of continuing with random sampling of *H. zea* populations. Sentinel plots should be set up in regions with high risk factors of resistance, taking into consideration greater Bt adoption, fields with use of same trait year-after-year, areas with low refuge compliance for Bt corn, and high *H. zea* pest pressure. The primary objective of this proposed change is to actively target areas of resistance concern based on high risk factors, gain insight into the resistance development in the same population year-after-year, and thereby identify resistance problems more proactively.

The Panel believed that under certain circumstances sentinel plots have value in collecting insects for resistance identification (Venette et al., 2000; Dively et al., 2016). The Panel noted that some industry registrants supported the development of sentinel plots (ABSTC Written Comments, Table 4; Corteva Agriscience™ Written Comments, page. 2). The Panel noted ABSTC's conclusions as: "costs of gathering data must be supported by the benefits," "…the spatial distribution of sentinel plots must match the pest's population geography," and "…sentinel plots for a non-high dose trait will be subject to, and confounded by, a lot of environmental variation and may not capture relevant areas (those with highest resistance allele frequencies)." The Panel believed there were many challenges with the creation of sentinel plots for it to be considered as the sole or most appropriate sampling option and that one major non-high dose insect, *H. zea*, is a facultative migratory pest that moves from overwintering areas each season. Some of these populations in the southern U.S. may be local; however, during the crop production season, populations can migrate into the northern-most states of grain production. The Panel suggested using sentinel plots at the same location year-to-year would only provide a snap shot of that population that year, which may not emerge from the same overwintering source. The Panel had several reservations as to whether sentinel plots can detect Bt-resistant populations in a timely manner to implement mitigation or management practices. The design of the plots (how large), location (where and how many locations per year), and management of these plots (who does the planting, how is damaged assessed) had not been fully investigated. The overall costs of this technique appeared to the Panel to be prohibitive.

A second option was to focus on fields with UXI. UXI fields are, in principle, timely because they are directly linked to control failures in real time. One concern was that there are currently no regulatory thresholds for damage or infestation to objectively identify UXI fields, which would be most likely found by growers or consultants. However, the Panel is suggesting UXI thresholds for *H. zea* larval density in cotton (see charge question 2Cb). There can be a delay in relaying the information of a UXI field to university or registrant staff. The Panel believes there are measures than can be taken to solve this issue through better communication practices such as social media and electronic reporting portals accessible to the public. The advantage of targeted sampling of populations in UXI fields is that it can be an indication of a potential problem. The Panel believed this sampling approach required expansion. Because corn fields are not normally scouted, separating damage by tissue (stalk versus ear) and pest detection may not be achieved.

Two Panel members believed that standardized pheromone trapping (same traps, lures, timing) could be used to indicate population trends for a regional area and provide information on when to sample UXI corn and cotton fields (Leonard et al., 1989; Hoffmann et al., 1991; Adamczyk and Hubbard, 2006; Jackson et al., 2008). Though there is evidence that pheromone trapping to assess larval populations in specific fields can be successful (Chowdhury et al., 1987; Coop et al., 1992; Latheef et al., 1991, 1993), the Panel argued that because pheromone traps provided a density dependent measurement, they were inherently unable to correlate larval damage to moth traps (Schneider et al., 1989).

One Panel member posed a comment regarding the lack of expedient confirmation of Bt resistance from sampling UXI fields (sentinel plots) in adequate time to mitigate against the populations that were currently present. Further, a comment submitted to the public docket by the Arthropod Working Group posted at https://www.regulations.gov (document ID number EPA-HQ-OPP-2017-0617-0064) stated, “they were not aware of compelling examples where resistance monitoring resulted in actionable efforts to effectively mitigate resistance.” The Panel agreed that new techniques needed to be developed using molecular or genetic methods that can quickly determine the presence and levels of Bt resistance in a population so that remedial actions can be taken.

One Panelist alerted the other Panel members of recent reports indicating the consistent and continuous decline of insect populations (Baxter-Gilbert et al., 2015; Hallmann et al., 2017; Young et al., 2017). The precise reasons were unknown; however, if this decline continued, it may impact the previously discussed insect sampling options including mitigation options for the survival of existing pests. The Panel agreed that the preservation of the Bt PIP program should be a long-term and important goal, and the EPA should take into consideration the issue of insect population decline in its overall insect resistance management plans.

B. Diet Bioassays

When target pests are exposed to a high dose of the Bt toxins, resistance becomes functionally recessive. Therefore, heterozygous resistant genotypes experience mortality like susceptible genotypes during diet bioassay testing with a diagnostic concentration. Conversely, when the Bt toxin is not high dose, resistance effectively becomes more dominant, and heterozygous resistant genotypes should experience less mortality than susceptible genotypes in a diagnostic concentration assay. F2-screens with sib-mating have shown to be more effective at determining resistance than F1-screens. However, fitness costs associated with resistance could eliminate resistant genotypes during rearing processes and increase the likelihood of false negatives with these types of diet bioassays.

Recently, a Single Nucleotide Polymorphism genotyping assay was utilized to screen for Cry1F resistance in different populations of *S. frugiperda*. This assay was used on extracted DNA of field-collected insects. These types of in-field DNA testing assays could expedite the resistance-confirming process and have the benefit of assessing insects isolated in the field, obviating the lower sensitivity of diet bioassays and associated challenges with testing offspring generations.

> **a**. Describe any measures that could improve the current diet bioassay methods to increase the detection of resistant genotypes and account for fitness costs to resistance. What are the advantages and limitations associated with each measure?
>
> **b.** Discuss the advantages and limitations of applying a DNA assay system to both high dose and non-high dose pests. Would such an assay system produce resistance determinations equally well for high dose and non-high dose pests? Can the SAP suggest other types of in-field assay systems that would produce the desired result of quick, reliable assays that obviate the potential for loss of resistant genotypes through rearing?

Panel Response 2B:

a. The Panel agreed that diet bioassays have been used for many years by academia, government, and private industry scientists to observe effects of PIP proteins on insect pest populations and for resistance monitoring. Such diet bioassays have been highly optimized, probably with limited options to provide minor improvements in performance for the detection of resistant genotypes and account for fitness costs to resistance. The Panel, in consensus, primarily recommended the standardization of diet bioassays for key lepidopteran pests such as *H. zea* and *S. frugiperda*. The Panel also recommended that diet bioassays be standardized across laboratories such that diet contents, Bt-PIP proteins, and other materials/methods (e.g. diet incorporation assays) allow for better direct comparisons and consistent assessment of susceptibility. Diets sometimes differ greatly from lab-to-lab and in some cases are confidential. Comparing results under such conditions is often difficult; it is equivalent to comparing "apples to oranges." One Panelist suggested the use of toxin overlay diets, as a low cost alternative to toxin incorporated diets. Another Panelist indicated that there may be inconsistencies associated with diffusion of the PIP in diet overlays. Another Panel member noted that diet preparations may be more suitable than others and may vary among insect species. Three recent publications from Deans et al. (2015, 2016, and 2017) suggested the carbohydrate to protein ratios in some diets are not optimal and can lead to spurious results when testing for resistance to Bt toxins. Furthermore, some insects (such as western bean cutworm) are more difficult to assay than other species better adapted for laboratory bioassays. With regard to current insect resistance monitoring efforts, one Panelist questioned the efficiency of random sampling followed by testing progeny of collected insects for resistance with diet bioassays. Other monitoring methods considered by the Panel, e.g., UXI and genetic methods, may be better investments of resources. The remaining Panelists posed a question if it would be advantageous to focus on UXI as a way to detect resistance, because it would be cost effective and less resource intensive to use this methodology. The Panel was concerned with the availability and access to PIPs currently produced in transgenic crops. Historically, it has been difficult to obtain from industry or to produce sufficient amounts of PIPs necessary for diet bioassays.

Additional recommendations provided by Panel members include:

1) Establish common, publicly accessible susceptible insect strains for the purpose of standardizing diet bioassays across locations and field-collected strains (see above).

2) Where possible, collect insects from Bt fields (and not just non-Bt fields) that show UXI to enhance odds of detecting presence of rare resistant populations. Note that the collection of insects from non-Bt fields has been argued by some industry scientists to be effective. However, one Panelist supported increased collections directly from Bt fields to increase the chances of detecting true resistant insects. Note, that a Panelist provided modeling data in Figures 2D.1, 2D.2, 2D.3-General Model Description to Simulation to Resistance Monitoring, which indicated that collections in either Bt or non-Bt fields may be equivalent.

3) Attempt to collect "larger" populations to improve size of lab-reared colony to increase chances of detecting rare resistance alleles and minimize potential fitness costs.

4a) Consider periodically out crossing field-collected "resistant" strains with field insects to maintain genetic variability and improve fitness.

4b) Consider introgression of field-collected strains with susceptible lab-adapted strain (e.g. use *H. zea* Benzon strain which has adapted well to lab rearing conditions).

One Panelist indicated that the introgression and/or out crossing of strains (either lab strains with field-collected strains or vice versa) could complicate bioassay results by possibly shifting baseline susceptibility measures.

5) Integrate new detection technologies (e.g. molecular methods such as genomics/transcriptomics or others such as metabolomics) as they become available and validated.

Furthermore, the panel acknowledged that pests such as *H. zea* are extremely challenging insects to manipulate in the laboratory (due to inherent challenges with inbreeding depression, fitness costs, high-dose requirements, apparent sudden shifts in susceptibility to PIPs, and others). Hence, bioassays of such pests carry inherent variability and are likely to continue to be recalcitrant in providing consistent patterns of susceptibility to PIPs in diet bioassays. One Panelist proposed that a possible solution to avoiding the uncertainties associated with diet bioassays would be to shift monitoring efforts to "on-plant" bioassays, although some similar uncertainties may also exist using plants and standardization would be required. Another Panelist indicated that the primary purpose of the bioassays should be to provide biological relevancy such that data most accurately reflect what is happening in the field.

b. The Panel agreed that there were clear advantages to molecular detection methods, including increased sensitivity of the assays, the ability to pool samples (coverage of population), lower labor and associated costs, detection of rare heterozygotes when resistance is recessive, detection of resistance across multiple developmental stages, and proactive use before loss of efficacy is detected in the field. However, any monitoring system such as molecular monitoring carries inherent limitations. A key disadvantage to currently employed polymerase chain reaction (PCR) detection methods is that knowledge of resistance genes or molecular markers is needed as a pre-requisite for developing such molecular detection technology. Unfortunately, this characterization often occurs only after resistance has evolved in the field. It is also possible that resistance can evolve independently of the known resistance alleles or markers (specifically in heterozygous field populations). Furthermore, experimental evidence suggests that resistance in low-dose pests such as *H. zea* may involve multiple mechanisms of resistance, thereby complicating the methods required to accurately detect multigenic resistance. Other complicating factors associated with resistance involve changes within genetic information that is not necessarily reflected within genomic deoxyribonucleic acid (gDNA). For example, molecular analysis in Cry1Ac-resistant labs and field strains of pink bollworm from the U.S. and India show that resistant individuals carry multiple, different mutations in the same gene (e.g. cadherin) and a more troubling problem is that some resistant field populations use ribonucleic acid (RNA) splicing and do not harbor mutations in their gDNA (Fabrick et al. 2014). Hence, molecular resistance monitoring may require assessing differences not only in gDNA, but also RNA (that is, in complementary deoxyribonucleic acid (DNA) or complementary DNA (cDNA).

The Panel discussed at length additional limitations regarding developing molecular markers for monitoring resistance that may involve genetic diversity of migratory pests such as *S. frugiperda* and *H. zea*. Population genetic studies based on highly variable microsatellite markers indicate *H. zea* originating from different geographical regions have moderate to high genetic diversity, but low genetic divergence among populations (Perera and Blanco, 2011; Seymour et al., 2016). Single nucleotide polymorphism (SNP) markers for *H. zea* also indicate low to moderate genetic diversity (Perera, unpublished). To date, one resistance mechanism each to Cry1A and Cry2A toxins, mediated by deletions in *ABCC2* and *ABCA2* genes, respectively, in *H. zea* have been identified (Perera, unpublished), but data suggest that there are other loci conferring resistance to both classes of Bt toxins in *H. zea.* A large number of genes implicated in Bt resistance in other species have been identified and annotated in the genome sequences of *H. zea* for developing genetic markers for detecting mutant alleles. Therefore, markers for interrogating both gDNA and RNA using high-throughput sequencing technology may be available in the near future.

For *S. frugiperda*, a DNA-based quantitative PCR (qPCR) genotyping method was developed and used by Banerjee et al. (2017) to track a potential molecular marker (*ABCC2mut* allele) for Cry1F resistance in Puerto Rico. The method demonstrated that the *ABCC2mut* allele was present in samples from Puerto Rico but had not yet detected the allele in 246 *S. frugiperda* samples from the U.S. mainland (e.g. Florida) or the Dominican Republic. The Panel felt unanswered questions remained. For example, if Cry1F-resistant *S. frugiperda* from the continental U.S. and other locations (e.g. Brazil) harbored this specific mutation and/or whether this qPCR-based method is sufficient for *S. frugiperda* resistance monitoring in the U.S. Unfortunately, the Panel noted that like *H. zea*, a reliable molecular genetic approach that can accurately differentiate resistance and susceptible *S. frugiperda* individuals (and/or heterozygotes harboring single *r* allele) for resistance monitoring from field populations in the continental U.S. has not yet been fully vetted. The Panel recommended that alternative methodology could be developed for resistance monitoring purposes. The use of high-throughput next generation sequencing to detect either resistance alleles or other markers linked with resistance to PIPs is currently being explored by several laboratories, but only one related peer-reviewed publication for *Heliothis virescens* is available at this time (Fritz et al., 2016). Such genomics/transcriptomics strategies may be less dependent on a single, specific mutation/allele/gene. The Panel recommended the use/validation of alternative high-throughput sequencing strategies in known high-dose pests may help to serve as model system for the development of tools in non-high dose pests (e.g. *H. zea* and *S. frugiperda*). In addition to DNA/RNA-based molecular approaches, novel techniques (such as proteomics and metabolomics) could also provide means to differentiate biological signatures for resistance. One Panel member recommended that in some instances, it may be possible to integrate cross-resistance to chemical insecticides (abamectin) to aid in detection of Bt resistance (see Xiao et al., 2016). However, a Panelist felt that this approach may be a limited scenario and would require that the same gene is mutated in a way that affects both modes of action and hence likely to be very limited in application/rare in different insect systems.

C. Unexpected Injury Threshold in Bt Corn and Bt Cotton

As described in the issue paper, EPA identified the need to adopt uniform standards to identify field damage in Bt corn and Bt cotton from potentially resistant insects. Exceeding these thresholds would trigger follow-up investigations (collections of insects and bioassays) and mitigation of the putatively resistant pest population. Without established regulatory thresholds for unexpected injury in these crops, it is unlikely that timely collections of insects and bioassays occur, and resistance could spread unchecked in the interim before mitigation actions are initiated. EPA expects that (1) UXI thresholds may need to be toxin, pest, and crop specific; (2) different thresholds will be needed for damage caused in Bt cotton and Bt corn by the same pest since different tissues are affected; and (3) a higher threshold may be needed for a non-high dose than for a high dose trait, simply because some damage could be expected in the former.

> **a.** Discuss any criteria beyond those described in the Agency issue paper and briefly reiterated here which the SAP believes would inform the use of thresholds for field resistance in Bt corn and Bt cotton for non-high dose lepidopteran pests. What are the most relevant factors to consider for establishing thresholds for such pests like *H. zea* in Bt corn and cotton, while reducing the likelihood of 'false positives', for example, caused by high pest density?
>
> **b.** Provide recommendations for scientifically sound threshold values for UXI caused by *H. zea* in Bt cotton where no reference comparison can be made to non-Bt cotton refuges. Discuss the benefits and limitations of using bolls and other tissues to measure UXI.

Panel Response 2C:

a. The Panel acknowledged the goal of a UXI is to detect practical resistance using a protocol for reports of suspected Bt field control failures. The Panel recommended the development of regulatory thresholds to investigate reports of field-evolved resistance to PIPs and the opportunity to implement defined UXI in IPM/IRM strategies. Presently, industry registrants use their own independent injury thresholds to trigger an investigation of stakeholder reported field control failures in Bt crops and in the interpretation of results (Berman et al., 2017; Payne et al., 2017; US EPA, 2018). The Panel recommended that UXI's should be developed, regardless of dose classification (low or high), for target pests and included as information during the product registration process. This concept is supported by the work of Andow et al. (2016) using western corn rootworm as a model. The study suggested the use of pro-active IPM strategies in an IRM plan prior to approval of release of new/novel PIPs that are not classified as high-dose.

The Panel agrees with the Agency's current (2018) issue paper that UXI thresholds should include toxins, pests, and/or crop specifications (US EPA, 2018). The Panel recommended that during the PIP registration process the following should be proposed as a regulatory standard and in-turn coordinated with the stakeholder community: the UXI target level, the protocol for establishing this trigger, and the protocol for sampling the specific pest. Development of UXI's thresholds should be considered for all target pests which should be included in the product registration materials. Currently there is considerable overlap in the PIPs used in field corn and cotton; therefore, UXI's should be crop specific for pests that utilize both crops (e.g. *H. zea* and *S. frugiperda*). For non-high dose target pests in Bt crops, some level of crop damage may be expected when the trait(s) are implemented in the landscape. An example of this scenario continues to be observed with *H. zea* in cotton (Luttrell and Jackson, 2012; Kerns et al., 2018).

The Panel recommended the evaluation of bioeconomic models to establish UXI thresholds (Fackler et al., 2014; Fan et al., 2016), particularly since there is a long history of using mathematical models to inform Bt refuge regulations. In general, the more stringent the threshold, the greater the option value of being able to reverse/mitigate resistance. This also includes forecasting the potential magnitude of the expected cost of the threshold if the events occurred more frequently in generating costly responses. The Panel explained that the final results of the decision analysis exercise along with bioeconomic modeling could indicate either (a) the threshold injury level should be set more stringently to permit effective responses and mitigation or (b) the use of such thresholds in terms of crop injury are generally inefficient, then an economic case could be made for discarding them entirely.

The Panel also recommended additional relevant factors for establishing UXI and non-high dose pests. The UXI should consider specific pest status (time and space) if refuge fields are available. Sampling a non-Bt standard cultivar such as a refuge or non-Bt sentinel plot would offer some indication of field infestation which could be related to historical levels. A consistent sampling protocol to support the UXI should to be developed. For example, *H. zea* in field corn has not been recognized as an economic target pest and rarely are corn fields sampled for infestations (Reisig et al., 2015; Bibb et al., 2018).

The Panel discussed in-depth how using an UXI based solely on crop tissue damage is not sufficient to support the investigative process. Additionally, the Panel discussed how untimely collection of field samples and reporting may damage Bt corn and/or Bt cotton long-term even after the target pests have cycled. For an investigation based upon plant tissue damage to proceed efficiently, the Panel recommended larval infestation levels as part of the sample information to support the UXI. A detailed identification of larvae will further discriminate among damaged tissues that can be associated with multiple species in field corn and cotton. More importantly, a minimum number of insects are required for confidence in laboratory bioassay results. Larval data will verify the presence of the target pest infestation at the site and increase the probability of collecting sufficient numbers for continued investigation of the reported field control failure. Examination of sites that have reported UXI further offers the opportunity for additional collections of target pests and can provide more diverse monitoring results to support IRM. To promote and support sampling of PIP crops by stakeholders, the Panel recommended that education and incentive programs be formally initiated as part of the overall IRM program for PIP's. The current Agency issue paper (US EPA, 2018) further provides a summary of the issues associated with establishing collections from Bt fields with reported control failures.

b. Used in the context of PIPs, the UXI threshold offers a warning that a target pest may have evolved practical resistance to the PIP. The Panel recognized the Agency had not established specific crop damage thresholds for UXI investigations in the terms of Bt crop registration for *H. zea*. The terms of Bt cotton registrations did not establish specific damage thresholds for UXI investigations. It is the responsibility of each registrant to coordinate sampling efforts and crop damage reports from suspected Bt field control failures. Initial information may be obtained from growers, Extension agents, consultants, or company agronomists, but currently there is no formal and consistent process established across industry registrants of Bt crops. Subsequent laboratory testing of larvae may reveal the occurrence of localized resistant populations from these fields (US EPA, 2010a; 2010b). If resistance to the PIP is confirmed and after all formal steps in the investigation have been completed, a mitigation (remedial action) plan is triggered as defined by the terms of registration (US EPA, 2010a; 2010b).

The UXI threshold is not to be mistaken with "economic injury level" (EIL) or "economic threshold" (ET) which are terms and tools of IPM (Stern et al., 1959). The Panel recognized that the UXI threshold should be higher than the ET, and in most instances, surpass the EIL for crops that expressed PIPs.

There is evidence that the UXI system worked with reference to the 2012-2017 southwestern corn borer (SWCB), *D. grandiosella*, Bt field corn control failures in the Cochise Valley of Arizona. This was in spite of not detecting a "SWCB resistant population" with standardized laboratory bioassays. However, this success was demonstrated for a high dose pest in a localized, geographically-restricted area (Clint Pilcher, Pioneer stakeholder presentation, US EPA SAP, July 17-19, 2018, public e-docket, Docket No. EPA-HQ-OPP-2017-0617, accessible through the docket portal: http://www.regulations.gov).

The Panel recognized the need to develop a standard UXI for *H. zea* in Bt cotton and identified several important challenges associated with establishing this critical level. The injured plants must be confirmed as Bt-expressing varieties and confirmation is needed to ensure that no other factors (e.g., population pressure, climate, cultural practices, or other non-targeted pests) are responsible for crop damage. A uniform regulatory threshold for UXI crop injury in Bt cotton should be independent of any comparison with a non-Bt cotton refuge because of the natural refuge paradigm for pyramided Bt cotton. Establishing a UXI for cotton PIPs will support the concepts addressed in many of the charge questions (sampling, resistance confirmation, mitigation/management, etc.) posed to the Panel.

During the Panel's introductory discussion of Bt toxins in cotton, *H. zea* was considered a secondary pest and was clearly documented to be less susceptible than the primary target in cotton, tobacco budworm, *Chloridea* (formerly *Heliothis*) *virescens* to Cry toxins (Stone and Sims, 1993; Luttrell et al., 1999). Initially, in the late 1990's and early 2000's pyramided-Bt PIP cotton technologies (Bollgard II and WideStrike) demonstrated improvements in field efficacy against *H. zea*, thereby reducing larval survivorship and crop damage (Chilkowski et al., 2003; Jackson et al., 2003; Jackson et al., 2006; Luttrell and Jackson, 2012), but occasional infestations exceeded EIL and supplemental insecticide treatments were warranted. From 1997–2011, 43 reports were presented in the National Cotton Council's Beltwide Cotton Conferences Proceedings which showed field efficacy of Bt cotton against *H. zea* was highly variable with *H. zea* damaged fruiting forms (bolls and squares) ranging from 1–60%, largely depending upon the intensity of *H. zea* infestation (Luttrell and Jackson, 2012). Action thresholds for initiating insecticide treatments targeting *H. zea* have remained the same for Bt and non-Bt cottons (Cry toxins) across the cotton belt which further indicated expectations of crop damage.

Recent reports of crop damage have suggested practical resistance of *H. zea* populations to selected Cry proteins in southern U.S. cotton fields. Samples for within-season and end-of-season damaged PIP cotton bolls were compared to Cry1Ac diet-based bioassay data in collections of larvae from fields with suspected Bt field failures. Bioassay results indicated a range of Cry1Ac susceptibilities across southeastern U.S. collections and suggested that some *H. zea* populations were demonstrating resistance (Reisig et al., 2018). Results from *H. zea* collections collected in 2017 throughout the southern U.S. states indicated a further shift in reduced susceptibilities with numerous populations exhibiting resistance to the Cry1Ac toxin. Many of these populations (70-75%) exhibited reduced susceptibility to Cry2 toxins. In spite of these levels of survivorship, significant *H. zea* infestations were usually required to produce economic yield losses. However, there were reports of significant yield losses (up to 40%) from *H. zea* in selected commercial fields in 2017 (David Kerns, Texas A&M University, personal communication and public article, http://www.plantmanagementnetwork.org/pub/crop/news/2018/IncreasedBollwormPressure/). Field efficacy trials of Bt cotton varieties against *H. zea* were implemented across eight locations of the southern cotton belt in 2017. *H. zea* infestation levels varied considerably by location, but were generally classified as high and prolonged. Seasonal means of damaged flower buds (squares) and bolls in non-Bt cotton varieties ranged from 4-52% and 1.25-20%, respectively across locations. For Bt cultivars not containing Vip3A, seasonal means of damaged flower buds (squares) and bolls ranged from 0-16% and 0-11%, respectively across locations. For Vip3A cultivars, seasonal means of damaged squares and bolls ranged from 0-6% and 0-<1%, respectively across locations (Kerns et al., 2018). Cotton cultivars expressing Cry toxins (especially Cry1A proteins) experienced significant and economic crop damage in several of these tests which will likely accelerate adoption of Vip3A cotton varieties.

There are currently no Bt PIPs classified as high dose for *H. zea* in cotton with the exception of Vip3A. Populations of *H. zea* have been exposed to Bt toxins for over two decades (multiple annual generations) with considerable selection pressure in field corn and cotton. The Vip3A toxin is considered a “near high dose” product. The Panel acknowledged that establishing a UXI from a simple binomial (presence/absence) sampling of crop damage or infestation for any currently available PIP is not possible for *H. zea*. Reports of *H. zea* populations expressing resistance to Cry toxins associated with crop damage across a range of geographies will necessitate a UXI for non-Vip3A cotton varieties to be sufficiently high (Kerns et al., 2018; Yang et al., 2018). Most cotton industry stakeholders have expectations of finding *H. zea* damaged fruiting forms during the production season.

Therefore, with an expectation of significant crop damage to occur on Cry1 cotton cultivars, the Panel recognized that a practical UXI cannot be established without creating a significant regulatory burden on stakeholders, industry, and the U.S. EPA. The Panel suggested that developing a strategy to phase out cotton varieties that express only Cry1A proteins in pyramids would have greater success in cotton IRM plans.

The Panel offered the following recommendations for establishing a UXI for other PIPs in cotton: UXI for cotton varieties which included a Cry 2 toxin in the pyramid should be reported when a sample of fruiting forms (squares and/or bolls) are damaged at 12-18% and larvae (L3 stage or greater) are present. Vip3A cotton cultivars have not currently been widely adopted. *H. zea* remains more susceptible to Vip3A compared to the Cry proteins. The UXI recommended in Vip3A cotton varieties should be when a sample of fruiting forms are damaged at 9-12% and larvae (L3 stage or greater) are present. These crop damage levels conform to 1.5-2x and 2-3x above the action threshold of 6% damaged fruiting forms currently being used to trigger an insecticide overspray for non-Vip3A and Vip3A cotton varieties. Action thresholds in Texas and the mid-southern U.S. to initiate supplemental insecticide treatments for *H. zea* after the initiation of flowering (reproduction) is when fruiting form (squares and/or bolls) damage is $\geq$ 6% and larvae are present (Vyavhare et al., 2018; https://cottonbugs.tamu.edu/fruit-feeding-pests/H.zea-and-tobacco-budworm/; Delta Agricultural Digest, Cotton Insect Control Guide [pages 60-63], Farm Press, Clarksdale, MS, 38614). Based upon the results of 2017 field trials (Kerns et al., 2018), the UXI’s of 12-18% and 9-12% would have triggered investigations of field control failures in at least three instances.

The Panel also recommended that as new traits/technologies are developed and crop/pest specific EIL and ETL’s are established, an initial UXI should be 1.5-2x the ETL or action level to initiate an insecticide overspray. The Panel noted that these are general recommendations, with respect to: 1) variation in target pest population levels; and 2) environmental conditions favoring larval survivorship and crop damage in Bt cotton.

Additional information should be collected to support the interpretation of the recommended UXI. The Panel recognized that general reports from Land Grant University entomologists on timing of *H. zea* occurrence, egg and larval infestation levels, and duration of adult/larval populations (generation times) across state and regions should be collected as part of the assessment. Historical and current pheromone-baited trap capture data should be included to document general population trends and adult activity.

A sampling protocol to confirm UXI should follow similar methodology to that currently being used by IPM practitioners and was recommended by the Panel. This process is relatively efficient, well understood by industry, and is anticipated to reduce the probability of mistaking non-damaged fruiting forms in the final UXI sample. However, it was noted that *H. zea* damaged fruiting forms detected during in-season sampling events may abscise from the plant. *H. zea* damage to these forms can be underestimated if the larval infestation is not detected timely. The Panel recommended the sampling process be initiated as soon as possible after the initial UXI reports have been received by the registrants to document infestations and crop damage.

Although *H. zea* adults oviposit throughout the cotton plant canopy, early stage larvae typically have the highest survivorship when feeding on young tissue (plant terminal region) and immature fruiting forms (young flower bud) and white flowers. In many instances, feeding by young *H. zea* larvae on the youngest plant tissues is inconsequential and may not result in EIL. However, these larvae may be detected prior to intoxication with Cry toxins, but will soon stop larval development and expire. The Panel recommended a protocol based upon sampling older fruiting forms (squares and bolls). Damage to these forms usually indicates an established infestation later stage larvae that have survived the expressed dose of Bt toxin in the plant. The Panel also recommended recording crop damage on older fruiting forms (squares and green bolls).

D. Definition of Resistance

EPA's definition of pest resistance is based on heritability of the resistance trait, higher survival of resistant individuals compared to susceptible individuals on Bt crops, and the likelihood that the pest can cause economic damage to Bt crops in the field. As described in the issue paper, however, this definition does not allow for proactive mitigation of resistance (e.g., prior to field failure). EPA is considering an 'early warning resistance' trigger (1-6% of individuals in population resistant) that would initiate mitigation strategies based on the tenets of Integrated Pest Management before resistance is confirmed in the field (practical resistance, >50% of individuals in population resistant).

> **a.** Discuss the criteria and additional considerations for defining resistance beyond those listed in the issue paper that enhance the ability to detect resistance faster and implement effective mitigation to extend the durability of Bt trait(s).

Panel Response 2D:

a. The Panel agreed with the Agency's argument presented in the issue paper that the current EPA definition of resistance is inadequate and does not clearly set appropriate thresholds of resistance needed to manage resistance evolution and phenotypic resistance once it evolves. A working definition should be clear so that appropriate actions can be taken in response to observational and monitoring data.

There is growing stakeholder consensus that resistance of pest insects to PIPs should be placed into three or more categories (Tabashnik et al., 2014; Andow et al., 2016; Tabashnik and Carrière, 2017). This consensus is also reflected in the Agency's issue paper. The Panel recommended the following three categories of resistance:

1. At Risk of Resistance: Resistance allele frequencies less than 2%, and no population shows a detectable increase in genotypic/phenotypic resistance.

A population is defined as the insects within a region which dispersal and mating are sufficient to produce little genetic heterogeneity. Thus, for highly mobile noctuid pests, populations can be as large as Sampling Regions 1-4 (defined in the Agency's issue paper, page 15) currently used for monitoring for resistance in corn pests. The definition of a population also includes much smaller areas, including single farms, as the noctuid population within a single farm represents a collection of potentially interbreeding individuals. There are special situations, such as isolated valleys in the southwestern U.S. and populated islands (such as Puerto Rico), where lepidopteran have relatively low dispersal between surrounding regions and therefore can be considered a separate population for IRM purposes.

The 2% threshold for resistance allele frequencies is consistent with the 1-6% threshold proposed by Tabashnik et al. (2014) (although it sets a single value for regulatory action). The Panel considered 2% an appropriate value in light of the observed cases of resistance that have evolved throughout the world (Tabashnik and Carrière, 2017). At this resistance frequency, damage due to phenotypically resistant individuals is likely to evolve rapidly if there is no cost of resistance or changes in IRM.

2. Heightened Risk of Resistance: The lower threshold is crossed when resistance allele frequencies exceed 2% in any population, or at least one population shows a detectable increase in genotypic/phenotypic resistance. The higher threshold to "observed practical resistance" is crossed when UXI is observed and is shown to be caused by heritable resistance.

The Panel discussed the category of "early warning resistance" as described in the Agency's issue paper and in the literature by Tabashnik et al. (2014) and Tabashnik and Carrière (2017). The Panel overall did not agree with the description of "early warning," and deliberated that "heightened risk" would be a more appropriate terminology. "Early warning" implies that there are effective mitigation plans that can be enacted that will stop the spread of resistance. Because the appearance of phenotypic resistance is likely to be sudden due to the nature of the evolutionary process, even extensive phenotypic monitoring may not provide adequate early warning.

The heightened risk category would initiate action to manage resistance under the expectation that resistance could rapidly lead to extensive crop damage. These actions could include (i) heightened monitoring of resistance, (ii) ensuring compliance with existing IRM, (iii) enacting heightened IRM by, for example, increasing the proportion of refuge, (iv) removing at-risk PIPs from regional markets, (v) integrating IRM with alternative resistance management strategies such as tilling, insecticide sprays and mating disruption, (vi) bolstering IPM, and (vii) increasing communication with stakeholders such as Extension agents, grower organizations, and public and private-sector scientists. These actions will likely be necessary at broad geographical scales, because resistance will likely represent a regional rather than local threat.

The appropriate responses for pests within this category depend in part on the amount known about the history of resistance within the population. For example, if long-term genetic monitoring has shown little increase in resistance despite levels of genotypic resistance in the population at >2%, then the appropriate response would be more limited than the response would be in the absence of this information. Such high but constant genotypic resistance has been the case for *Helicoverpa armigera* and *H. punctigera* in Australia; despite resistance allele frequencies (as determined by F1 and F2 screens) being in the 1-5% range for Cry1, Cry2, and vegetative insecticidal protein (Vip) PIPs, there has been little change in resistance over 15 years of monitoring, and the appropriate response has been strict adherence to IRM. The case of *H. armigera* and *H. punctigera* in Australia, however, should not be used to set guidelines for other pests, because numerous other pests (and *H. armigera* in other countries) have shown rapid increases in resistance.

3. Observed Practical Resistance: The threshold into this category is crossed when UXI is observed and shown to be caused by heritable resistance.

Observed practical resistance for a pest implies that resistance has been observed with practical consequences for pest control and may occur at either the local or regional scales. The appropriate responses include (i) IPM, and (ii) removing PIPs from the market. Responses should be informed by historical monitoring. For example, if genotypic resistance has risen slowly and locally, then this might imply a cost of resistance, in which case mitigation strategies to reduce genotypic resistance may be possible.

Inadequacy of the Current Definition

In the current EPA definition, the inclusion of the criterion involving “the likelihood that the pest can cause economic damage to Bt crops in the field” removes the possibility of responding to resistance when it can still be managed. Furthermore, defining resistance in terms of damage introduces uncertainty, because damage depends not only on resistance of the pests, but also on the abundance of pests which fluctuates naturally among years. Thus, by the current definition a pest could potentially be judged as resistant even though it has low genotypic/phenotypic expression of resistance. This would occur if favorable weather conditions created large populations of susceptible insects. Resistance should be defined in terms of the genotype and phenotype of the insects (Tabashnik and Carrière, 2017). This allows the definition to be used in tests for resistance.

Impracticality of "Early Warnings"

To explore the possible use of definitions of resistance in monitoring for early warnings of resistance, the Panel produced a model of resistance evolution (see Appendix 1: Supplement to Figures 2D.1, 2D.2, 2D.3- General Model Description to Simulation to Resistance Monitoring) based on the model of Ives et al. (2011). This model is similar to other models of resistance evolution with structured refuges, such as Martinez and Caprio (2016, 2018). The model is parameterized for a near-high dose pest, in which susceptible homozygotes have a survival of 0.05 on Bt plants, and heterozygotes have a survival of 0.0975. The model is spatially explicit on a 50 x 50 field grid. There is a central region of 10 x 10 fields with reduced refuge amount (a 2% structured refuge), while the remaining landscape has a 20% structured refuge. In simulations, resistance always occurs more rapidly in the central 10 x 10 field region. Resistance is monitored at 25 sites spread evenly across the landscape consisting of a pair of Bt and refuge fields. Monitoring consists of insect density (assumed to indicate damage), average phenotypic resistance (as would be determined by population-level feeding assays), and resistance allele frequency (as would be determined by genetic screening). The output of the model can thus be used to address the information available in the different types of monitoring for resistance evolution and the possibility of providing early warnings of resistance.

Figure 2D.1 shows the output of the model for the density of larvae in each of the 25 monitoring sites; the top panel indicates the density in Bt fields, the middle panel indicates the density in refuge fields, and the bottom panel provides the ratio of density of larvae in the Bt relative to refuge fields. Resistance (an average resistance allele frequency of 50%) in this simulation occurred in generation 31. The density of larvae within fields is assumed to correspond to the injury experienced within fields. This simulation suggests that neither the density of larvae in Bt fields, nor the density in Bt fields relative to the density in refuge fields, predicts the appearance of resistance in generation 31.

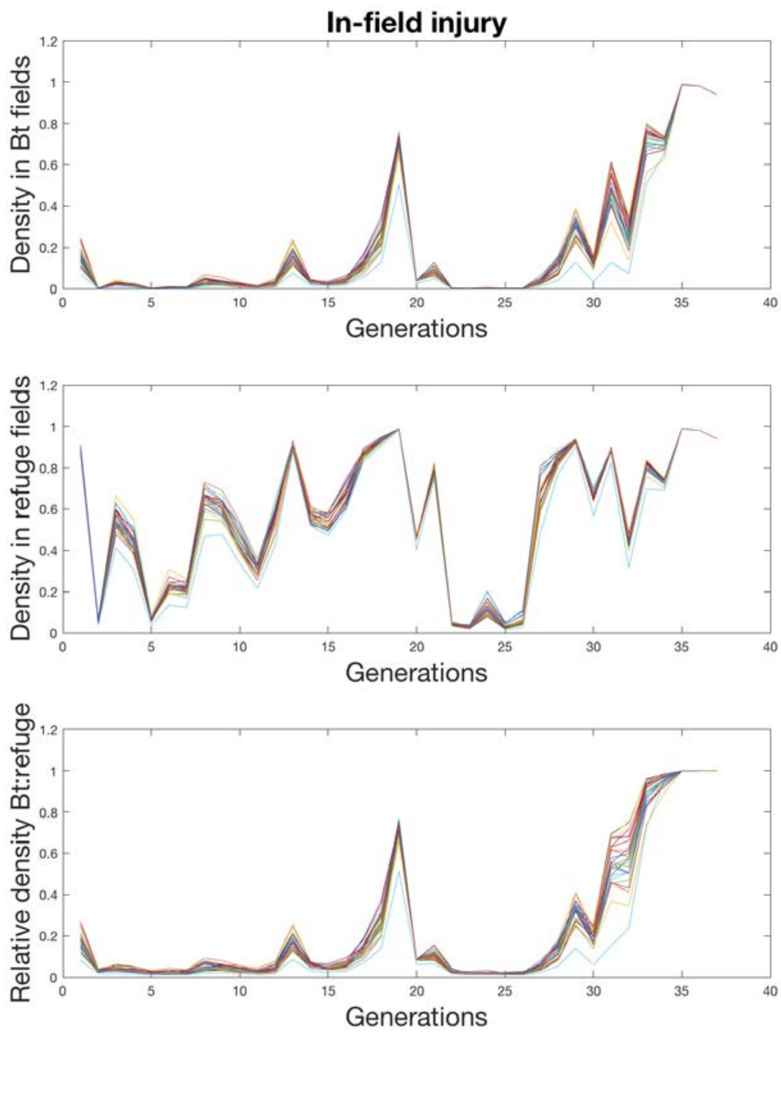

Figure 2D.1: Simulation to Resistance Monitoring-Larvae Density in Various Fields. This demonstrates the simulation of resistance monitoring (see also Appendix 1 for Supplement to Figures 2D.1, 2D.2, 2D.3-General Model Description to Simulation to Resistance Monitoring), larvae density in 25 Bt fields (top panel), 25 refuge fields (middle panel), and the relative densities in Bt to refuge fields (bottom panel).

Figure 2D.2: demonstrates the output of the model for the average survival of larvae feeding on Bt versus non-Bt plants; this is the type of information that could be collected from population-level feeding assays. Genetic resistance (resistance allele frequency >50%) is reached at generation 31, and there is little change in average phenotypic resistance (survival of larvae on Bt plants relative to non-Bt plants) until generation 29. Therefore, average phenotypic resistance does not provide an early warning of resistance. The reason for this is that average phenotypic resistance accelerates more rapidly than exponentially (Figure. 2D.2, bottom panel), which can be explained as follows. The simulation considers the case of a near-high-dose PIP, as is appropriate for the lepidopteran evaluated by the Panel. In the model simulation, the survival of susceptibles on Bt plants is 0.05. Therefore, when the resistance allele frequency is low, all of the survivors on Bt plants are susceptible. These susceptible larvae obscure the rise in survivorship of the rare heterozygous resistance individuals, and the much rarer homozygous resistant individuals. It is only when the resistance allele becomes relatively common that the higher survival of resistance heterozygous and homozygous larvae is observed.

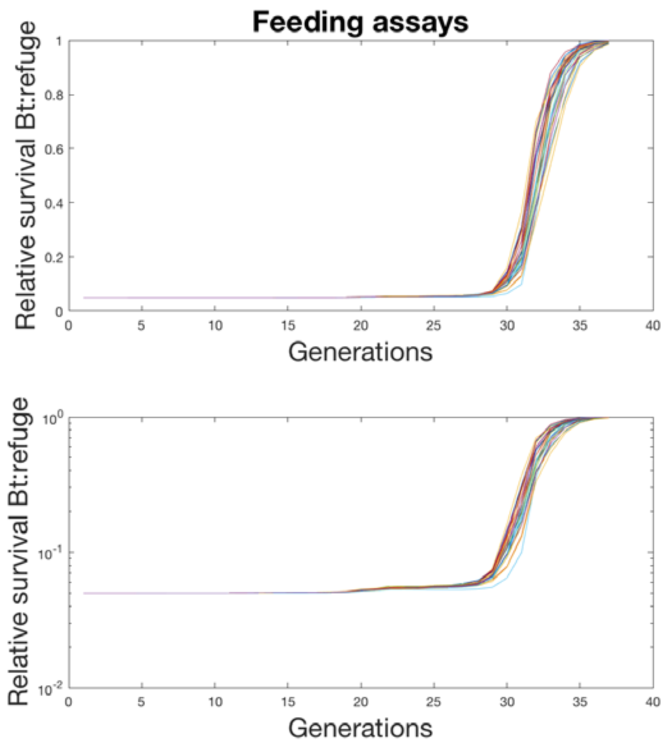

Figure 2D.2: Simulation of Resistance Monitoring-Average Survival of Larvae Fielding on Bt Versus Non-Bt Plants. This demonstrates the simulation of resistance monitoring (see also Appendix 1 for Supplement to Figures 2D.1, 2D.2, 2D.3-General Model Description to Simulation of Resistance Monitoring), the average survival of larvae on Bt relative to non-Bt plants or media at 25 simulated sites on the landscape. The top panel gives the relative survival on an absolute scale, while the bottom panel gives the same values on a log scale.

Figure 2D.3 demonstrates the output of the model for the resistance allele frequency in the 25 monitored Bt and refuge fields. In contrast to phenotypic resistance (Figure 2D.2), genotypic resistance increases roughly exponentially. Furthermore, there is little difference in the allele frequency among fields, either Bt or refuge. Thus, even though resistance appeared first in the 10x10 field region at the center of the modeled landscape with only a 2% proportion of refuge fields, there was little spatial variation in resistance allele frequency. This lack of variation is not due to the rapid spread of resistant individuals among fields; in the model, adults can fly only to the adjacent 8 fields and therefore have very low dispersal rates. Instead, the lack of variation in resistance allele frequency among fields is due to the fact that resistance evolution occurred throughout the landscape. This conclusion is confirmed in simulations in which, once resistance allele frequency in the central 10 x10 field region reaches 0.01, this region is removed. Resistance evolution proceeds at the same rate in the surrounding region even without this central region.

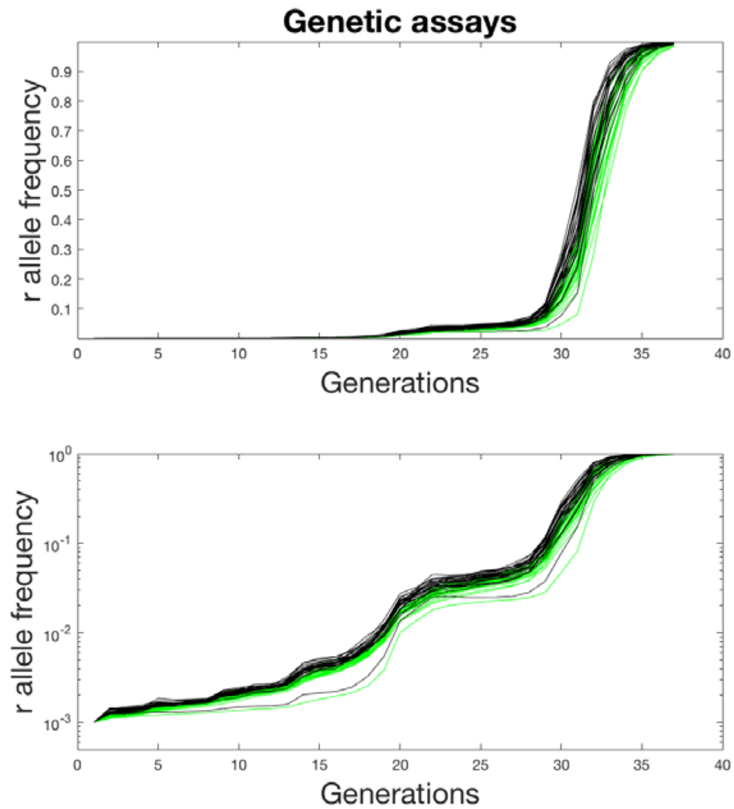

Figure 2D.3: Simulation of Resistance Monitoring- Frequency of the Resistance Allele in 25 Pairs of Bt and Refuge Fields. This demonstrates the simulation of resistance monitoring (see also Appendix 1 for Supplement to Figures 2D.1, 2D.2, 2D.3-General Model Description to Simulation of Resistance Monitoring), the frequency of the resistance allele in 25 pairs of Bt (black) and refuge (green) fields. The top panel indicates the resistance allele frequency on an absolute scale, while the bottom panel provides the same values on a log scale.

To summarize the model results:

(i) UXI is useful to document the presence of resistance in a population. Comparing damage between Bt and refuge fields does not provide better predictions than are provided by the damage in Bt fields. The strongest patterns that can be documented are levels of damage before and after the emergence of resistance.

(ii) Phenotypic resistance is necessary to confirm that increased damage in Bt fields is due to resistance, because even in the absence of resistance, damage to Bt fields can be high under favorable conditions to susceptible pests (Figure 2D.1). Nonetheless, the average phenotypic resistance of a population likely increases suddenly and only when genetic resistance is high, making population-level phenotypic resistance a poor predictor of resistance evolution.

(iii) Genetic resistance monitoring will give information about the evolution of resistance in a population. This is the information that is needed to confirm that a pest is "at risk" of resistance, instead of "heightened risk." It will also give information about the nature of resistance -- in particular, whether there is a cost to resistance -- that could be a great help in designing management strategies for cases of heightened risk of resistance and observed practical resistance.

Genetic Resistance Monitoring

Genetic resistance monitoring often requires either an F2 screen (Andow and Alstad, 1998) or, if a resistant lab colony is available, an F1 screen. These screening methods have been applied effectively for *H. armigera* and *H. punctigera* in Australia. F2 screens have the advantages that they can identify rare resistance alleles with novel modes of action, and they do not require a resistant lab colony. They have the disadvantage, however, of possibly giving underestimates of resistance allele frequencies due to reduced survival or mating of resistant heterozygotes and/or homozygotes (e.g., there is cost of resistance). F1 screens are simpler to perform and can be more accurate, although are limited to detecting resistance that has the same mode of action as resistance in a lab colony. It is also possible that molecular screens will be developed that can be used in the future to identify specific resistance alleles (see Panel Response to Charge Question 2B). However, in the current absence of these molecular tools, current genetic screening methods should be used.

Genetic monitoring is essential for categorizing the risk of resistance evolution. It also gives fundamental information about the nature of resistance that can be used to potentially mitigate resistance. This is the case, for example, if genetic monitoring reveals a cost of resistance. Genetic monitoring could allow statistical predictions of resistance evolution. For example, monitoring data and models could be integrated by applying Bayesian population modeling approaches to iteratively update and improve the probability distributions of various parameters in the models as monitoring data become available so as to continuously improve and refine the models. The Panel suggested that the Agency look into the Bayesian model forecasting for the long-term benefits of establishing more accurate predictions of insect resistance problems. This approach is currently used extensively in modeling in human toxicology and risk assessment (Bernillon and Bois, 2000) as practiced by the EPA National Center for Environmental Assessment in probabilistic mouse and human physiologically based pharmacokinetic (PBPK) models for human risk assessment (US EPA, 2010c).

Incorporating Mathematical Models into Monitoring Programs

The field of resistance modeling has grown to include more models, yet with greater model transparency it has become clear that the models have similar structures and, for the same parameter values, give similar results. IRM modeling has matured. The Panel recommended that the EPA maintain in-house IRM modeling expertise that can be used to evaluate monitoring procedures for different lepidopteran pests. This could not only help to establish effective monitoring strategies, but could also evaluate their cost-effectiveness and the possible advantages of using multiple types of monitoring. In-house modeling expertise will also help in future evaluations of IRM plans.

Goals of Monitoring for Resistance

Either explicitly or implicitly, monitoring for resistance is most often discussed in the context of stopping or mitigating the spread of resistance. This assumes that resistance occurs as a rare, localized event like a forest fire or an outbreak of Ebola virus. However, such point-source "outbreaks" of resistance are unlikely for lepidopteran pests for three reasons. First, these pests have high dispersal, so that resistance alleles are likely to spread rapidly throughout a population. Second, initial (natural) resistance appears to be widespread in some lepidopterans, so the potential for resistance evolution is equally widespread. Third, conditions that lead to resistance evolution, in particular, low refuge abundance (either natural or mandated), are likely to be widespread. These three reasons suggest that a more appropriate conceptual equivalent to controlling resistance evolution is equivalent to controlling the influenza virus. Monitoring for resistance evolution will provide critical information for how to respond to resistance when it emerges. However, it will not provide much early warning that could be used to stop the spread of resistance. These factors argue for an emphasis on preventive approaches as opposed to remediation approaches to manage resistance in lepidopterans.

<u>Implementation of Panel Recommendations for a Definition of Resistance</u>

The Panel recognized that defining different categories of resistance goes hand-in-hand with monitoring for these categories. The Panel provided the following recommendations for implementing the definition of resistance.

1. At Risk of Resistance

Establishing that resistance allele frequencies are less than 2% requires genetic monitoring of a population. Currently, the best available method for monitoring is an F2 screen. If resistant laboratory strains of a pest are available, then F1 screens will be possible.

Detecting an increase in genotypic/phenotypic resistance requires sampling to have sufficient statistical power to detect a change. The Panel recommended that sampling levels be sufficient to give statistical power to detect a two-fold increase in genotypic/phenotypic resistance at the alpha = 0.05 confidence level (see for example, Roush and Miller, 1986).

2. Heightened Risk of Resistance

Current IRM plans for PIPs are based upon models that assume initial resistance allele frequencies to Bt toxins are less than 1%, which corresponds to populations "at risk of resistance" (US EPA, 2009, 2011). After EPA regulations for PIP IRM were established, however, it was found that some noctuid pests had resistance allele frequencies >1% before the PIPs were extensively used (e.g., *H. armigera* and *H. punctigera* in Australia). Resistance allele frequencies were also relatively high for pink bollworm in the first two years preceding the introduction of to Cry1Ac Bt cotton in Arizona (Tabashnik et al., 2000, 2005). This implies that background levels of resistance could place pests in the heightened risk of resistance category before PIPs are available on the market.

The high natural resistance observed in some lepidopteran pests implies that, for registration of new PIPs, the initial assumption should be that target pests be classified to the "heightened risk of resistance" category. If registrants provide data showing that resistance is below the heightened risk category, then EPA can classify them as "at risk of resistance."

In the absence of data on current levels of resistance allele frequencies for *H. zea*, *S. albicosta*, *S. frugiperda*, and *D. grandiosella,* the Panel recommended that the EPA consider these pests for listing under "heightened risk of resistance."

3. Observed Practical Resistance

Observed practical resistance could be determined in at least three ways: (i) data provided by monitoring programs for resistance that are used to distinguish "at risk" from "heightened risk" cases, (ii) academic or other independent sources of information on resistance, or (iii) reports from farmers that are confirmed by registrants. Implementation of this category in general follows the procedure currently employed for the determination of resistance by EPA when unexpected injury is found (see Charge Question 2A-C).

Specifically, once unexpected damage is found, it is first necessary to rule out that plants are not expressing Bt. If they are expressing Bt, resistance to Bt toxins in most lepidopterans can be measured by a diet-based or plant-based laboratory bioassay sensitive enough to distinguish Bt resistant from Bt-susceptible individuals. A sensitive assay is necessary to avoid misclassifying a resistant population as susceptible. Any assay used to confirm resistance of field populations must be freely available to all public-sector scientists, and ideally, all public and private-sector scientists would use the same assay method. The keys to quick analyses are having the infrastructure established to quickly test insects and to having publicly available lab assays, plants or both.

The Panel's response to charge question 8 discusses more generally possible strategies for monitoring for resistance to place pests and PIPs into the three categories proposed here.

Charge Question 3. – *Resistance Risk of Seed Blend Corn in the Southern U.S.*

Theoretical and laboratory studies provide supporting evidence that seed blend refuges increase the risk of resistance development in ear-feeding pests of corn, such as *H. zea*, particularly in the southern U.S. Cross-pollination events between the Bt and non-Bt plants result in mosaics of Bt expression in kernels throughout Bt and refuge ears. Bt corn ears may contain kernels with full, partial, and no Bt expression, while refuge ears likely consist of kernels with partial Bt and no Bt expression. Partially resistant genotypes may passively benefit from or even actively exploit such a mosaic by tasting and rejecting toxic kernels and moving to less toxic ones, while susceptible insects die. Sub-lethal Bt exposure shifts the functional dominance of resistance and provides a pathway for greater survival of heterozygous resistant larvae, which have a fitness advantage compared to susceptible individuals under this scenario.

Non-hemizygous Bt corn plant cultivars combined with non-Bt pollen incompatibilities or corn self-fertilization capabilities through gene editing approaches could prevent Bt and refuge ear cross-fertilization. Use of seed blends composed of such varieties could theoretically present technical solutions to the identified resistance risk and make "Refuge-in-the-Bag" (RIB) products a viable option in the southern U.S. Solutions of these types would provide adequate refuge compliance and sound resistance management for ear-feeding pests of corn.

a. Discuss and evaluate the resistance risk of RIB products for ear-feeding pests of corn in light of the corn pollination dynamics discussed above and the biology of ear-feeding pests (e.g., multiple generations per year and overwintering capacity).

b. Discuss and evaluate the scientific and economic feasibility of developing:

i. Non-hemizygous Bt varieties to avoid kernel mosaics during Bt x Bt pollination;
ii. Parental corn hybrids with genetic incompatibilities to limit or eliminate the potential for cross pollination between Bt and non-Bt corn plants; and/or
iii. The development of selfing Bt and non-Bt lines to avoid cross-pollination and mosaics of Bt expression in kernels.

Panel Response 3:

a. The Panel agreed with the conclusions in the Agency's issue paper that seed blends in RIB decreased the durability of PIPs to resistance evolution in ear-feeding lepidopteran pests. The potential for RIB to reduce durability was initially identified as a problem caused by plant-to-plant movement of larvae when using seed blends (e.g., Mallet and Porter, 1992, Tabashnik, 1994); this concern involves both stalk and ear-feeding pests. For ear-feeding pests, there is an additional problem of pollen exchange between Bt and non-Bt plants, and the resulting mixing of Bt and non-Bt kernels within the same ear (Chilcutt and Tabashnik, 2004). For *H. zea* this problem is a greater concern than plant-to-plant movement due to the relatively low movement rate of larvae among plants. In contrast, the high expected prevalence of Bt kernels within non-Bt plants will mean that many larvae on non-Bt (refuge) plants will encounter Bt expressed in kernels. Because larvae must eat many kernels to survive to adulthood, this means that heterozygous resistant rS larvae will be exposed to moderate doses of Bt toxin that might be sublethal for these larvae, yet lethal for susceptible homozygotes. This causes potentially strong selection for resistance on the refuge plants, leading to faster resistance evolution (Chilcutt and Tabashnik, 2004; Caprio et al., 2016).

The Panel recommended the use of seed blends in southern, cotton-growing areas of the U.S. It is addressed in greater detail in charge question 4.

The Panel recommended permissibility of seed blends where ear-feeding lepidopteran pests do not overwinter, but with a minimum of 10% non-Bt seed within the blends for functionally pyramid varieties. This recommendation addressed regions outside the cotton-growing region. It is designed to compensate for the loss of durability due to selection for resistance in seed blends due to cross-pollination. Previous FIFRA Scientific Advisory Panels addressed the issue of seed blends for pests of corn and cotton (US EPA, 2009, 2011), and concluded that a seed blend of 5% provided less durability than a structured refuge of 5%. The focus of previous FIFRA Panel recommendations was on lepidopterans that do not feed on corn ears. Growing evidence suggested that the risk of decreased durability for ear-feeding lepidopterans in seed blend refuges were greater than for stalk and root-feeding pests. The current Panel's recommendation for increasing the proportion of non-Bt seed in seed blends were therefore consistent with previous Panel recommendations, especially in light of new evidence about the risks of cross-pollination in seed blends and the documented cases of resistance evolution in ear-feeding pests throughout the world.

The Panel acknowledged that pollen flow between Bt and non-Bt plants in seed blends represented a serious concern for resistance management. Simultaneously, the Panel recognized the advantages of seed blends, because they are easier for farmers to manage than structured refuges, and compliance will be higher. Given the critical issue of compliance, the Panel acknowledged that seed blends should remain an option for IRM in regions where ear-feeding lepidopterans do not switch between corn and cotton host plants. To compensate for the increased resistance evolution in seed blends, however, it is necessary to increase the proportion of non-Bt plants within the blend.

Increasing the proportion of non-Bt plants in seed blends involves a trade-off between managing for resistance evolution and controlling pest damage. The Panel did not want their recommendations to lead to seed blends that are not utilized by farmers because they provide inadequate pest control. Relative to structured refuges with the same proportion of non-Bt plants in the landscape, seed blends will provide better control because non-Bt plants containing Bt kernels will kill some susceptible larvae (Tabashnik, 1994; Carrière et al., 2004; Carroll et al., 2013). In other words, if cross-pollination leads to increases in the rate of resistance evolution relative to structured refuges, it will also provide relatively greater protection against damage to non-Bt plants.

The impact of pollen flow between Bt and non-Bt plants can be addressed using a simple mathematical result that explains the rate of resistance evolution of pests to Bt PIPs. For a single-toxin PIP in which there is no cross-pollination, the rate of resistance evolution is

$$p(t+1)/p(t) - 1 = R(1-Q)s_{\mathrm{Bt.rS}} \qquad \text{(equation 3.1)}$$

where $p(t)$ is the frequency of a resistance allele r in generation t, R is the maximum per capita population growth rate, Q is the proportion of refuge plants, and $s_{\mathrm{Bt.rS}}$ is the survival of rS heterozygotes on Bt plants relative to the survival of SS susceptible homozygotes on refuge plants. This approximation is based on six assumptions: (i) resistance is not completely recessive, (ii) the initial allele frequency is low to moderate (<0.01), (iii) there is no cost of resistance (e.g., the survival of resistant insects on non-Bt plants is the same as susceptible insects), (iv) dispersal of male and/or female adults is sufficient to genetically mix the population, (v) almost all of the susceptible population breeds from non-Bt rather than Bt plants, and (vi) there is no selection for resistance on non-Bt plants. Assumption (v) is appropriate for high-dose cases of resistance, when the survival of susceptible SS individuals on Bt plants is <0.01. For moderate-high dose, the approximation begins to be less accurate when the refuge Q is small. Nonetheless, the approximation is used to compare cases with and without pollen flow, for which the approximation is useful even for the non-high-dose case. A formal mathematical derivation of this equation was given by Ives and Andow (2002), and can be extended for the case of pyramid PIPs, for which $s_{Bt.rS}$ is replaced by $s_{Bt.rSSS}$, the relative survival of heterozygotes for one resistance trait (Ives et al., 2011).

Cross-pollination between Bt and non-Bt plants violates the last assumption (vi) used to derive the approximation above. For the case when there is selection for resistance on the non-Bt plants due to cross-pollination, the equation becomes

$$p(t+1)/p(t) - 1 = (s_{refuge.rS}/s_{refuge.SS} - 1) + R(1-Q)s_{Bt.rS} \qquad \text{(equation 3.2)}$$

where $s_{refuge.rS}$ and $s_{refuge.SS}$ are the survivals of rS heterozygotes and SS homozygotes on non-Bt plants. This mathematical result clarifies the main consequences of cross-pollination for resistance evolution. Without cross-pollination, all selection for resistance occurs on Bt plants. With cross-pollination, there is also selection on non-Bt plants that are contaminated with Bt pollen and therefore express Bt in some of the kernels.

Essential information needed for understanding resistance evolution when there is cross-pollination is the survival of rS heterozygotes on refuge plants expressing Bt in some kernels. Unfortunately, this information is not available for *H. zea* for the PIPs used for controlling this pest. However, Yang et al. (2017) experimentally determined the relative resistance of rS and SS individuals for *Spodoptera frugiperda* using a resistant colony. From their results, $s_{refuge.rS}/s_{refuge.SS}$ is 4.9 (=0.423/0.087) and 5.0 (=0.50/0.10) for a 5% and 20% RIB-refuge, respectively. These values imply strong selection for resistance on non-Bt plants.

The Panel continued to discuss how studies on *H. zea* in the form of survival of SS susceptibles on Bt cross-pollinated refuge plants. Without information on the survival of rS heterozygotes, it was impossible to make detailed predictions about the effect of cross-pollination on resistance evolution for *H. zea*. Nonetheless, the Panel notes that it is informative to consider data on the survival of susceptible *H. zea* under different scenarios for the survival of rS heterozygotes. For the case of *S. frugiperda* (Yang et al., 2017), heterozygous rS individuals had the same survival on contaminated non-Bt plants as rr homozygotes. This gives the worst-case scenario for resistance, because this implies that the value of $s_{refuge.rS}$ will be large (resistance is dominant). As an alternative, better-case scenario, the Panel assumed that $s_{refuge.rS}$ is midway between $s_{refuge.SS}$ and $s_{refuge.rr}$ (assumed to equal 1). Onstad et al. (2018) summarized values of $s_{refuge.SS}$ from 14 studies in six articles. Using these values, the average of $s_{refuge.rS}/s_{refuge.SS}$ is 1.32 (range 1-2.70) and 1.16 (range 1-1.85) for the assumptions of complete and intermediate survivals of the rS heterozygotes. The lowest survival of SS susceptibles on cross-pollinated refuge plants, 0.37, was found by Yang et al. (2014) for a Cry1A.105/Cry2Ab2/Cry1F pyramid, which gives $s_{refuge.rS}/s_{refuge.SS} = 2.70$ under the first scenario that assumes survival of rS heterozygotes equal to the survival of rr resistant homozygotes on cross-pollinated non-Bt plants.

For assessing seed blends in IRM, it is appropriate to compute the proportion of non-Bt plants in seed blends, Q', that is equivalent to a structured refuge covering a proportion Q of crop area. This comparison should be made to address two questions. First, how does cross-pollination affect the durability of the PIP to resistance evolution of the pest? Second, how does cross-pollination affect the control of susceptible pests? The first question can be answered using equations 3.1 and 3.2. Figure 3.1 plots the equivalent proportion of refuge (non-Bt plants) in a seed blend when there is cross-pollination (equation 3.2) against the proportion of refuge when there is a structured refuge (equation 3.1) for four cases: (i) the case of *S. frugiperda* for which $s_{refuge.rS}$ and $s_{refuge.SS}$ were estimated (Yang et al., 2017), (ii) the worst case for *H. zea* given in the study by Yang et al. (2014), (iii) the average case from the studies reported in Onstad et al. (2018) assuming full survival of rS heterozygotes, and (iv) the average case from the studies reported in Onstad et al. (2018) assuming intermediate survival of rS heterozygotes. These give values of $s_{refuge.rS}/s_{refuge.SS}$ = (i) 5.0, (ii) 2.70, (iii) 1.32, and (iv) 1.16. To make this comparison, it is necessary to compute the survival of rS larvae as the proportion of Bt-pollinated kernels within an ear of non-Bt corn changes due to changes in the proportion of non-Bt plants in the blend. These computations were based on the simplest case considered by Caprio et al. (2016); the model assumed that larvae randomly consume 25 kernels within an ear and that their overall survival is the product of their survivals on each kernel (see also Appendix 2 for Supplement to Figure 3.1-Rate of Resistance Evolution).

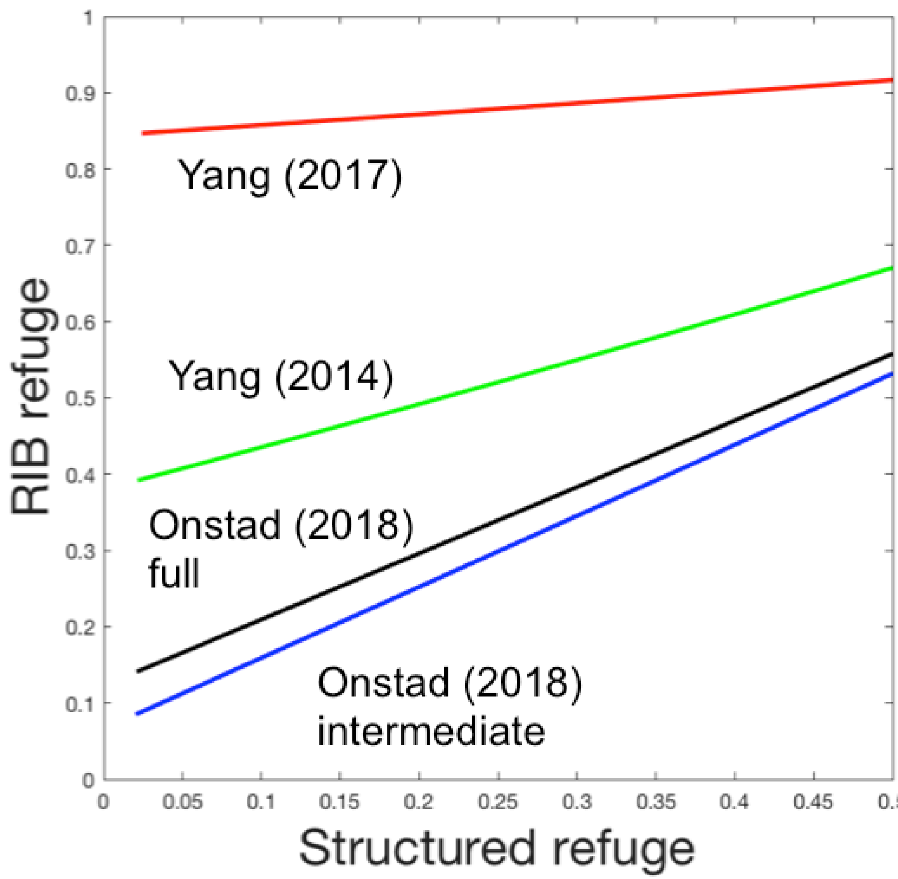

Figure 3.1: Rate of Resistance Evolution: Comparison between seed blends (RIB) and structured refuges that give equivalent rates of resistance evolution. Cases differ in the relative survivals of heterozygous rS and homozygous SS individuals on ears of non-Bt plants that express Bt toxins due to cross-pollination. Values of $s_{refuge.rS}/s_{refuge.SS}$ are 5.0, 2.70, 1.32, and 1.16 from, respectively, Yang (2017) (for *S. frugiperda*), Yang (2014), the average from Onstad (2018) assuming full survival of heterozygotes and assuming intermediate survival of heterozygotes between SS and rr homozygotes. The proportion of Bt pollen in non-Bt plants is 0.5*(1-*Q*) under the assumption that Bt plants are hemizygous and pollination is random within seed-blend fields, where (1-*Q*) is the proportion of Bt plants.

For the case of *S. frugiperda* for which the survival of heterozygous rS individuals can be calculated from the data (Yang et al., 2017), the proportion of non-Bt plants in a seed blend with cross-pollination would have to be >80% to have the same durability as a structured 5% refuge, implying that seed blends are not a viable option for IRM. For the best case considered -- using the average SS survival of *H. zea* from the studies reported in Onstad et al. (2018) assuming intermediate survival of rS heterozygotes -- a 10% seed blend has the same durability as a 5% structured refuge.

In addition to decreasing the durability of PIPs, cross-pollination increases the suppression of susceptible insects when cross-pollination decreases the survival of susceptibles in refuges. Quantifying this effect, however, is not simple. The effects of cross-pollination on short-term suppression are given by the decreased survival of SS susceptibles on non-Bt plants containing Bt-expressing kernels. However, even though susceptible larvae die, they might still have caused damage to plants. Cross-pollination could also affect cannibalism among *H. zea* larvae and affect plant damage (Chilcutt, 2006). There is also the possibility of reduced survival caused by cross-pollination to increase long-term, area-wide suppression (Hutchison et al., 2010). Although the effects of cross-pollination to increase the suppression of pests are difficult to quantify, when the effects of cross-pollination are large enough to decrease PIP durability to resistance evolution, the effects will also be large enough to increase suppression.

It is also possible that contamination of Bt plants with pollen from non-Bt plants will increase the survival of heterozygous rS larvae on Bt plants; this will be another avenue through which cross-pollination could increase the rate of resistance evolution that is not included in the analyses above. However, this might be expected to be less potent than the contamination of non-Bt plants by Bt pollen, because even for Bt plants pollinated with pollen from Bt plants, 25% of kernels will not express Bt. Because the proportion of non-Bt plants in current seed blends is low, the increase in non-Bt kernels in Bt plants caused by cross-pollination will be small. Furthermore, experimental evidence for *H. zea* from Burkness et al. (2011) showed zero survival of large larvae on Bt plants pollinated with non-Bt pollen, suggesting limited effects of cross-pollination. This experiment, however, could not address the effects of cross-pollination on the survival of heterozygous rS larvae, which is the critical information for assessing seed blends.

The Panel acknowledged the challenge of quantifying the possible loss of durability caused by cross-pollination between Bt and non-Bt plants in seed blends. Nonetheless, even a "good-case" scenario (case iv) showed that to have the same durability of a 5% structured refuge, a seed blend would need to contain 10% non-Bt plants. These conclusions are consistent with the results obtained by Caprio et al. (2016) using a model incorporating many other features of larval feeding behavior. Pan et al. (2016), and model results presented to the Panel by Monsanto and Dow DuPont, also addressed the effects of cross-pollination in seed blends, although it is difficult to assess their conclusions because their treatment of the survival of rS heterozygotes on non-Bt plants with Bt-expressing kernels ($s_{\mathrm{refuge.rS}}$) is unclear.

In addressing the second part to the current charge question concerning strategies for limiting cross-pollination within seed blends, the Panel concluded it would likely be a long time for these strategies to become practical. Given the potentially large loss of durability caused by cross-pollination in seed blends, and the cases of emerging resistance in ear-feeding lepidopterans, the Panel suggested immediate implementation of its recommendations under Charge Question 3a.

Scientific Feasibility Evaluation

b.i. As described in the public comment submitted by Corteva Agriscience™ (2018), the Panel discussed in detail how the elite performance of maize hybrids in the last century was based (on heterozygocity) in maize hybrid vigor. Based on available literature, insertion of non-hemizygous Bt transgene(s), with a given condition of preserving the intact maize genetic makeup of an elite maize diploid inbred could be a plausible approach to preventing kernel mosaics during Bt x Bt pollination. In particular, the new CRISPR-Cas9 genome editing technology in maize (Nannas and Dawe, 2015; Dong et al., 2018) could develop Bt transgenic diploid lines with only transformed non-hemizygous Bt transgene(s) via a doubled haploid technique. While public comments to the Panel during the in-person meeting expressed the concern that the technology could impede technical development, the Panel's view was that this was a promising technology and hoped that long-term investments in its development would extend PIP durability and stimulate further advances in new PIP products.

One Panel member expressed concern regarding the utilization of non-hemizygous Bt hybrids in a corn seed blend. The Panelist described how there were two possible drivers of increased resistance evolution (decreased durability) due to cross-pollination of non-Bt plants by Bt pollen. First, cross-pollination generates Bt-expressing kernels that cause selection for resistance on non-Bt plants that are supposed to act as refuges. Second, cross-pollination generates kernels that do not express Bt in Bt plants that are supposed to act as Bt plants. Hemizygous Bt varieties produce only 50% pollen containing Bt genes, whereas non-hemizygous Bt varieties would produce 100% Bt pollen. Therefore, for the case of contamination of non-Bt plants by Bt pollen, non-hemizygous Bt varieties will likely increase the rate of resistance evolution, because these varieties would produce twice the amount of Bt pollen that could contaminate non-Bt plants. However, for the case of Bt plants, non-hemizygous varieties would reduce the proportion of kernels that express no Bt in Bt plants by reducing non-Bt pollen, and this will likely decrease the rate of resistance evolution. The relative magnitudes of these two effects of non-hemizygous Bt varieties to increase (by contaminating non-Bt plants) and decrease (by reducing contamination of Bt plants) resistance evolution may depend on the particular pest and PIP. However, available data from Burkness et al. (2011) for *H. zea* on Cry1A corn showed zero survival of susceptible larvae on Bt plants crossed with non-Bt pollen. In this case, the effect of producing non-hemizygous Bt varieties would not slow resistance evolution by ensuring that all kernels in Bt plants express Bt, because with current cultivars the survival is zero. In contrast, non-hemizygous Bt varieties would increase contamination of non-Bt plants by Bt pollen and speed resistance evolution. Thus, the net effect of a hypothetical non-hemizygous variety would likely be to increase the rate of resistance evolution.

Due to the possible negative effects of non-hemizygous Bt varieties on PIP durability, additional research as part of the integrated effort (such as, in combination with pollen incompatibility between Bt and non-Bt hybrids) would be required to explore the possible benefits of this strategy before this technology is pursued.

In addition, another Panel member also noted Thakare et al. (2017) reported successful results of utilizing RNAi technology to develop transgenic maize plants that produce aflatoxin-free maize kernels. This Panel member pointed out that host-induced gene silencing could be another viable strategy for developing Bt hybrids without kernel mosaics.

b.ii. The Panel discussed in detail the gametophyte factor genes (*Ga and ga*) which underscored the incompatible pollination between popcorn and field (dent) corn. Nelson (1994) reported 10 distinct gametophyte factor genes. Bloom and Holland (2012) reported in detail that gametophyte factor 1 gene (*ga1*) in field corn was not compatible with *Ga1-s* gene in popcorn; popcorn plants with the *Ga1-s* gene can only be pollinated by *Ga1-s* pollen. Additionally, according to an online news article in 2014 by CivilEats.com, Dr. Frank J. Kutka (North Dakota State University) was working on utilizing the *Ga2-ss* gene identified from teosinte - a corn relative - for organic corn production and preventing Bt pollen contamination (CivilEats.com 2014). The incompatibility between *Ga* and *ga* genes could be utilized to develop genetic incompatibility, and eliminate cross pollination between Bt and non-Bt plants.

A Panelist made additional comments on this approach and described how gametophytic incompatibility was commonly used as a genetic barrier to prevent pollen contamination in popcorn from field corn. Three of these include gametophyte factors 1 and 2 (*Ga1*, *Ga2*) and teosinte crossing barrier (*Tcb1).* It is scientifically possible to develop hybrids and to limit pollen contamination and reduce Bt/non-Bt mosaics. Information from a maize breeder who worked in this area suggested it was scientifically possible, especially with gene editing technologies. Such a system could have two mutually incompatible lines that probably would take 5-10 years to develop. One line could be PIP maize (Gametophytic factor 1a) it would only be able to fertilize plants that have the same genetic background with *Ga1* gene. The second line could be the non-Bt near isogenic maize line to be used as refuge (Gametophytic factor 2). Similarly, the refuge plants would only fertilize plants with the same genetic background, in this case, the non-Bt near isoline plants, with *Ga2* gene. If these factors were incorporated into multigene cassettes, the subsequent time to develop would be the same as any new genetically engineered trait. The incompatibility factor could be stacked with other genes of interest into a cassette. Again, for this system to work, it would require two separate cassettes, one for PIP product and the other for refuge maize. There was a concern, however, that for the 5% RIB product (or 1 refuge plant out of 20 corn plants in a field), there might be pollination issues. For example, if it were windy on days of pollination, low fertilization could have occurred. This could be compensated if refuge plants were selected to produce large amounts of pollen over an extended period of time. This Panelist suggested the system deserved consideration; however, it was questionable as to whether this, approach, would be practical.

b.iii. The Panel agreed in general that the review article entitled "Genetic and Genomic Toolbox of *Zea mays*" by Nannas and Dawe (2015) provided an inventory of exciting historical discoveries ranging from transposons, imprinting, and chromosome biology to recent advancement in molecular tools in maize research. These diverse approaches and tools described in the review may be attempted for developing selfing Bt or non-Bt inbred lines to prevent cross pollination, and mosaic kernels for Bt gene expression.

Economic Feasibility Evaluation

One Panelist discussed in detail and suggested corporate incentives for investing in a technological development to generate new profit centers and to protect existing profit centers. Profit expectations are essential to this plan including costs; cost of product multiplication/marketing and of working with regulatory authorities. This Panelist posed the following question: if a company were to assume that the three posed innovations that were technically feasible and decided to bring them to market, would the company have reasonably expected to earn sufficient revenue and recover all of its investment cost? That is, would the expected revenue cover not only post-technology development phase (or seed production) cost, but also the initial investment on technological innovations where the investment might see little return, should another company claim the intellectual property right of an innovative technology first. Given the importance of seed technology as a set of tools for managing insects, the major industry stakeholders that market Bt seed should be well-motivated to examine the merits of these technologies as a means of maintaining Bt susceptibility in natural insect pest populations. It is also possible that these technologies carry with them seed replication or regulatory costs, including challenges with seed purity and related matters (which are atypical for corn seed). Another factor for consideration is that the new corn seed products embodying the aforementioned technologies might be in demand throughout the world as a replacement of the existing products because the proposed technologies would lead to less damage caused by partially Bt resistant insect populations. Overall, given the general nature of the underlying biological mechanisms, seed companies might view investments in these technologies as providing opportunities for generating competitive advantage and protecting positions in seed markets.

This Panelist discussed the notion that industry would be interested in examining the aforementioned technologies and that it would be a different discussion as to whether or not these innovations would be available in time to protect/preserve Bt traits. In addition, the perceived need for these technologies to protect Bt traits will depend on prospects for exploring and developing new toxins. Since 1996, at least six Bt toxins have been registered with the U.S. EPA for corn and at least eight registrations were granted for cotton. A recent review by Tabashnik and Carrière (2017) cites work on developing toxins other than from Bt bacterium and on developing genetically modified Bt toxins that may preserve the technological approach beyond the failure of current Bt toxins. If commercially viable new toxins are imminent or prospects are propitious, commercial motives to develop alternative technologies to protect Bt susceptibility among insects will be diminished.

Alternative Approaches for Consideration:

The Panel understood that, in any large-scale seed development and production setting, the challenges of developing and integrating the above three new plausible technologies would demand significant long-term investments, as well as significant changes in the currently optimized seed development and production systems. Thus, a Panelist described alternative approaches in genetics and breeding (for long-term) and seed production (for short-term) that could be utilized to expand a seed manufacturer’s product portfolio to mitigate Bt resistance in lepidopteran pests. This includes maintaining the RIBs in corn crop production with improved efficiency in the southern U.S. states. The Panelist specifically discussed the main goal for us was to improve IRM efficacy by utilizing/integrating available genetic resources with multiple modes of action and diverse IPM tactics in seed development and production phases, respectively. The Panel recommended two available approaches to be considered for seed development and production as long- and short-term approaches, respectively, for management of Bt resistance in *H. zea*.

A) Manipulation of Crop Genetics to Mitigate Bt Resistance in Seed Development (Long-Term)

1) Diversify seed development tactics by integration of native insect resistant corn hybrid as non-Bt refuge choice. The selection of non-Bt hybrid in the RIBs can increase pest mortality with an additional mode of action that would not only extend /enhance Bt technology durability, but also eliminate the current concerns by growers relating to low yield and grain quality caused by non-Bt hybrids. In a similar manner, utilizing a hybrid with high level of native insect resistance for Bt transgenic hybrid development would also be a good strategy to increase insect mortality, and reduce Bt resistance in a pest population.

   There are many secondary metabolites in maize plants that can be utilized for this purpose, e.g., phytoallexins and silk maysin. High level of corn silk maysin (a flavone glycoside) had been examined extensively from 1970's to 1990's as a key phenotypic trait for *H. zea* resistance in corn production in southern states, because fresh silk is the main corn tissue that female moths prefer to oviposit, and corn silk is the only tissue that is critically important for neonate and early instars of *H. zea* larva survival and penetration into corn ears. The Panel agreed the Agency or industry stakeholders can exploit the combination of high level of silk maysin and Bt toxins in corn silks to significantly increase neonate and early instar larval mortality before they penetrate in corn ears feeding on developing corn cob or kernels. Laboratory bioassays demonstrated maysin can cause high *H. zea* mortality (Wiseman et al., 1985; Wiseman et al., 1992). However, a field screening by Ni et al. (2008) showed no negative inverse correlation between insect damage and silk maysin levels. The possible cause of the discrepancy between diet bioassay and field data might be the dynamic nature of silk maysin depletion after pollination. It was suggested that the use of molecular tools to further examine the possibility of manipulating the pathway(s) by not only increasing high maysin levels with maize p1 gene (Cocciolone et al., 2005), but also preventing maysin depletion, could be a valuable tactic for Bt resistance management in *H. zea* in the southern U.S. states.

2) Expand the transgene portfolio by utilizing diverse transgenes from other bacterial/fungal insect pathogens for new toxins/insecticidal metabolites in developing new PIP products.

   While seed manufacturing companies have admirably introduced a series of Bt transgenes in the PIP products in tandem in recent decades, further diversification of the existing transgene portfolio would be beneficial to reduce/eliminate Bt resistance (Tabashnik and Carrière, 2017). For example, *Streptomyces avermitillis* and *Saccharopolyspora spinosa* are soil bacteria that have been utilized for developing insecticides abamectin and spineotram, respectively. The OldWorld bollworm (*H.*

armigera) with over 1000-fold Bt toxin Cry1Ac resistance showed increased susceptibility for these two insecticides (Xiao et al., 2016), rather than cross resistance to insecticides reported in other pests (e.g., Bt resistance in Cry1F resistant fall armyworm from Puerto Rico showed cross resistance to organophosphate insecticide – acephate; Zhu et al., 2015). The Panel recommended that seed manufacturing companies examine Bt resistant *H. zea* populations for abamectin and spineotram susceptibility increase in comparison with the findings from *H. armigera*. Should the results from *H. zea* be consistent with the findings in *H. armigera* as reported by Xiao et al. (2016), this would fit perfectly for Bt resistance management in *H. zea*. The Panel suggested that exploiting Bt resistance with increased susceptibility to toxins/insecticidal metabolites from other bacterial or even fungal pathogens had great potential to be used as one of the innovative IRM tools.

B) Integration of New Tactics for Bt Resistance Mitigation in Seed Production (Short Term)

1) Adopt a blend of cytoplasmic male sterile (CMS) Bt hybrid with an unrelated non-Bt hybrid not only prevents cross pollination between Bt and non-Bt plants, but also leads to yield increase.

Munsch et al. (2010) reported a Plus-Hybrids system, which is a blend of a cytoplasmic male-sterile (CMS) hybrid and an unrelated male-fertile hybrid ensuring pollination of the whole stand with a ratio of 80:20, has led to a 10-20% yield increase in Europe, because of the xenia effect. The xenia effect, also known as allo-pollination, is defined as the direct effect of an unrelated pollinator hybrid on the developing kernel of pollen-receiving hybrid, which results in increased kernel weight and yield. The Panel recommended that seed manufacturing companies examine this approach, which has some potential in improving the current RIB technology as a sustainable IRM tool.

2) Provide seed treatment with endophytic entomo-pathogens to increase *H. zea* and other insect pest mortality on Bt and non-Bt plants.

Recent literature has demonstrated that establishing mutualistic plant-microbe relationship, and inparticular, using either endophytic or mycorrhizal fungi can increase crop plant tolerance to biotic and abiotic stresses in general. For example, seed treatment inoculant Myco-Gold® Corn Blend is comprised of five key ingredients: 1) an entomo-pathogen *Beauveria bassiana* to control insect pests; 2) mycorrhizal fungi for a better root system; 3) biostimulants in chelated form for accelerated plant growth; 4) *Azospirillum/ Azotobacter* bacteria for nitrogen fixation in non-legume plants; and 5) chelated micronutrients for boosting initial plant growth (Myco-Gold.com, 2018). Utilizing endophytic entomo-pathogens could be a short-term strategy for managing Bt resistant pest populations.

Charge Question 4. – *Bt Traits Expressed in Corn and Cotton*

A number of biological conditions known to lead to the rapid evolution of resistance in *H. zea* exist in the southern U.S., for example, climatic conditions permitting several generations per year and polyphagous feeding preference. *H. zea* has between 4 and 6 generations per year in these southern areas. The first two generations utilize wild hosts in spring, while the next two funnel preferentially through corn during summer. This is where population densities and the risk of resistance development are greatest. Generations 5 and 6 feed on cotton, soybean, sorghum, and other cultivated and natural hosts in late summer and early fall. Since selection in Bt corn drives resistance in *H. zea*, the IRM focus has been on actively mitigating resistance in generations 3 and 4 (on corn) with a higher non-Bt refuge than in the northern Corn Belt (20% for pyramids and 50% single traits).

In addition to the discussed biological conditions favoring resistance development, other risk factors such as shared Bt traits expressed in corn and cotton and cross-resistance exist. These likely will lead to continuous selection to all traits on up to 4 generations of *H. zea* in some areas of the south. There have been reports of *H. zea* field resistance to Cry1A and Cry2A toxin groups in recent years**.**

> **a.** Given the temporal and spatial cropping patterns in the southern U.S., describe options that would result in reduced selection pressure on Bt traits that are expressed both in corn and cotton. Please rank the options in order of effectiveness at mitigating resistance risk, and please explain the rationale behind the ranking.

Panel Response 4:

a. The Panel discussed how effective PIP traits and susceptible genes in pests to PIP traits should be considered as a "public good" of value to the public, producers and the environment. The Agency's stated goals for current FIFRA Panel members was provided in the issue paper as follows. "… to reduce resistance risks for lepidopteran pests, increase the longevity of currently functional *Bt* traits and future technologies, and improve the current insect resistance management program for lepidopteran pests of *Bt* corn and cotton" (Martinez et al., 2018). Consistent with these goals, effective IRM plans are imperative to improve the durability and increase the benefits of these technologies to society.

Jackson et al. (2008) identified two generations of *H. zea* in corn in the southern U.S. (generations 3 & 4) as the part of the annual cycle during which a high percentage of the individuals in a region are selected for resistance to *Bt* toxins. As *H. zea* populations emerge after going through corn, some are migratory and move north to infest and be selected for resistance in later planted corn (Hartstack et al., 1982; Lindgren, 1994; Westbrook, 2008). Some move only short distances (Fitt, 1989) where they may infest non-*Bt* host plants or infest cotton and would be once again selected for Bt resistance to virtually the same toxin groups as in corn (Von Kanel et al., 2016). Cross-resistance among some of the Cry toxins contributes to reduced effectiveness of the traits (Welch et al., 2015). In the cotton generation, parts of the population move onto non-cotton host plants (Harding, 1976; Knipling and Stadelbacher, 1983) which serve as non-*Bt* refuge and reduce selection pressure in these generations (Brèvault et al., 2011).

With regard to crop damage, *H. zea* is generally considered an occasional or minor pest of field corn (Buntin et al., 2004; Reisig, et al., 2015). Most of the damage is at the tip of the ears and losses are not typically high (Bohnenblust et al., 2013; Farias et al., 2014). Exceptions to the "low damage from *H. zea*" paradigm in field corn occur in occasional years when conditions are right for the growth of the fungi responsible for mycotoxins (Diener and Davis, 1977; Jones et al., 1981; Payne, 1992). Corn earworm damaged ears have been associated with increased aflatoxin levels (Ni et al., 2011). The aflatoxin threat is currently being successfully mitigated in high risk areas by grower application of atoxigenic strains of *Aspergillus flavus* to fields planted to field corn (Weaver et al., 2017). Fumonosin, produced by *Fusarium verticillioides*, is another mycotoxin in field corn grown in the south that has been associated with insect damaged kernels (Isakeit, 2006). Field corn is not typically scouted or sprayed for *H. zea* and yield losses have not been demonstrated in the southern U.S. (Reisig, 2015; Bibb et al., 2018).

In contrast to its pest status in corn, *H. zea* is quite damaging to cotton. Cotton fields are routinely scouted for the larvae and damage. Economic thresholds designed to prevent economic loss in a cotton field call for treatment to be made pre-bloom when eight larvae longer than ¼ inch per 100 plants are present, or post-bloom when fruit damage above 6% is found with the presence larvae (Vyavhare et al., 2018). In recent years, cotton farmers have increasingly oversprayed Bt cotton to control above threshold populations of *H. zea*. Oversprays occurred initially on Cry 1Ac single toxin cotton varieties (Luttrell et al., 2004), and have been applied in later years on pyramided Cry toxin cotton varieties (Little et al., 2017).

The Panel noted, the highest priority need for the Vip3A protein is in cotton where relatively low populations of *H. zea* cause significant losses and economic damage if not controlled (Masud et al., 1985). In contrast, *H. zea* in field corn is a minor to occasional pest where it is unusual for *H. zea* to be an economically important concern (Buntin and All, 2017), provided corn is planted at the appropriate time (Buntin, 2008).

Stalk borers are the target pests against which *Bt* toxins have been deployed in corn (Siebert et al., 2012; Hutchison, 2010). Stalk borers are high dose pests for several Cry toxins and little to no evidence of resistance to the toxins has been detected (Siegfried and Hellmich, 2012; Castro et al., 2004). Cry toxins continue to provide effective stalk borer control.

PIP technology can be considered a host plant resistance tactic within the larger suite of IPM tactics that provide suppression of *H. zea* and other pests in crops. However, availability of effective toxins (and their deployment in pyramided seed), and the presence of effective refuges are the most critical IRM components that we can manage to retard resistance development.

Panel members recommend that EPA make every effort to quickly assess and approve effective PIPs, especially those that are “high dose traits” against pests for which only “low dose traits” have been available up until now.

Panel members gave high priority to the maintaining and lengthening the effective life of insecticidal traits. Southern corn growers have failed to comply effectively with block refuge requirements (Martinez et al., 2018). A majority of the Panel members were convinced by literature that demonstrates blended refuge or RIB to be a flawed resistance management strategy for ear feeding caterpillars. Many kernels of ears on refuge plants in blended refuge fields are pollinated by pollen grains from *Bt* plants carrying *Bt* genes. Because some kernels are pollinated by pollen from non-*Bt* plants and some are pollinated by *Bt* plants, individual kernels may express none of the toxins or any of the possible combinations of toxins (e.g., one, two or three in three gene pyramids). The presence of kernels with full and compromised *Bt* expression in refuge ears negates the effectiveness of the refuge in blended refuge RIB fields. Studies of *H. zea* feeding on these “refuge” ears have documented negative effects on the growth, vitality and survival of “refuge” *H. zea* immatures and adults (Yang et al., 2014; Yang et al., 2015; Yang et al., 2017). Written comments from the public docket from southern entomologists and consultants did not communicate support for blended refuge in corn in the southern cotton zone. The Panel’s consensus was that blended refuge should not be a part of the refuge strategy for corn in the southern zone.

The Panel supported the concept of incentivizing growers to establish block non-*Bt* refuge crops. The consensus of the Panel is to support industry development and marketing of high yield non-*Bt* corn hybrids for use in refuges. The Panel suggested ideas for increasing block non-*Bt* refuge in corn could include blocks of land on large farms planted to non-*Bt* corn, cost-free or reduced cost refuge corn seed and improved marketing alternatives for non-*Bt* refuge corn. One Panel member suggested a whole farm approach to block refuge or on-farm block refuges, suggesting such an approach would be superior to blended RIB refuge. Additionally, the Panel supported planting and conservation of crop and non-crop natural refuge plants on non-crop land, in marginal areas, and in arable riparian areas on farms. Panel members also supported the concept of the United States Department of Agriculture/ Natural Resource Conservation Service (USDA/NRCS) or other agencies providing financial support for planting and conservation of crop or non-crop *Bt* refuge plantings. One Panel member suggested a system where dealers would provide farmers who purchased non-*Bt* corn with vouchers which would be required at the time Bt corn seed was purchased. The idea of community refuge among small farmers was discussed with panel support. One Panel member recommended crop rotation (*Bt* and non-*Bt* crops) to aid in compliance with block refuge requirements and that the communities be involved in discussions concerning refuge requirements to gain support for the concept. Members of the Panel recommended studies be conducted to better understand the reasons why many southern field corn growers have been non-compliant with structured refuge requirements. And, the Panel supported strengthened enforcement of requirements for structured refuge in field corn.

The Panel unanimously recommended prohibition of sales and use of field corn cultivars which produce Vip3A insecticidal proteins in the southern cotton zone. Corn plants with pyramided Cry proteins but lacking the Vip3A proteins provide control of the major corn pests. Under this strategy, field corn pyramids with Cry proteins alone will produce moths that have not been selected for Vip3A resistance. These moths will serve as part of the refuge for the Vip3A technology in cotton. This approach will break the previously described “bottleneck” selecting *H. zea* for resistance for the Vip3A toxin in corn. It will help protect Vip3A technology in southern cotton where it is a high priority need to control damaging populations of *H. zea* which have already developed field-evolved resistance to cry toxins (Luttrell et al., 2004; Ali et al., 2006; Ali and Luttrell, 2007; Luttrell et al., 2007; Tabashnik et al., 2008) and which occur annually in damaging numbers in cotton in many areas of the south. This strategy involves rotation of traits in time across *H. zea* host crops to deliver the highest priority benefits. It subjects as few generations of *H. zea* as possible to selection for resistance to the Vip3A toxin each year while maintaining the high priority benefits. This strategy has the best chance to deliver optimal economic benefits to producers over time. It has the best chance to extend the durability of the Vip3A protein with the least diminution of insect control in any crop. Not deploying the Vip3A toxin in southern field corn is not expected to affect the efficacy of corn pyramids against the primary pests of field corn in the south – the stalk borer complex. Modeling of this strategy for resistance durability including when new PIPs become available and economic impact was recommended by the Panel.

Corn plants with the MIR 162 event for Vip3A, express a “near high dose” against *H. zea*, and, when pyramided with Cry1Ab in corn it appears to provide the first high dose toxin against *H. zea* (Burkness et al., 2010). The Panel supported the concept of natural refuge in cotton which, along with pyramided Cry toxin corn, will serve as sufficient Vip3A refuge for cotton without additional refuge requirements. The Panel expected that this approach would increase the durability of the Vip3A toxin in cotton. The Panel suggested that *H. zea* selected for Cry toxin resistance in field corn that subsequently move to cotton will be killed in Vip3A cotton, potentially improving the durability of Cry toxins in both crops.

Some members of the Panel recommended combining the strategy to prohibit Vip3A plantings of field corn in the southern cotton zone with limiting refuge requirements for field corn expressing Cry toxins to levels sufficient to curtail resistance development in stalk borers and secondary pests. The Panel suggested that this concept be modeled to provide projections on its effectiveness in extending the durability of PIPs in corn and cotton. In addition, the Panel suggested that future requirements for registrations of PIP technologies include modeling for best fit of the PIP against the pest, for the crops in which PIP deployment is being considered, and to evaluate economic and IRM concerns.

The Panel suggested that single gene corn products should be removed from the market. However, some Panel members did not favor removal of “non-functional pyramided PIPs” that act as single events in corn from the market. A PIP may be non-functional against a low-dose pest such as *H. zea*, but a very important part of the pyramid against high dose pests such as borers in corn and tobacco budworm and pink bollworm in cotton.

The Panel suggested that use of carefully targeted and timed insecticide sprays with modes of action which are independent from those of PIPs be encouraged when pest populations exceed economic thresholds. Effective insecticides will protect PIP cotton without contributing to selection of pests for resistance to the PIP.

Members of the Panel suggested a monitoring network be developed to assay *H. zea* populations for resistance to PIPs in field corn. This effort would work in concert with educational efforts through state Extension services and commodity groups to inform growers about changes in resistant pests, and pest and resistance management practices. The Panel supported empowering growers and consultants to manage *H. zea* and resistance in *H. zea* (along with other lepidopteran pests) in corn and cotton by providing them with information through state Extension services and commodity groups.

The Panel encouraged the seed industry to continue working to develop refuge field corn hybrids that cannot be pollinated by pollen from surrounding *Bt* plants. Such self-pollinating non-*Bt* plants would be very valuable in RIB (blended refuge) resistance management. If this technology can be developed, it would eliminate many of the risks associated with blended refuge. This would make the concept more acceptable for application in the south against ear feeding lepidopteran pests.

One of the important components of IPM is post-harvest stalk destruction. The Panel supported actions to achieve elimination of post-harvest volunteer plants and crop residue destruction in corn and cotton to limit late season development of pest populations and PIP resistance, as a component of on-going IPM and IRM strategies.

Charge Question 5. – Resistance Management for *S. albicosta*

S. albicosta has historically been a secondary pest of corn but was added to the label of Herculex® (Cry1F) Bt corn products in 2003 as a sporadic pest (at best) suppressed by the toxin. As early as of 2010, damage in Bt corn was detected in the northeastern U.S. following the insect's range expansion across the northern Corn Belt. In recent years, resistance was suspected to have evolved or was confirmed in parts of the U.S. and Canada. A novel trait, Vip3A, provides good control and is currently the only effective Bt Plant Incorporated Protectant for this pest. As discussed in the issue paper, Vip3A is commercialized together with other traits as pyramids with a 5% refuge in the northern Corn Belt, although the other Bt traits in these pyramids have not shown activity against *S. albicosta*. EPA is considering the development of an IRM plan for *S. albicosta* because the Vip3A trait is at risk of resistance**.**

> **a.** Discuss how *S. albicosta* would fit into the current refuge-based IRM paradigm for Bt corn PIPs considering life history of the pest, crop management, agronomics in the northern Corn Belt, and limited Bt trait availability, monitoring, and IPM options. What additional factors should EPA consider for resistance management of *S. albicosta*?

Panel Response 5:

a. Consensus was reached by the Panel to prohibit the development ofan IRM plan for WBC. Such a plan would likely be burdensome for producers and registrants and would interfere with resistance management for other, more widespread primary pests, particularly European corn borer and corn rootworm. The Panel recognized that WBC is a significant pest in localized areas, especially areas with sandy soils that are commonly found in the Great Plains and Great Lakes areas. In these areas the Panel recommended locally-appropriate best management practices (BMP) should be developed that focus on PIP traits and IPM. The Panel noted the importance of Vip3A because it is the only highly effective Bt protein for the WBC. There were concerns by the Panel about resistance evolution to this trait, especially because of RIB (refuge) that could compromise assumptions of the high-dose/refuge IRM strategy. In this case, larvae could avoid high dose by moving among PIP and non-PIP plants and by feeding on a mosaic of PIP expressing tissues in corn ears. Due to these challenges several members of the Panel recommended EPA consider implementing a monitoring program for Vip3A resistance and an action/education plan for if/when Vip3A susceptibility shifts in problematic areas. The Panel also recognized that because of the recent range expansion, the pest status of the WBC is dynamic. If the WBC becomes a serious pest over a larger area, then the Panel recommended current guidance should be reevaluated. One member of the Panel noted that the problem of managing insect susceptibility is inherently dynamic in the sense that management actions now have implications for costs and benefits at later points. Thus, in addition to current benefits and costs, at least some attention should be paid to the probability that a problem will increase in scale.

The Panel developed the following issues and recommendations for the EPA to consider related to control of the WBC and managing resistance of this important pest.

The Panel decided there was convincing evidence that WBC has evolved resistance to Cry1F in many areas of the Corn Belt. Currently the only efficacious Bt trait is Vip3A. This is problematic because Vip3A is planted with a 5% refuge and is only available in pyramided varieties. Thus, there is high selection pressure for WBC resistance because available pyramids are functionally equivalent to a single trait product. Regarding the biology of the WBC, one positive attribute is that there is only one generation per year. Negative attributes include: overwintering throughout much of Corn Belt (especially areas with sandy soils), common movement between plants, and ear feeding (at least part of larval feeding occurs in the ear). One Panelist noted that early instars often are found near leaf-enclosed tassels but some subsequently move to and feed on ear tissues. In general, the plant-to-plant movement of the WBC larvae and their within plant movement are problematic for mixed PIP and non-PIP refuge scenarios because of the well-documented threat to high-dose with seed mixtures (larval movement and mosaic toxin expression in ears). Thus, the Panel determined there is good reason for the EPA to be concerned about possible resistance development to Vip3A by WBC.

The recent documented history of WBC provided the Panel with insights into addressing the questions. Over the past 15 years the WBC has had a range expansion from the Great Plains and Western Corn Belt regions across the northern Corn Belt, including the Great Lakes region and southern Ontario, then finally to northeast U.S. and Maryland. There were also reports of WBC in northeastern Mexico (near Saltillo) (Sánchez-Peña et al., 2016**)**. Reasons for this range expansion is not known but could include reduced tillage leading to greater overwintering survival, reduced chemical pesticide use with expanding Bt corn adoption, the pest's propensity for long-distance dispersal, reduction of other ear-feeding lepidopteran competitors by Bt traits, and possibly climate change facilitating the expansion (Hutchison et al., 2011). In its native range WBC was controlled with IPM approaches that included scouting (Paula-Moraes et al., 2011) and well-timed insecticide application when economic thresholds (eggs) were reached (Paula-Moraes et al., 2013). Larval survival is a critical element in the WBC colonization success of corn. A four-year study in Nebraska showed the WBC larval colonization success is variable, ranging from 4-20%, depending on regional conditions (Paula-Moraes et al., 2013). Growers in the WBC's native range in Nebraska are currently facing challenges, where IPM practices, such as Bt traits (Cry1F) and scouting plus the use of insecticides, are not as effective as they were prior to 2013 (Archibald et al., 2017). After commercialization of the Cry1F trait in 2001, WBC was added to the trait's label in 2003. Some growers in areas with WBC problems integrated Cry1F corn with IPM. Most of these problematic areas had sandy soils, which facilitates overwintering of WBC pre-pupae. Likewise, the Great Lakes region has many areas with sandy soils, which is problematic because high overwintering survival in these sandy-soil areas promotes high WBC populations that commonly exceed economic thresholds. Consequently, many growers in the Great Lakes region and southern Ontario have been challenged to control this new pest (Smith et al., 2018).

The Panel noted EPA's statement "Cry1F has never fully controlled *S. albicosta* but was added to the label in 2003. EPA concluded at the time of review that Cry1F at best suppressed the pest (US EPA, 2003). Since *S. albicosta* has historically not been considered a major pest of Bt corn but a sporadic pest, registrants may not have developed Bt toxins with this pest in mind. This would explain why there are so few Bt tools available today" (EPA issue paper, page 61).

Throughout most of the Corn Belt, the WBC is generally classified as a secondary pest (occasional or localized economic significance) in field corn but can cause EIL that leads to yield loss and reduced grain quality. A fourteen-year light trap study in western Nebraska indicated six to eight years between high populations, indicating a cyclical population fluctuation (Hagen, 1976). Larval survival is a critical element in the successful colonization of field corn field by WBC and has been documented to be variable depending on regional conditions (four-year study in NE indicate a pest survival range from 4 to 20 %) (Paula-Moraes et al., 2013). One Panel member argued for classifying the WBC as a primary pest for the western Great Plains and the Great Lakes regions. Most of the severe injury occurs in areas with sandy soils but there are outbreak years where high populations occur outside these sandy-soil areas. The Panel noted that from an IRM perspective there is a problem, because for any corn planting generally only one IRM strategy is employed. One plant may have multiple pests, but deploying appropriate IRM strategies that are effective against all pests is challenging, or not possible. So throughout much of the Corn Belt, use of pyramided corn hybrids with two or more traits against pests is effective against European corn borer and corn rootworm, but inadequate for other pests such as WBC where Vip3A is the only trait and the IRM plan is not focused on this pest. As the EPA has pointed out, there are many uncertainties and challenges in these situations.

A Panel member recommended that better or more defined terms are needed when assessing whether to implement new IRM regulations on pests considered as "secondary pests," such as *S. albicosta*. Industry representatives made strong recommendations that IRM is not necessary for secondary pests, and that changes to current regulations could threaten to disrupt strategies currently in place for maintaining Bt traits against "primary pests." Although this may be true for some pests, another Panelist provided a word of caution that should be considered when characterizing pests as "primary" and "secondary" pests based on historical data. An example of this was provided by this Panelist where the pink bollworm has long been considered a pest of secondary importance in India, but its pest status has changed considerably now based on its level of management. The pink bollworm formerly was considered a primary pest of cotton in the southwestern U.S. before the eradication program, but it still is considered a major global pest. Pink bollworm is considered a "secondary pest" in most cotton growing areas of Asia (cotton bollworm, *Helicoverpa armigera* is considered the primary threat). However today, after the widespread evolution of resistance to both single- and dual-toxin Bt cotton in India, the pink bollworm has gained status as a severe "primary" pest of cotton there. Hence, EPA should consider that the pest status of insects can change and should not necessarily be based primarily on the "historical" context of the pest but rather on current activities in the field.

The Panel considered two general approaches for addressing the WBC issue. First, determine whether it is warranted to develop an IRM plan for WBC. Second, utilize the BMPs (Bt traits one tool of many in the tool box) to address WBC issues.

As stated in the Agency's issue paper "EPA is considering whether western bean cutworm should be viewed as a primary pest of corn and if a Bt corn IRM plan is warranted for the insect. Such a plan should consist of the following post-registration requirements: monitoring for and mitigating resistance (insect collections, assay development, and mitigation strategies) and grower education." EPA also noted that "IPM with IRM is another option that would delay resistance development in *S. albicosta*."

EPA recognized the following uncertainties:

1) "Since western bean cutworm feeds on maternal corn tissue first as a young instar larva, it should, therefore, always be exposed to Bt expressed in maternal tissue after hatching. Therefore, it could be argued that selection occurs early and on the leaf tissue rather than the kernels and that the potential for sub-lethal expression in a mosaic environment may not be relevant. Seed blends would then not pose an additional resistance risk to western bean cutworm." (One Panel member pointed out that WBC does not feed on the leaves as an early instar, except if that is the only tissue available, but the mortality is very high compared to when tassels are available. Tassels are the preferred tissue for early instars.)
2) "If western bean cutworm is elevated to a primary pest of corn, then refuge proportion may need to be reconsidered for pyramided Bt products that express only one functional toxin for the control of this insect. Modeling analyses may be necessary to assist with this deliberation/decision." Two Panelists pointed out an additional IRM risk to include in the modeling analysis is when early instars feed on a non-Bt tassel and then move to adjacent plants. This is in addition to larvae moving down to the ears where they might be subject to selection pressure from the mosaic expression of toxin in ears.

The Panel considered whether an IRM plan should be developed for WBC and developed the following list of positive and negative points.

Positives:

1) Growers from Great Plains and Great Lakes regions where the WBC is an important pest would gain major benefits from prolonged use of Vip3A and future genetically engineered traits effective against the WBC.
2) Increased education (part of IRM plan) increases awareness of WBC status as a pest.

Negatives:

1) Mismatched IRM plans with other pests could compromise existing IRM plans for European corn borer and western corn rootworm (e.g., 20% refuge requirement could make seed blends untenable, reversion back to block refuge could increase grower noncompliance). In this case, effective IRM plans for primary pests that historically occur throughout the Corn Belt could be compromised.
2) Growers from most of the Corn Belt will gain no or limited benefits.
3) Burden for industry to develop an IRM plan for this pest because of "post-registration requirements: monitoring for and mitigating resistance (insect collections, assay development, and mitigation strategies) and grower education." However, two Panel members pointed out that the best approach in WBC problematic areas is to pursue both adoption of IPM and increase education targeting crop consultants and growers.

Written public comments related to a BMP strategy for WBC from several industry registrants indicated that Bt traits are considered one tool in a tool box of control methods. One Panel member was concerned that this tool box is very small and is continually shrinking due to insecticide resistance and the possibility of Vip3A resistance. Consequently, sustainable management of WBC may be compromised, especially in the Great Plains and Great Lakes areas.

The Panel also considered whether BMP should be encouraged for WBC and developed the following list of positive and negative points.

Positives:

1) Scouting methods for IPM control of WBC are available (e.g., see scouting recommendations and economic thresholds developed by the University of Nebraska https://cropwatch.unl.edu/2017/scouting-and-treatment-recommendations-western-bean-cutworm, and speed-scouting mobile app https://itunes.apple.com/us/app/western-bean-cutworm-speed/id543341625?mt=8).
2) In this case, Bt traits are considered one tool in the tool box and recognizes IPM as another important tool.
3) BMP could be tailored to regions and different pest pressures.
4) In general, EPA recognizes importance of a dual IRM and IPM approach to resistance management for other corn pests. IRM with IPM is an emerging strategy for many corn pests.

Negatives:

1) IPM would encourage use of chemical insecticides.
2) Possible resistance issues with chemical insecticides.
3) Not known whether Great Lakes growers will easily adapt to BMP procedures.
4) Vip3A trait could be compromised.

The following excerpts from Industry comments focused on BMP were noted by the Panel:

Corteva Agriscience™: "Growers need practical and economically favorable programs that are not limited to any specific pest management tactic, but rather protect the entire sustainable pest management system. Best management practices call for improved agronomic practices, planting of pyramided Bt trait products, and scouting for economic injury that can trigger insecticide applications or other management practices."

More specifically related to this charge question:

Corteva Agriscience™: "For secondary pests such as *S. albicosta*, and for those against which Bt traits do not provide complete protection, Corteva Agriscience™ supports the development of locally-appropriate best management practices that maximize the benefits of Bt traits against the entire pest complex in the area."

Syngenta: "economic damage during the past decade are either sparse, or entirely absent throughout most Corn Belt states. This absence of broad geographical establishment and the lack of annual economic damage solidifies the status of western bean cutworm as an occasional or secondary pest of field corn, which is best managed through an IPM approach."

Also, EPA noted in its issue paper (page 30): "IPM may need to be an integral part of managing this pest in Bt due to lack of availability of different traits (e.g., monitoring for adult densities, increased scouting for eggs in Bt corn and use of conventional insecticides) (Smith et al. 2017)."

The Panel considered that a BMP approach could follow IPM practices adopted by growers in the native range of WBC. Below is a possible approach that is practical and reasonable based on Syngenta's input and modified by Panel members.

In Non-Bt Corn (and Bt hybrids without Vip3A)

1) Use degree-day model to inform timing of pheromone trapping and scouting (Hanson et al., 2015).
2) Monitor adult flights with pheromone traps.
3) Scout corn for egg masses at plant stages V16—VT when traps indicate moth flight, typically during early-July to mid-August.
4) Use economic thresholds (typically 5-8% of plants with egg masses, see also Paula-Moraes et al., 2013).
5) Apply a foliar insecticide with residual activity when economic threshold is reached, before a majority of eggs have hatched, and before larvae have entered the silks and ears.

In Bt Corn (Vip3A hybrids)

1. Monitor fields for performance and unexpected damage during August through September.

The Panel noted the following uncertainties:

1) It is not known if Cry1F resistance occurred once and spread across the country or if resistance has occurred in multiple locations. This is relevant for the Vip3A trait and future PIP traits.
2) Some growers choose a prophylactic approach to some pests/pathogens rather than IPM. These growers may spray pesticides even when they are not warranted. Such an approach should be discouraged. It is unknown how recommendations made by the Panel would influence this unwanted tactic.
3) If 5% blended refuge strategy is deployed, it is not clear whether field monitoring for performance and unexpected damage by WBC would occur.
4) Finally, if a 5% blended refuge strategy is deployed, it is not clear how this will impact ear damage that could lead to fungal contamination and challenges with mycotoxins, especially Deoxynivalenol (DON). Related to this, one Panelist noted availability of Vip3A hybrids in a structured refuge for those regions with both heavy WBC pressure and risk of mycotoxins (e.g., the Great Lakes) would allow growers to separately harvest Bt and non-Bt blocks to reduce mycotoxin contamination in the harvested grain.

Charge Question 6. – *Mitigation of Resistance*

EPA's regulatory goals for mitigation of Bt resistance are to limit or extirpate (if possible) the resistant population in the site(s) of concern and maintain the durability of the affected traits in areas where they are still effective. Depending on the life history, ecology, and population dynamics of the organism and timing of mitigation, extirpation may be an option if resistance is localized; failing that, it may be possible to manage pest densities to maintain Bt PIP efficacy.

To develop effective mitigation strategies, knowledge of some critical pest parameters is required. One main focus should be on the spatial scale of a randomly mating population, the typical fraction of the population dispersing beyond such a spatial scale, and the typical distance traveled by Noctuid moths. The first parameter should aid in delineating a minimum size of MAA to address local resistance. The other two factors provide information related to percent of resistance genes escaping from a resistant population and how rapidly resistance could establish in other areas.

a. Please discuss the delineation of a Mitigation Action Area for resistant Noctuid populations considering:

i. Typical dispersal propensity as well as the fraction of a population that could engage in longer distance dispersal; and

ii. Other characteristics essential to determine such an MAA and limit the spread of resistance genes.

b. Please discuss population management approaches for cases of widespread field-resistance.

Panel Response 6:

ai. The Panel concurred with data from literature reporting typical dispersal propensity of *H. zea*. Short-range movement or crop-to-crop dispersion is variable and can range from 10 m to 10 km (CABI, 2013). Observations from airborne radar indicated long-range movement, reporting migration of *H. zea* in a single night at altitudes up to 900 m. Migrating moths from south Texas were reported departing early evening and moving around 400 km per night (Westbrook and Lopez, 2010). Migratory trips of at least 750 km can occur based on pollen found on moths of *H. zea* in Arkansas (Hendrix et al., 1987). In the Caribbean, *H. zea* moths moved over open water 55-65 km in a single night (Haile et al., 1975). Return southward migration of *H. zea* has also been detected by radar, providing potential additional sources of genetic variability (Westbrook and Lopez, 2010). However, more information is needed to establish the frequency and patterns of migration as well as the environmental and physiological cues that trigger this return migration. The Panel agreed that additional studies are critical to better understand the genetic structure and proportion of migratory moths in populations of *H. zea* that could engage in longer distance dispersal. Variation of allozyme frequencies, over three years, in populations of *H. zea* from Mississippi were tested and indicated gene flow in local and regional scales (Han and Caprio, 2002). Analysis of the microsatellite DNA variation in samples of *H. zea* collected from the states of Alabama, Louisiana, Mississippi and Texas and ranging from 150 to 900 km apart, did not identify population differentiation by geographical distance (Perera et al., 2011). Although this study, based on a single collection from each location, could not accurately predict temporal variation in genetic composition of the populations, a later study assessing the extent of genetic structure attributed to migratory populations of *H. zea*, during different seasons, indicated a high genetic diversity within migrant populations and low genetic differentiation among migrant populations with no genetic structure in populations within or between seasons (Seymour et al., 2016). *H. zea* is a facultative seasonal nocturnal migrant, with no delay in egg maturation after adult emergence (Fitt, 1989) and no commitment from moths to migrate at a specific time. Moreover, most long-range migration of *H. zea* is nocturnal (Westbrook and Lopez, 2010), therefore mating and oviposition are incompatible with migratory behavior.

aii. The Panel acknowledged that there are many factors that must be considered when determining MAAs, particularly for the highly mobile noctuid moths. The Panel recognized that facultative seasonal migration of *H. zea* is likely a response to deteriorating environmental conditions and/or weather conditions. The spatial and temporal dynamics of host plants could offer cues to determine the temporal frequency of adult movement in the landscape. Oviposition on host plants by *H. zea* is complex, and often coincides with the peak of flowering and nectar production. *H. zea* is a polyphagous species and host plants from the botanic families of Poaceae, Malvaceae, Fabaceae and Solanaceae are listed as its primary hosts (e.g. CABI, 2013). Assessments of *H. zea* populations in the southern U.S. have documented the existence of non-cotton crop hosts supporting generations of *H. zea* (e.g. Stadelbacher, 1981; Gould et al., 2002; Jackson et al., 2007; Head et al., 2010). In general, moth migration is initiated when the natal host becomes unable to support the next generation.

Overwintering survival also could affect genetic population structure in a specific region. Pupal diapause during the winter can be a carry-over of resistance alleles between seasons. Depending on the severity of the winter, *H. zea* can survive at about 40 degrees north latitude (Capinera, 2017). According to Morey et al. (2012), the amount of time in which *H. zea* pupae are exposed to near-zero temperatures is more important for overwinter survival than if "extreme" low temperatures are reached within a specific region. Although near-zero temperatures occur in the southern U.S., they often vary from region to region and between years. The density dependent mortality factors during different life stages likely also influence area wide population dynamics in pests, such as *H. zea*.

Finally, the impact of cross-resistance to Bt traits should be considered when determining the MAA and limiting the spread of resistance genes in a specific region. The registered Bt-corn toxins include Cry1Ab, Cry1F, Cry1A.105, Cry2Ab2, and Vip3Aa20. The registered Bt cotton toxins include Cry1Ab, Cry1Ac, Cry1F, Cry2Ab2, Cry2Ae, and Vip3Aa19. With the exception of Cry1Ac and Cry2Ae, all toxins expressed in Bt cotton are also expressed in Bt corn products. Based on documented cross-resistance between some of the toxins, all toxins are expressed in both Bt crops (topic also discussed in charge question 4).

b. The Panel agreed that there are two overriding approaches when selecting tactics for mitigation of resistance within an IRM plan. A proactive approach is one in which the objective is to prevent or delay the evolution of PIP resistance of a target species and subsequent field failure. Whereas proactive approaches aim to preserve the efficacy of technology as long as possible, reactive approaches only attempts to mitigate resistance after it has occurred and avoid the spread of the problem. The Panel agreed that proactive approaches are preferred and likely necessary for the highly mobile pests such as *H. zea* and *S. frugiperda*. Reactive management of such resistant pest populations will likely be very difficult to mitigate/contain. The Panel agreed that management of widespread field-evolved resistant populations should be the last alternative for an IRM program, and the feasibility of success of mitigation tactics are dependent on factors such as regional vs. local outbreak, detection and response timing, fitness costs associated with resistance, and others. Fitness costs may influence life history traits of *H. zea*, such as larval and pupal survivorship, life stage duration, and adult reproductive rate. The absence of fitness costs may result in stable field populations of resistant insects and compromises the management approach to decrease resistance selection pressure (Gassmann et al., 2009; Bernardi et al., 2015).

The Panel recognized however, that adoption of IPM tactics to reduce pest populations in Bt-crops could contribute to a mitigation plan to reduce selection for resistance. A study with *Chloridea virescens* recommended a delimitation of 15 km for an area-wide management during the crop season (Schneider et al., 1989). The implementation of a mitigation plan for resistance in *D. grandiosella* to Cry1F was presented in the EPA issue paper as a successful example. Area-wide IPM tactics were recommended with communication among stakeholders and intensification of resistance monitoring. The Panel noted that resistance to Cry1F in SWCB was in a relatively isolated area (Cochise Valley of Arizona), where sub-freezing temperatures and natural enemies (Popham et al., 1991) impact the distribution of this species.

Charge Question 7. –*Grower Non-Compliance with Refuges in the Southern U.S.*

Historically, compliance with structured refuges has been low in the southern corn growing regions relative to other corn production areas. Despite the disparate refuge compliance in the northern and southern U.S., southern growers have consistently acknowledged in anonymous surveys that they are aware of IRM requirements and have sufficient information to comply with IRM requirements. This suggests that non-compliance is likely due to multiple factors, such as economics, agronomics, pest diversity and pest pressure, etc.

a. Discuss and identify (1) the agronomic and economic dynamics and human factors contributing to this relatively high level of non-compliance in the southern U.S., and (2) recommend approaches that could incentivize southern corn growers to improve refuge compliance.

Panel Response 7:

a. EPA concluded in its review of The Agricultural Biotechnology Stewardship Technical Committee's or ABSTC's 2014-2016 Compliance Assurance Program Reports (Martinez and Reynolds, 2017) that refuge compliance in the southern U.S. is lower than desired and far lower than in the northern Corn Belt. Further, compliance in the South has decreased over the past several years. It attributed the lack of compliance relative to the north as due to:

> *"1) seed blend adoption in that region; 2) the mandate for a greater refuge requirement in the south for single trait and pyramided products (50% and 20%, respectively) compared to the northern Corn Belt; 3) lack of desired hybrids in refuge products (Reisig, 2017); 4) smaller field sizes; 5) more diverse cropping systems; and 6) greater complexity of operations."*

In addition, research and experience with successful pink bollworm control and high compliance rates in Arizona suggests that compliance is obtained when there is a strong, organized grower response to manage the pest and delay Bt resistance (e.g. Tabashnik et al., 2010). That is, a strong norm exists for cooperating toward a common goal of delaying resistance to Bt cotton.

In the southern U.S., Reisig (2017) found that owners of smaller farms were less likely to plant a refuge. This could be due to different labor and management constraints on smaller farms than larger farms, as well as the fact that larger growers might derive more direct (personal) benefit from planting a refuge so are more likely to do so (Brown, 2018). That is, the pool of susceptible insects is less a public good and more a private good for larger acreages than smaller acreages. Operators of smaller farms might be less likely to believe their non-compliance contributes negatively to the common pool of susceptible insects due to their small size; and, it might be more difficult to economically justify planting a refuge on their farms. Additionally, there are anecdotal reports from Extension personnel that the quality and diversity of refuge (non-Bt) seed is lower than for Bt seed. If this is the case, that is another disincentive to plant non-Bt seed.

Also, Hutchison et al. (2010) found that once adoption rates surpassed 40% (as they did in northern states), most economic benefits accrued from planting non-Bt crops due to area-wide pest suppression combined with the fact that non-Bt corn seed is less expensive than Bt corn seed. Therefore, under these conditions, northern growers could plant a refuge and save money, providing an incentive to plant a refuge not present in the southern states. Hutchison et al. (2010) and Tabashnik et al. (2010) also argue that growers' cooperation in maintaining refuges in areas where Bt crops are grown are critical for realizing economic and agronomic benefits to all farmers in the region.

In other work, Hurley et al. (2005) found that in Minnesota and Wisconsin, corn farmers were more likely to comply with refuge requirements if they farmed larger acreages and were aware of the requirements. The three reasons provided most often by farmers why farmers did not comply with refuge IRM requirements were: farmers use neighbors' fields as refuges (natural refuges); it is inconvenient to change planters in order to plant non-Bt as well as Bt seed; and IRM requirements are not considered sufficiently important.

Therefore, in the more heterogeneous southern states, the greater variation in farm size, cropping systems, pest pressure, complexity of operations and other factors, made it less likely that the social norms for IRM would emerge (Brown, 2018; Reisig, unpublished data). However, it is well understood by the Panel that southern farmers have cooperated in the past on projects where the threat was accepted as real by the vast majority of actors and a common interest was recognized, such as boll weevil eradication. Thus, corn growers may not deem corn earworm enough of a threat to justify the trouble, cost and yield loss associated with planting block refuges; or they believe a new technology will be introduced that forestalls the need to plant block refuges.

The difference in outcome between the southern U.S. and other regions dovetails with the findings of Elinor Ostrom (e.g. 1990). Her empirical work on the management of common pool resources suggests that the chances of effective cooperation towards community-driven management of these resources are increased in the presence of: (i) a small (in terms of number of actors), relatively homogeneous group with common interests; and (ii) established social norms, reciprocity and trust within the group.

It is critical, therefore, to establishing such norms and conditions for cooperation in order to address non-compliance. In this vein, Brown (2018) finds a social marketing program by Monsanto was particularly effective at getting growers to use any refuge, but had no statistical significant effect on compliance with regulatory mandates.

Based on the extant literature, the Agency recommended interventions for EPA to explore to increase compliance in the southern U.S. including:

- Tailored marketing programs for smaller and larger farms. That is, small farms, in total, control a substantial amount of acreage and their compliance is necessary to realize beneficial outcomes. And, as acreage of a farm increases, the benefits of delayed resistance become more direct. These facts could inform future IRM marketing initiatives.
- Listening sessions or focus groups led by Extension to dialogue with growers about the need for establishing and maintaining refuges, and the constraints growers face to do so.
- Fees on Bt seed, discounts on refuge seed or subsidies for planting refuge (Ambec and Desquilbet, 2012). This approach would require quantitative evaluation of product quality and variety of Bt versus non-Bt traits in privately collected seed sales data on corn hybrids across the U.S. This data could be used in econometric modeling to compute how large the relative price discount or subsidy for refuge seed would have to be in order to achieve different refuge targets (Ciliberto et al., 2017).
- Investigate measures within the U.S. crop insurance program, or other agricultural subsidies, to compel compliance. Recent efforts to incorporate cover crop incentives into crop insurance (O'Connor, 2013) might be a model. In the case of refuges, that would require EPA coordinating with USDA, specifically the Risk Management Agency.
- Implement a 'refuge in time' policy, e.g. withhold Vip3A from corn in the south.
- And, finally Brown (2018) asserts that improved compliance among growers may be achieved by:

  "…inducing growers to adopt technology that reduces variable costs of planting refuge. This could include adoption of multi-hybrid corn planters, which would reduce growers' variable costs of planting multiple corn varieties with different soil and sowing density optima, as well as greater use of "precision ag" services, for example in calculating efficient refuge configurations."

The Panel believed compliance with IRM requirements is in part a function of perceived probability of receiving a sanction, and the severity of any sanction from non-compliance (Hurley and Mitchell, 2014). Problematic in this regard is the reliance on a consortium of seed firms (ABSTC) to monitor regulatory compliance and sanction growers. The regulatory enforcement regime is indirect (Brown, 2018) and the sanction is denial of Bt seeds to the grower, thus resulting in a loss of sales to the seed firm. According to the EPA's review of ABSTC data from 2014-2016 Compliance Assurance Program (CAP) Reports for Bt Corn Products Targeting Corn Borer and Corn Rootworm (2017):

> "*the number of growers who lost access to the block refuge Bt technology has remained relatively low and unchanged over the three years discussed in this document.* ***This might*** *reflect ABSTC's success in bringing the growers back into compliance (*emphasis added)."

This finding raises the question of whether the Agency knows the extent to which non-compliant growers are made compliant, or merely assumes that any farmers who were sold seed are either in compliance or in the compliance grace period. For example, turning to the Agency's interpretation of ABSTC data from 2014-2016, Table 3: *On-Farm Survey, Significantly Non-Compliant Growers, and Loss of Access to Bt Technology,* the EPA indicates that:

> *"In 2014, 372 growers who were out of compliance in 2013 were re-assessed. Of these, 224 were significantly non-compliant in 2013, and nine were non-compliant once more in 2014. These growers lost access to the Bt technology requiring a block refuge"*

The implications for compliance and technology access are unclear from this data. Does this mean that: i) 215 growers came into compliance in a relatively brief time, and therefore were not denied access to the technology; or, ii) that only 9 of the non-compliant growers sought to purchase the technology and the remainder either did not wish to buy the technology or went to a different vendor to avoid the sanction of losing access? Without reliable data on the enforcement and regulatory outcomes from non-compliance with refuge requirements, it is difficult to structure an effective response to non-compliance. The Agency is encouraged to require more detail on reporting of regulatory outcomes (or, if they have it, provide more of this data to the Advisory Panel in preparation for the meeting), especially denial of seed sales to non-compliant growers.

Charge Question 8. – *New IRM Framework for Lepidopteran Pests of Bt*

Based on scientific concerns for risks of resistance development in non-high dose pests of Bt corn and cotton, EPA is considering changes to the current IRM program for lepidopteran pests to decrease the selection intensity and improve the resistance monitoring and mitigation approaches. Some of the available options to address the current limitations are:

- Limiting or otherwise managing the use of single trait corn products;
- Managing the use of:
  - Bt corn RIB in the southern U.S.; and
  - Non-functional pyramids;
- Developing
  - Grower incentive programs to increase compliance with refuge planting;
  - Molecular assays to test in-field insect samples and expedite resistance confirmation; and
  - An agreed-upon Mitigation Action Area;
- Use of sentinel plots to monitor for resistance;
- Use of an early resistance threshold for resistance followed by early intervening mitigation actions based on IPM;
- Implementation of standardized UXI levels in corn and cotton;
- Implementation of immediate mitigation actions in response to UXI fields relying on an appropriate MAA and before resistance is confirmed; and
- Implementation of grower incentive programs to improve refuge compliance in the southern U.S.

a. Discuss the advantages and limitations of the stewardship options discussed in the Agency issue paper and any additional options not identified by EPA that could be used for proactive resistance management of non-high dose pests of Bt corn and Bt cotton; and

b. Discuss the potential strategies to improve durability of Bt pyramids and functionally single trait products (i.e., pyramids with resistance to one or more toxin) targeting lepidopteran pests in regions at high risk of resistance, which could include areas with low refuge compliance, high trait adoption, heavy pest pressure, etc.

Panel Response 8:

a. The Panel endorsed broad-based, multi-tactic, biologically sound IPM principles as the basis for stewardship and the foundation on which IRM strategies are built for both non-high dose and high dose pest of Bt corn and cotton. General consensus was reached for strategies that mitigate resistance development versus strategies that manage resistance after development. This was deemed especially important in non-high dose and highly mobile pests such as *H. zea*. In consensus, the Panel supported the concept that the most effective strategies for slowing/suppressing the development of resistance among non-high-dose pests are PIP pyramids and refuge strategies. A consensus of the Panel supported expedited EPA review of novel PIP technologies and, if appropriate, prompt labeling of safe, effective new PIP technologies. The Panel agreed that deploying new PIPs in pyramids with previously approved PIPs would result in increased durability of individual PIPs. There was consensus that this action would provide farmers with the products they need to protect their crops.

While the Panel recognized blended refuge in field corn in the south has the advantage of higher compliance than a block refuge, none of the Panelists voiced support for the scientific merits of blended refuges to increase the durability of PIP toxins. The EPA issue paper provided the overview, scientific status and reasons for the organization of this Scientific Advisory Panel. It states EPA's overall goals are "to reduce resistance risks for lepidopteran pests, increase the longevity of currently functional Bt traits and future technologies and improve the current resistance management program for lepidopteran pests of corn and cotton." The available scientific studies, the consensus of public docket responses from southern field entomologists and the consensus of the Panel did not support deployment of blended refuge – RIB – for toxins targeting ear-feeding pests in southern field corn. The consensus of the Panel for a lack of support of deployment of RIB in the South included: cross-pollination and segregation of PIP toxins in individual kernels of "refuge" ears and their effects on *H. zea* survival and selection for resistance in the refuge (Yang et al., 2014; Yang et al., 2015; Yang et al., 2017). The consensus of the Panel was that deployment of RIB against ear-feeding pests in field corn in the south was not consistent with EPAs stated goals.

Southern growers of field corn have been non-compliant with structured refuge requirements to increase the durability of PIPs deployed against ear-feeding pests (US EPA, 2018). Additionally, researchers, the FIFRA SAP Panel and southern IPM practitioners lack confidence in the effectiveness of the blended refuge approach to providing refuge. Therefore, the traditional means of providing refuge that is essential to maintaining the durability of PIPs – specifically the Vip toxin – currently fall short of meeting the EPA goals stated in the issue paper. For that reason, the Panel was unanimous in its support for a prohibition on the use of Vip field corn in the southern zone (discussed thoroughly in charge question 4). The consensus of the Panel was that the proposed strategy of trait rotation in time and among crops offers southern farmers the best chance of meeting EPA's goals – reducing the risk of resistance and extending PIP durability – while providing southern corn and cotton farmers with traits that provide crop protection and enable them to produce crops profitably.

b. The Panel supported IRM approaches that are consistent with IPM principles in mutually reinforcing ways. Specifically, steps to delay and suppress resistance development should be the primary focus of IRM. An important, but secondary, focus should be on detection and mitigation of resistance after it has been detected. Currently available monitoring techniques rarely allow time for mitigation actions before highly migratory non-high-dose pests such as *H. zea* and *S. frugiperda* can complete their development and move out of the area.

Members of the Panel cautioned EPA to consider all pests – both those considered high- and non-high-dose – in the discussion of "functionally single trait" pyramids. While a trait may be non-functional against a non-high-dose pest, it may be providing valuable suppression of resistance in high dose pests and should continue to be used in pyramids for that reason. There was consensus among the Panel that the trait rotation strategy discussed in the response to charge question 8.a (above) provides the best opportunity to forestall the development of resistance in lepidopteran pests in regions of high risk for resistance, in areas with historically low compliance with block refuge requirements, in areas of high trait adoption, and in areas with high pest pressure.

Rationale: Interdisciplinary science to support a new IRM framework for lepidopteran pests of Bt crops

The Panel believes Bt PIPs generate significant benefits for agricultural producers, consumers and the environment (NRC, 2010, 2016). A surge in insect resistance to Bt crops around the globe during the past decade jeopardizes the sustainability of those benefits (Tabashnik and Carrière, 2017). To address this threat in the U.S., the Agency desires to reduce resistance risks for lepidopteran pests, increase the longevity of currently functional traits and future technologies, and improve the current IRM program for lepidopteran pests of Bt corn and cotton (US EPA, 2018). The following comments by the Panel provide scientific advice on a series of activities to accomplish those goals.

Insect resistance to Bt crops evolves due to selection pressure, an ecological process shaped by biological conditions, technology and human behavior. Therefore, interdisciplinary science that explains how natural and social systems interact to produce temporal or spatial patterns of resistance must be used to develop a new IRM framework. However, the body of science, especially on individual and collective human behavior, is incomplete. Some Panel members argued that efficient management of insect or weed resistance pose a "wicked challenge" that not only requires the integration of frontier biophysical and social science, but also taps stakeholder experiential knowledge to fill information gaps (Gould et al., 2018; Jussaume and Ervin, 2016; Shaw, 2016). Due to the heterogeneity of the natural and social systems that define specific insect resistance challenges, effective strategies will vary by: (1) place (ecosystem setting); (2) specific insect(s); (3) crop or rotation; (4) community or region, and; (5) other salient biophysical, technical, and social conditions. Hence, a standard template for IRM strategies and tactics does not apply to all settings.

Uncertainty pervades wicked problems. The development of new Bt crops requires large R&D investments and long lead times. Hence, relying on the discovery and commercialization of new Bt PIPs to solve current resistance problems introduces a risk that the new discoveries will not arrive in time to avert significant damages. The long lead time and uncertainty place more emphasis on conserving the extant pool of pest susceptibility to current Bt crops. Effective resistance management remains formidable because of the interwoven biophysical and social mechanisms that shape the evolution of resistance across varying crop landscapes. Negotiating progress will require continuous learning and adaptive management to tailor IPM approaches that can sustain crop production system resilience and possibly deliver other valuable ecosystem services from agricultural operations. Nevertheless, the Panel recommended a proactive resistance management approach in which IPM measures are applied "before, during and after" insect resistance occurs. A good example of being proactive is the case of WBC and Vip3A—by monitoring for Vip3A shifts in susceptibility, a proactive rather than reactive approach is taken.

Principal elements in IRM reform

The Panel envisioned that IRM reform entails five steps that encompass the available options listed under question 8 and additional options publicly identified by Panel members.

1. **Engage key stakeholders to identify the problem causes and dimensions, barriers to IRM, and technically, economically and socially feasible remedies.**

Public participation in environmental assessment and decision-making has a strong scientific foundation (NRC, 2008). Engaging stakeholders serves multiple purposes, from gathering intellectual and experiential knowledge to define the insect resistance dimensions, to building trust and education among the affected parties to implement management options. Insect susceptibility to Bt PIPs provides a valuable ecosystem service for agricultural production. Research has documented the importance of obtaining stakeholder input to inform ecosystem service management (e.g., Haddaway et al., 2017). For example, stakeholder engagement helps frame the appropriate management context and tailor inputs to local needs and data availability (Ruckelshaus et al., 2015). Comprehensive engagement helps assure appropriate output metrics and credible knowledge production processes, while constructing a full picture of the values and tradeoffs involved (Cash et al., 2003; Cowling et al., 2008; Iniesta-Arandia et al., 2014).

Anyone who has a valid interest in the resource management process or outcome qualifies as a stakeholder. Hence, government agency staff responsible for managing the resource, in this case insect susceptibility to Bt PIPs, should participate (Haddaway et al., 2017). Appropriate stakeholder groups likely differ in composition for different IRM program phases. For example, the Panel served as a key stakeholder group to inform timely development of regulation, whereas stakeholders to aid program implementation will require more expertise in education and community institutions.

As noted, resistance challenges vary considerably across regions and cropping systems due to differing biological and social conditions. This central artifact of wicked problems emphasizes that appropriate stakeholder engagement will vary by distinct production-pest-social situations. The Agency should consult grower organizations, including non-Bt crop producers, industry representatives, including crop advisors, Extension personnel and other scientists to help identify the distinct areas or zones in which to engage relevant sets of stakeholders. Decentralized, county-based Extension programs are uniquely qualified and geographically and educationally suited to aid in this engagement process to aid IRM education.

The Panel was not familiar with EPA's current process of eliciting stakeholder input for Bt resistance management. If comprehensive engagement occurs regularly, this comment supports the need to continue such intelligence gathering and social capital or trust building. At each stakeholder meeting, a trusted party, e.g., Extension educator, should lead off the session by conveying the importance of proactively conserving pest susceptibility to current Bt crops, given the scientific and financial uncertainties of commercializing new Bt crops. Panel members recommend that Extension professionals engage early with growers and other stakeholders to proactively implement IRM strategies and communicate regularly with all parties about Bt PIP product problems that may be associated with UXI or other issues. Several panel members noted that the success of Bt crops in Australia and Arizona have underlying similarities, with grower engagement critical for IRM.

2. **Strengthen comprehensive scouting and monitoring program to detect Bt PIP resistance and communicate real time for proactive management.**

The Panel concluded that it is unlikely to be able to detect resistance early enough to enable effective mitigation actions that would stop the spread of resistance once it arises. Nonetheless, implementing an effective resistance monitoring program is a fundamental part of effective IPM. The Panel believed that a comprehensive system of scouting and genetic monitoring (e.g., F2 screens) of croplands in corn and cotton will pay multiple rewards. Ideally, such a system would identify the adequacy of management conditions that slow the evolution of resistance, such as land planted to non-Bt crops near Bt crop fields that serve as refuge for rearing non-resistant insects. It could also be used to detect genetic resistance at low allele frequencies (<0.02) that could be used to encourage compliance with existing IRM, and detect allele frequencies corresponding to heightened risk of resistance (>0.02) that should elicit increased IRM. Genetic monitoring will also provide information, such as quantifying any cost of resistance, which could be used to design more effective management strategies. Finally, this rich information base could be used to establish population and damage thresholds that trigger IRM actions.

The importance of a scouting and monitoring program for Bt resistance received strong support in public comments to the Panel. Of special note, the National Association of Independent Crop Consultants proposed a system of real time communication to keep all stakeholders informed (NAICC 2018). Their proposal focused on the importance of early detection of resistance:

> *"Early detection of populations showing signs of resistance is critical to implementation of any insect resistance management strategy. Unless resistance carries a fitness cost, mitigation and/or resistance management strategies need to be initiated when the frequency of resistant individuals in the population is low. (NAICC, 2018, page 2)."*

The Panel endorsed the development of an efficient real-time communication system on emergent Bt PIP resistance among producers, crop advisers, Extension professionals, government and Bt crop industry representatives and other stakeholders. Effective scouting requires the commitment of human resources and technology, both of which can carry significant costs. Due to the mobile nature of insect resistance, the benefits of an early detection monitoring and real-time communication system will be spread over the entire farming community impacted by the resistant insects. In effect, the benefits are public goods as all parties share the rewards of controlling resistant insects. It would seem appropriate, therefore, for all farmers in the management region to contribute to the financing of those costs. One possible approach to implement such a system would be to work with producer associations that offer training on efficient scouting and monitoring practices. Given the public nature of benefits, government staff and university Extension faculty could assist this process by facilitating the formation of the associations to lower start-up costs. For example, local and statewide commodity groups with web-based outreach could collaborate with Extension to provide the real-time information on resistance. There are likely important lessons from the effective boll weevil, *Anthonomus grandis*, and pink bollworm, *Pectinophora gossypiella*, eradication programs that could inform cost-effective approaches.

An effective genetic monitoring system (e.g., F2 screening) should be supported by PIP registrants, for example through the Agricultural Biotechnology Stewardship Technical Committee (ABSTC). As a best-practice approach to genetic assays, it is appropriate for at least two labs to conduct genetic screening independently but using the same protocol. One possible model would be to have a lab from within the ABSTC membership and a second lab at a university perform the screening.

The NAICC's public comment proposed that an independent third party should be responsible for confirmation of insect resistance, presumably to remove any potential conflicts of interests by Bt crop sales firms. The Panel concurred with this recommendation. This recommendation raised the issue of what method should be used to confirm whether resistance exists. The Agency's issue paper mentioned the use of diet bioassays and that issue is discussed in the response to charge question 2. The Panel believed obtaining good data on allele frequencies would be important, although it recognized the challenge for genetic methods to detect novel resistance alleles. The Panel was hopeful that implementation of molecular/genetic monitoring for resistance to Bt crops may be possible in the near future. However, the Panel cautioned that EPA should not be completely reliant on any one method for resistance monitoring, particularly early in the validation and use of such methods. For example, resistance monitoring before and during the Pink Bollworm Eradication Program in Arizona used both diet bioassays and PCR molecular monitoring to observe field populations. Only after several years of corresponding monitoring data from both methods was the molecular monitoring method entrusted to serve as the primary methodology.

Finally, the Panel suggested that EPA evaluate the use of big data analytics of social media postings to determine whether it can serve as a potential early warning mechanism to identify developing UXI. This approach could be accomplished by tracking key words used on different social media related to issues of crop damage by insects. Such an effort could use methodologies modeled after those developed to observe the spread of human diseases such as influenza (e.g., Paul and Dredze, 2014; Yang et al., 2013). Scientists within EPA have used big data for human health studies and could be an internal resource on how to approach gathering and using social media big data. (Isaacs et al., 2014) The EPA Office of Environmental Information (https://www.epa.gov/aboutepa/about-office-environmental-information-oei) may be able to provide technical assistance as well.

The analysis of social media postings could be linked to the geographic location where the posts were made. If done, the general location of the observations suggesting crop damage can be overlaid with meteorological information to potentially distinguish whether the damage being discussed on social media may be due to adverse meteorological conditions (e.g. lack of rain) and/or from insect damage. A critical step in the process is to determine the key words or abbreviations that are being used on the social media discussing crop damage, the type of crop and presence of insects. Two approaches that may assist in identifying the correct words to search for are: 1) meeting/surveying farming community members to ask them if they use social media to relay information about the conditions of their farms and if so how they would refer to insect damage to crops and access social media (Such intelligence-gathering activities could be part of the stakeholder engagement process.); and 2) in regions where UXI have been documented use mining big data techniques to evaluate social media (tweeter, blogs, farming Facebook groups etc.) for the several months prior to the UXI's identification for words and phrases (or pictures) related to crop damage and insect presence. This is analogous to what has been done showing that social media post can be related to the occurrence of breakout of influenza or other disease in a particular community. Several studies have analyzed the utilization of social media by agricultural communities (e.g., Valsamidis et al., 2013). Spatial information from social media is also being used to understand agricultural management decisions. (Golubovic et al., 2016).

3. Evaluate resistance management strategies and tactics on an <u>areawide basis</u>

The Panel recommended the adoption of an areawide approach for the new IRM framework. Mutually reinforcing biophysical and social science rationales support this recommendation. From the biophysical side, some insect pests are geographically mobile, sometimes traveling very long distances, as discussed in the responses to charge question 6. Therefore, resistance monitoring should be conducted at the regional or areawide level to capture the mobile insect population. Due to mobile pests, the pool of insect susceptibility to Bt crops crosses farm boundaries which necessitates an areawide approach for effective management (Brown, 2018; Ostrom, 2009). Under such common pool resource conditions, individual farmers require some form of assurance that their stewardship will be reciprocated by neighbors in the region where insects regularly traverse. Otherwise, they will have little or no incentive to comply with recommended management actions, such as planting non-Bt crop refuges within proximity of Bt crop fields. Scientists have argued that similar common pool resource conditions for herbicide resistant weeds support the exploration of community-based management schemes (Ervin and Frisvold, 2016).

The Panel noted that multiple studies have documented benefits to other crops in a region from maintaining large amounts of Bt crop acreage that suppress insect populations including those that have evolved resistance in other regions, such as pink bollworm and *Helicoverpa* species. This effect reduces costs for all producers (e.g., Carrière et al., 2012; Hutchison et al., 2010; Wu et al., 2008; Dively et al., 2017). In effect, the persistent expression of Bt toxins can be seen as a tool to constrain population growth, facilitate the integration with other IPM tactics, and manage densities below damaging levels while moderating selection pressure with refuges that suffer damage below economic thresholds, e.g., pink bollworm (Roush, 1997). The key question is to determine the minimum level of Bt crop adoption in a region that confers insect protection and insecticide cost avoidance region-wide (Carrière et al., 2012). Especially with pyramids, adoption of Bt varieties for less than 80% of the crop may provide substantial delays of resistance (Roush, 1998). Because population dynamics may be difficult to estimate, an adaptive management strategy would be to restrain the crop adoption (such as by variety availability) to 80% and observe non-transgenic crops for levels of damage; little damage would be a strong indicator that a near optimal level of adoption had been achieved on an areawide basis.

Social science rationales also exist for areawide approaches. Adopting an areawide approach allows consideration of selecting the lowest cost or maximum net benefit refuge configuration within the region. Requiring that each farmer plant the same percentage of refuge in non-Bt crops does not minimize region-wide farm compliance cost unless all farms in the area are highly homogeneous in natural resources and crop production conditions. However, using such a uniform rule likely makes it easier for farmers to understand and implement the refuge. Heterogeneous natural resource and production conditions imply that some farms can meet the refuge requirements at lower cost or with higher net benefits than others. This heterogeneity in resource and production conditions is the basis for EPA and state pollution trading schemes, e.g., sulfur dioxide, that have significantly lowered pollution control compliance costs over uniform firm-by-firm standards (Field and Field, 2012). It also underpins USDA conservation targeting schemes for retirement of highly erodible lands (Conservation Reserve) and wetlands protection (Wetlands Reserve) (Classen et al., 2008).

The Panel believed that allowing more flexibility to construct structured community non-Bt plant refuges holds the potential to lower the costs of meeting minimum refuge size and optimal spatial configuration. This assumes that heterogeneous natural resource and production conditions coexist in the region. For example, it may be possible to target farmland not normally used for production of high value crops or marginal lands to serve as non-Bt refuges of varied plants. If the criteria for eligibility encompass other ecosystem services, such as wildlife habitat and flood control, then the non-Bt croplands or natural refuges could provide social values beyond fostering insect susceptibility to Bt crops. If the assumption of lower costs and/or significant co-benefits from structured refuge lands holds, then area wide compliance with desired refuge size and spatial configuration should improve, all else equal.

Economic science suggests alternative mechanisms to pursue improved refuge economics and compliance. The basic idea is to exploit potential cost savings or possible payments for farmers who have low productivity lands to satisfy the refuge requirements. Under one approach, farmers planting Bt crops who must deliver specified amounts of refuge, much as in current policy, are allowed to purchase the structured refuge acreage via approved contracts on other lands. Second, farmers could submit bids to deliver refuge acreage to a government agency or other entity, much as happened with the Conservation and Wetlands Reserve programs. If the government subsidized such programs, it would shift financial responsibility for maintaining the refuge from the private to public sector (which might be more economically justified if there are significant environmental co-benefits from such a program). Alternatively, it appears feasible to compel PIP registrants to coordinate (e.g. ABSTC), establish and fund such a program, perhaps as an option to satisfy refuge conditions in PIP reregistration.

4. **Develop IRM strategies that are more technically, economically and socially feasible at the grower level**

The NAICC public comment (2018) stresses the need to develop IRM program tactics that are feasible from the grower’s perspective. The Panel concurred that aligning resistance programs with the farmer’s technical complements, economic incentives and social compatibility will increase the probability of proactive management. Doing so will help achieve the long-term public good benefits that derive from effective resistance management. Several of the stewardship options listed under charge question 8 relate to technical, economic or social feasibility considerations and are discussed in turn.

Limiting or otherwise managing the use of single trait corn products: The Panel concurred that available science and data indicate that pyramided trait corn products delay resistance better than single trait products. The sequential adoption of single then pyramided trait products can also limit the future effectiveness of pyramided products for which some single traits have experienced resistance.

Managing the use of Bt corn RIB in the Southern U.S.: The Panel strongly recommended against the use of blended refuge (RIB) to protect traits targeting lepidopteran ear-feeding pests of corn in the South. In addition, none of the university faculty or the consultant group supported RIB in the materials they submitted to the docket for Panel review. In particular, the EPA should regulate against using the Vip3A trait in field corn in the South. The value of using Vip3A RIB in corn in the south is lower than the value of preserving the efficacy of the Vip3A trait in cotton. This conclusion reflects that *H. zea* is a minor pest in corn (Buntin and All, 2017) and a much more serious pest of cotton (Vyavhare et al., 2018).

As explained in the Panel's response to charge question 3, there is robust evidence that RIB would require a higher proportion of refuge seed as compared to structured refuge in order to achieve the same delay in resistance evolution. Because of this, one Panelist notes that if the EPA were to consider approving corn RIB products for use in South with a higher proportion of refuge seed (something the Panel advised against), this could negatively affect growers who would have preferred structured refuge, particularly if the mandated proportion of refuge was significantly lower in the structured case. Because of this, if the EPA were to permit RIB in the south, they should simultaneously allow the option of complying via structured refuge (which would seemingly erode the key advantage of using RIB to enforce the mandates, and thereby further argues against using RIB in this context).

Managing the use of non-functional pyramids. The Panel was cautious about the elimination of non-functional pyramids in cotton and corn in the south. The operative question is, "Non-functional against what pest?" Unless the toxins are proven non-functional against key pests such as stalk borers in corn and tobacco budworm in cotton, they should continue to be used in the pyramids in these crops.

Developing grower incentive programs to increase compliance with refuge planting: Incentives to increase compliance with refuge requirements can be structured in different ways. The most obvious is to provide temporary or recurring monetary payments if the refuge is established properly and maintained, similar to payments made for lands enrolled in the Conservation and Wetland Reserve Programs. A key question discussed by the Panel was, what private (e.g., industry) or public source (e.g., federal government) would be willing to fund those payments. An example of a private source for another pesticide resistance issue was the provision of payments by private industry to some farmers who agreed to rotate herbicide modes of actions to conserve the efficacy of popular herbicides. Those programs had restricted coverage due to funding limitations (Mitchell, 2011) and may have little effect due to the lack of new resistance management effort over what farmers would have done in the absence of the payments (Frisvold et al., 2017). The second basic approach is to reduce the cost of establishing and maintaining the refuge, e.g., lowering the price of non-Bt crop seed, and/or increasing the yield and return of non-Bt refuge crops.

A third approach is to make other government agricultural subsidies to growers conditional on complying with the refuge mandate. This could be done through the Federal Crop Insurance Corporation (FCIC), for example either by making refuge compliance a precondition for crop insurance eligibility (e.g. by incorporating refuge into the Good Farming Practices requirements for crop insurance, RMA, 2014) or by offering an insurance discount to farmers complying with mandates. These approaches would require EPA to at least coordinate their rulemaking with the relevant government agency administering the subsidy (the FCIC in the case of crop insurance). At most it would require Congress to act to modify this agricultural subsidy program. Finally, incentives could be non-pecuniary or non-monetary. For example, social marketing and moral suasion programs can be used to appeal to a grower's responsibility to be a good steward of the Bt PIP technology and conserve its efficacy for them and their neighbors. One Panelist noted that scientists have barely scratched the surface in terms of transparent, rigorous quantitative evaluation of the potential for social marketing, moral suasion to aid resistance management (e.g., Brown 2018). These non-pecuniary instruments could complement mandates or economic incentives (though some caution is warranted with unexpected interactions).

Multiple Panelists emphasized that econometric modeling can be useful to identify the magnitude of the discounts or subsidies required to achieve target refuge compliance levels. Two working papers illustrate this point with specific numbers. One by Ciliberto et al. (2017), through the Iowa State Center for Agricultural & Rural Development, uses the Kynetec seed sales survey data which has been used in other significant economic publications. They estimate an econometric model of demand for different corn and soy traits in the U.S. Results from one of their focal regression specifications appear to imply that discounting refuge seed by between 14-18 percent would put these varieties at parity with Bt products, in terms of their desirability with growers. (Estimating the actual discounts for obtaining a given level of refuge seed purchase would require additional analysis of their model.) As another example, another Panelist discussed an econometric analysis of Philippine commercial maize growers between 2007 and 2011 which suggests that discounting non-Bt hybrids by 43 percent would be necessary to put them on par with the economic returns from Bt seed (Brown et al., 2018). These are just examples, and as with any predictive model of human behavior, uncertainty accompanies the predicted grower response. It also is important to note that refuge seed discounts could be funded through both public and private means, e.g. by making refuge seed discounts (or vouchers) a condition of PIP reregistration.

Of note about the availability of non-Bt seed varieties, the paper from the Iowa State Center for Agricultural & Rural Development summarizes basic information along these lines in the Kynetec data (Cilberto et al., 2017, Table 5). The statistics show that in 2000 there were on average 10 corn varieties for sale in a given crop reporting district, of which 6 were not genetically engineered. By 2011, there were 17 varieties available on a given crop reporting district (CRD), of which only 2 on average were not genetically engineered. The table doesn't separate out Bt from other genetically engineered traits, but that information is in the underlying data, arguing for consulting the Kynetec information to assess the availability and variety in the types of refuge seed available.

The emphasis of the Panel's discussion on managing resistance on an areawide basis (see above) also highlights "intrinsic incentives" growers may have to opt out of the costly Bt technology (and thus plant refuge) and free-ride on areawide pest suppression of neighboring farms planting Bt (Hutchison et al., 2010; Brown et al., 2018). For example, Brown et al. (2018) estimates that Philippine corn farmers' willingness to pay for single-trait Bt (to control Asian Corn Borer) decreases by 18.9% in response to 10% increase in areawide use of the trait. These types of areawide feedbacks should be considered in implementing incentive-based policies for refuge compliance.

In concert with the response to charge question 4 on ways to reduce selection pressure in southern U.S. regions, the Panel supported the concept of incentivizing growers to establish block non-Bt refuge crops. In consensus, Panel members supported industry development and marketing of high yielding non-Bt corn hybrids for use in refuges. The Panel suggested ideas for increasing block non-Bt refuge in corn could include blocks of land on large farms, cost-free or discounted refuge corn seed, and marketing alternatives for non-Bt refuge corn. The Panel supported a whole-farm approach to block refuge as opposed to on-farm block refuges or blended (RIB) refuge. Under the whole-farm approach, industry, government or both would work with growers and incentivize a few of them to plant non Bt refuge crops across their whole farms. The rationale would be that a large farm planted to refuge crops could serve the refuge needs of a large region and be less expensive than a farm-by-farm approach. Concerns were noted about the spatial distribution of the "refuge farm" relative to the refuge-dependent, PIP cultivar farms in the area.

Also, the Panel supported planting and conservation of crop and non-crop natural refuge plants on non-crop land, in marginal areas, and in arable riparian areas on farms. Large-scale tests in China showed that natural refuges delayed resistance, but were not as effective as an equivalent area of non-Bt cotton refuges (Jin et al., 2015). However, natural refuge plants may deliver environmental co-benefits aside from conserving the susceptible insect pest pool. Members also supported the concept of USDA/NRCS or other agencies providing financial support for planting and conservation of crop or non-crop Bt refuge plantings. One Panel member suggested a system where dealers would provide farmers that purchase non-Bt corn with vouchers that would be required to be produced at the time Bt corn seed was purchased. The idea of community refuge among small farmers was discussed with Panel support. One Panel member recommended crop rotation (Bt and non-Bt crops) to aid in compliance with block refuge requirements and also that communities be involved in discussions concerning refuge requirements to gain support for the concept. Members of the Panel recommended that studies be conducted to better understand the reasons why many southern corn growers are non-compliant with structured refuge requirements. This issue is explored in depth in the response to charge question 7. And, the Panel supported strengthened enforcement of requirements for structured refuge in corn.

Finally, one Panel member suggested that grower incentive programs may be more effectively implemented and awarded to Extension scientists/service personnel in the following way to address the "non-compliance" problem and to further encourage and strengthen the relationship and trust between Extension scientists/service personnel and growers. Initially, the program would be aimed specifically toward "non-compliance" problems; however, the mechanism described below could also promote other aspects of working relationships between Extension scientists/service personnel and growers. This Panel member recommended that the EPA establish "Center Grants/Contracts" for Extension scientists/service personnel in universities and growers to work together, synergistically, in the problematic (e.g., non-compliance) areas with the special emphases of: (1) encouraging applicants to come up with innovative approaches to reach the growers so as to solve the "non-compliance" problems relevant to the local areas; (2) encouraging multi-university collaboration to cover the problematic areas; (3) encouraging creative ways to further strengthen the "trust" between the Extension personnel and the growers; and (4) establishing quantitative means to measure successes.

<u>Molecular assays to test in-field insect samples and expedite resistance confirmation</u>: The Panel acknowledged the potential benefits of molecular assays to test in-field insect samples to expedite resistance confirmation. However, the Panel also acknowledged that no functional molecular tests are available, and that developing a test would require knowledge of the mechanism of resistance and would assume that this mechanism is the same (rather than multiple, independently evolved resistance mechanisms). A molecular test might take many years to develop. Therefore, F2 screens should be used, along with targeted sampling of UXI fields. Panelists hoped that work on molecular resistance detection methods would continue and lead to an effective in-field test, although genetic monitoring now must be conducted with existing methods (F2 screens).

<u>Use of sentinel plots to monitor for resistance</u>: Sentinel plot monitoring could be conducted comprehensively using Bt and non-Bt sweet corn hybrids (which are generally the most attractive host plants to *H. zea*) in the spring and fall in both northern and southern states. The sentinel plot monitoring could also be performed in combination with seasonal insect migration monitoring (trapping) to assess *H. zea* population pressure at the silking stage of corn plants (which is critical for female moth oviposition) and prevent false negative or positive determination, and to predict the possibility of UXI field occurrence at a location or in a region in the near future. The larvae collected from these sentinel plots could be used in available molecular or diet bioassays for Bt resistance detection. In addition to detecting difference of Bt resistance in *H. zea* between the northern and southern states, such collaborative efforts on monitoring can provide critical information on reduced Bt susceptibility shift with intra-seasonal (the spring to the fall) data, as well as inter-seasonal (from year to year) data, at a given geographic location.

However, such a comprehensive monitoring system of sentinel plots would require considerable resources to implement. Lacking a quick turn-around resistance detection system and given the highly migratory nature of the noctuid pests, the consensus in the Panel was that the UXI field of either corn or cotton was more likely to be the first to detect resistance as every PIP field is a potential monitoring location for resistance. This conclusion reinforces the need to strengthen the comprehensive scouting and monitoring program to detect Bt resistance in noctuid pests and communicate in real time for immediate and proactive management within a given area, as well as across the regions with diverse cropping systems.

5. **Facilitate grower cooperation to implement community-driven IRM programs for common pool resource challenges.**

A rich scientific literature on common pool resource management exists to inform future IRM programs for Bt crops. This literature makes clear that alternative approaches can be successful depending upon the biophysical and social particulars. Hardin (1968) initially warned about the tragedy of the commons, e.g., ocean fisheries depletion, in a seminal essay published in *Science*. He argued that some form of regulation was necessary to remedy the inherent problems of open access in which users may ultimately deplete and exhaust the entire resource, e.g., groundwater aquifers. His essay prompted many scientists and activists to emphasize the need for government regulation to correct potential degradation of commons resources.

Ostrom (1990) subsequently added an important element to the scientific discussion of commons management. She collaborated with colleagues (Ostrom et al., 2012) to theorize and document how many communities around the globe had successfully countered the destructive tendencies of open unregulated access through voluntary cooperative community action to conserve common pool resources. The central hypothesis of this body of work is that community-driven efforts to manage common pool resources can be more cost-effective and sustainable than externally imposed solution approaches. It is possible that community-based efforts also can address social equity concerns if they arise from open democratic participation of all stakeholders. It is critical to emphasize that the community-based or driven efforts do not imply the absence of government involvement (Ervin and Frisvold, 2016). As happened in the boll weevil and pink bollworm eradication efforts, both state and federal governments played key roles e.g., technical assistance and education, to assist the grower and community-led efforts. Moreover, research has documented that the threat of government regulation can spur voluntary environmental management by business (Ervin et al., 2013). Such proactive environmental management responses by business likely embody more flexibility and innovation than prescriptive uniform technology standards, although the achievement of system-wide environmental performance targets remains uncertain.

Ostrom (2009) cautioned that private approaches were not always feasible. In analyzing over 80 cases of common pool resource management, it was concluded that the successful cases of private community self-organization were characterized by the following ten conditions:

1. <u>Size of the resource system</u>. Very large systems are unlikely to self-organize because of the high cost of defining boundaries, monitoring and assembling ecological knowledge. Small systems might not produce enough valuable services to induce such action. For these reasons, moderate sized systems are most conducive to self-organization.
2. <u>Productivity of resource system</u>. There is an inverted U-shaped relationship between resource productivity and self-organization. If a resource is either abundant or already exhausted, users perceive few incentives to pursue collective action for conservation. Some scarcity (value) is necessary to induce their efforts.
3. <u>Predictability of system dynamics</u>. Resource system dynamics need to be understood well enough so that the effects of management actions on the resource can be accurately predicted. Unpredictability can deter users from self-organization because of a lack of assurance of effective group actions.
4. <u>Resource unit mobility</u>. Self-organization is less likely for highly mobile resources, such as wildlife or water in an unregulated river, that range over great distances. As argued above, insect resistance exhibits spatial mobility. This means resistance-management organizations would need to cover a large enough area to encompass the major factors contributing to resistance. It is possible, however, that smaller management units nested within the larger area can coordinate their efforts to address the larger scale domains. Ostrom et al. (2012) terms this approach polycentric governance structures.
5. <u>Number of users</u>. Larger groups of resource users have higher costs to organize, but also can assemble labor and other resources to cover administration, monitoring, and other

costs. The effect of group size on self-organization depends on the particulars of the socio-ecological system. In the case of IRM, smaller more homogeneous groups have tended to be more successful at managing resistance, e.g. Australia and southwestern U.S., as compared to the southern U.S. (and Brazil and India). Research on Missouri farmers' willingness to cooperate to control pests found that simple, local cooperative efforts were more popular than more formal county-wide programs, again favoring small groups (Stallman and James, 2015). The most effective user configuration for IRM does seem to be a small number of large-scale growers with adequate resources.

6. Leadership. When some resource users have entrepreneurial skills and enjoy the respect of others, the likelihood of collective action increases. For example, the presence of college graduates and local elders in the resource system can exert positive effects. Extension professionals may be in a prime position to play leadership roles given their neutrality and extensive social networks.

7. Norms/social capital. Users of resource systems who share moral and ethical standards of group behavior, including the norms of reciprocity and trust, will have lower transaction costs in reaching agreement and be more likely to self-organize and carry out effective monitoring.

8. Knowledge of the socioecological system. When users share common knowledge of the resource system—its vulnerabilities to excessive use, how their actions affect each other, and the rules used in other systems—they expect lower costs of organizing. If this knowledge is lacking and the resource system regenerates slowly, the risk of excessive degradation rises.

9. Importance of the resource to users. If the resource in question plays a significant role in the welfare of the users, they are more likely to perceive that the gains from collective action to conserve the resource will outweigh the costs of organizing and maintaining.

10. Collective choice (governance) rules. If users have full authority and autonomy to take collective action to develop and enforce rules, they face lower transaction costs of organizing and less expense in implementing controls to defend the resource from exploitation by external parties.

The scientific insights, both opportunities and challenges, from Ostrom's work with colleagues are starting to be applied to weed and insect resistance issues (Ervin and Jussaume, 2014; Gould et al., 2018). Ervin and Frisvold (2016) analyzed several community-based programs in agriculture that identified six common themes that affect the degree of success.

- Requirement for a strong scientific basis
- Effective communication of scientific principles
- Active involvement of social scientists
- Need for strong leader/coordinator, e.g., champion
- Need for ongoing monitoring, reporting and evaluation
- Need to establish clear geographic boundaries

Several of these themes have surfaced in the Panel's analysis of insect resistance management programs and likely have application in IRM program reforms.

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APPENDIX 1: Supplement to Figures 2D.1, 2D.2, 2D.3- General Model Description to Simulation to Resistance Monitoring

Description of the model presented by Anthony R. Ives during the SAP meeting

The model is based on the model published in Ives et al. (2011). It keeps track of both allele frequencies and insect densities.

General model description

The model is discrete in time, with each time step corresponding to an insect generation. It assumes that mating within fields is random, with females producing *F* offspring. The model is designed for a pyramid of two Bt toxins, although it can also be run for a single-toxin crop. Resistance to each of two Bt toxins is governed by diallelic, independently segregating loci, with R_1 and S_1 denoting resistant and susceptible alleles to Bt toxin 1, and R_2 and S_2 denoting the resistant and susceptible alleles to Bt toxin 2. Thus, there are nine genotypes of offspring whose frequencies within fields are at Hardy-Weinberg equilibrium. The survivals of offspring with genotypes R_1R_1, R_1S_1, and S_1S_1 from Bt toxin 1 are given by s_{1RR}, s_{1RS}, and s_{1SS}, and similarly s_{2RR}, s_{2RS}, and s_{2SS} give the survivals associated with Bt toxin 2. Survivals on plants containing both of the Bt toxins are assumed to be multiplicative, as is expected if toxins have independent modes of action. For example, the survival of an $S_1S_1S_2S_2$ individual on Bt plants is s_{1SS} x s_{2SS}, and the survival of an $R_1S_1R_2S_2$ individual is s_{1RS} x s_{2RS}.

Following any mortality caused by Bt, we assume there is density-dependent survivorship given by $(1 + x)^{-1}$ where *x* is the density (all genotypes) of surviving larvae within a field. The specific form of this survival function makes little difference for any of our qualitative or quantitative conclusions. Because the model explicitly keeps track of the number of individuals of different genotypes, rather than just genotype frequencies, density-dependent survival changes the rate of resistance evolution. Density dependence occurs at the scale of individual fields. If density-dependent survival were caused solely by natural enemies, and if the natural enemies were globally dispersing, then density dependence could act at the global rather than local scale, giving results corresponding to the frequency-only model.

The spatially structured model is built on a 50 by 50 grid of same-sized fields, with a proportion *Q* being refuge fields and a proportion (1 – *Q*) being Bt fields. By assuming that the entire landscape is made up of refuge or Bt fields, the model ignores the possibility of fields of other crops or non-crop habitat. Biologically, this is equivalent to assuming that, even though different types of habitats may be available on a real landscape, these habitats are permeable to dispersing adults who move through them as if they were not there. Refuges are distributed randomly on the grid, and crop rotation is included by randomly rearranging refuges on the grid. The model assume that fields are rotated every 2 insect generations.

When males disperse from their natal fields, they do so before mating, whereas females disperse from natal fields following mating. The probability of dispersing a linear (Euclidean) distance *x* from natal fields is proportional to d^x, so dispersal drops off exponentially with distance; the fraction of adults remaining in their natal field is proportional to $d^0 = 1$. In the simulations presented, $d = 0.2$ and have a maximum dispersal distance of 12 fields. Finally, the grid of fields has “wrap-around” boundaries (i.e., the grid is on a torus), so that insects dispersing off one side of the grid appear on the opposite side; this assumption makes the dynamics on the 50 by 50 grid better approximate the dynamics expected for a much larger grid while remaining computationally manageable.

Simulating resistance monitoring

For the SAP, the model was run assuming one resistance allele was fixed in the population to give the case of an effectively single-toxin crop. The initial resistance allele frequency for the other allele is 0.001. The S_1S_1 susceptible individuals have a survival of 0.05 in Bt fields, and the survival of r_1S_1 heterozygotes is 0.0975 (the dominance is h = 0.05). To give realistic fluctuations in insect abundances, there is generation-to-generation environmental variation in fecundity/density-independent survival of insects in Bt and refuge fields. The background proportion of refuge fields is Q = 20%. To generate resistance evolutionary hotspots, the central 10 x 10 field region (4% of the 50 x 50 grid) has 2% refuge. Spread evenly across the grid, there are 25 pairs of Bt and refuge fields that act as sentinel fields in which insects are sampled; one sentinel pair of fields occurs in the middle of the region designed to be a hotspot with 2% refuge.

The model keeps track of the following in both Bt and refuge fields in each sentinel pair:

(i) Insect larval density, which would indicate crop damage.
(ii) The average survival of larvae, which would indicate survival in population-level feeding assays.
(iii) Resistance allele frequencies, which would be detected by genetic screens.

Model code (Matlab)

```
% This is for EPA use only and is copyrighted by Anthony R. Ives

% proportion refuge overall and in central square
Q=.2;
Qcenter=.02;
centerFract=.2;

costofresistance=0;

% include mitigation
mitigatethreshold=.01;
mitigateFract=.4;

increaserefugeflag=0;
Qmitigate=.2;

insecticideflag=0;
Sinsecticide=.3;

pyramidflag=0;
p2mitigate=.001;

eliminateflag=0;

% Pre-selection generations
Tstart=20;
Tmax=200;

%size of grid
N=50;

% rotation frequency
rotation_freq=2;

% Environmental variation
s_envir=1.5;

% reproductive rate in Bt fields (under flag2=1)
F1=50;
a=1;

%dispersal of males (rm) and females (rf) from Bt and refuge fields
rm1=1;
rm2=rm1;
rf1=1;
rf2=rf1;
rm=[rm1 rm2];
rf=[rf1 rf2];

% dispersal: (1) horizontal/vertical; (2) limited (n); (3) global dispersal
disperseflagm=2;
disperseflagf=disperseflagm;

% movement distance (if dispersalflag = 2)
dm=2;
df=2;

% n0flag=0 means dispersing individuals do not remain in natal field
n0flag=1;

% initial gene frequencies
p1start=.001;
```

```
p2start=1;

% survivals in Bt fields
h1=.05;
s1L=.05;
s1H=1;

h2=1;
s2L=1;
s2H=1;

s1=[s1L s1L*(1-h1)+s1H*h1 s1H];
s2=[s2L s2L*(1-h2)+s2H*h2 s2H];

sBt=s1'*s2;
sBt=sBt(:);

% survivals in refuge fields (to include a cost of resistance)
if costofresistance==1
    h1=.5;
    s1L=1;
    s1H=.2;
else
    h1=1;
    s1L=1;
    s1H=1;
end

h2=1;
s2L=1;
s2H=1;

s1=[s1L s1L*(1-h1)+s1H*h1 s1H];
s2=[s2L s2L*(1-h2)+s2H*h2 s2H];

srefuge=s1'*s2;
srefuge=srefuge(:);

% set up survivals
surv=[sBt srefuge];

% calculate V for mating of genotypes
VV(:,:,1)=[1 .5 0;.5 .25 0;0 0 0];
VV(:,:,2)=[0 .5 1;.5 .5 .5;1 .5 0];
VV(:,:,3)=[0 0 0; 0 .25 .5;0 .5 1];
for i2=1:3
    for i1=1:3
        V(:,:,3*(i2-1)+i1)=kron(VV(:,:,i2),VV(:,:,i1));
    end
end


% set up dispersal matrix Mm
if disperseflagm==1,
    %xx is movement rate to 4 nearest squares.
    %Distribution over squares is pseudo geometric (scale by 1/2)
    M=zeros(N,N);
    xx=(8*(1-.5^n))^(-1);
    for w=1:n
        xw=xx*.5^(w-1);
        M=M+xw*(diag(ones(N-w,1),w)+diag(ones(w,1), N-w));
    end
    Mm=M+M';
end
```

```
% set up dispersal matrix Mf
if disperseflagf==1,
    %xx is movement rate to 4 nearest squares.
    %Distribution over squares is pseudo geometric (scale by 1/2)
    M=zeros(N,N);
    xx=(8*(1-.5^n))^(-1);
    for w=1:n
        xw=xx*.5^(w-1);
        M=M+xw*(diag(ones(N-w,1),w)+diag(ones(w,1), N-w));
    end
    Mf=M+M';
end

ww=.5*F1;
x0=(ww-1)/(a*ww);

% fields for sampling
Btsamplegrid=zeros(N,N);
Btsamplegrid(5:10:N, 5:10:N)=1;
refugesamplegrid=zeros(N,N);
refugesamplegrid(5:10:N, 6:10:N)=1;
Nsamples=sum(sum(Btsamplegrid));
Btsample=zeros(Tmax, Nsamples, 3);
refugesample=zeros(Tmax, Nsamples, 3);

% initiate matrices
W=zeros(N,N,9);
Xm=zeros(N,N,9);
Xf=zeros(N,N,9);
Surv=zeros(N,N,9);
Wn=zeros(N,N,9);
P1=zeros(N,N);
P2=zeros(N,N);

mitigateflag=0;

output=[];
tempoutput=[];
for rep=1:1

    % initially assume no resistance
    p1=0;
    p2=0;
    wi=[(1-p1)^2 2*p1*(1-p1) p1^2];
    wj=[(1-p2)^2 2*p2*(1-p2) p2^2];

    w=wi'*wj;
    w=w(:);
    for i1=1:N
        for i2=1:N
            W(i1,i2,:)=w;
        end
    end

    t=0;
    Plist=[];
    Xplist=[];
    while t < Tmax && (p1 < 1-10^-3) & ((t <= Tstart) | (p1 > 10^-5))   % resistance
failure looking only at trait 1
        t=t+1;

        if t == Tstart
            p1=p1start;
```

```
            p2=p2start;
            wi=[(1-p1)^2 2*p1*(1-p1) p1^2];
            wj=[(1-p2)^2 2*p2*(1-p2) p2^2];

            w=wi'*wj;
            w=w(:);
            for i1=1:N
                for i2=1:N
                    W(i1,i2,:)=w;
                end
            end
        else
            p1=0;
            p2=0;
        end

        if mitigateflag==1 & pyramidflag==1
            p2=p2mitigate;
            wj=[(1-p2)^2 2*p2*(1-p2) p2^2];

            for i1=1:N
                for i2=1:N
                    wi=[W(i1,i2,7) W(i1,i2,8) W(i1,i2,9)];
                    w=wi'*wj;
                    w=w(:);
                    W(i1,i2,:)=w;
                end
            end
            pyramidflag=0;
        end


        if t > 1 && max(Btsample(t-1,:,2))>mitigatethreshold
            if mitigateflag==0
                'mitigation starts at gen '
                t - Tstart
            end
            mitigateflag=1;
        end
        % periodical rotation and mitigation
        if mod(t-1,rotation_freq)==0
            Btgrid=ones(N*N,1);
            rp=randperm(round(N*N));
            Btgrid(rp(1:round(Q*N*N)))=2;
            Btgrid=reshape(Btgrid,N,N);

            Btgridcenter=ones(round(N*centerFract)^2,1);
            rp=randperm(round(N*centerFract)^2);
            Btgridcenter(rp(1:round(Qcenter*N*N*centerFract^2)))=2;

Btgridcenter=reshape(Btgridcenter,floor(N*centerFract),floor(N*centerFract));

            range=(ceil((1-centerFract)*N/2):(ceil((1-
centerFract)*N/2)+round(N*centerFract)-1))+1;
            Btgrid(range,range)=Btgridcenter;

            if mitigateflag==1 & increaserefugeflag==1
                Btgridmitigate=ones(round(N*mitigateFract)^2,1);
                rp=randperm(round(N*mitigateFract)^2);
                Btgridmitigate(rp(1:round(Qmitigate*N*N*mitigateFract^2)))=2;

Btgridmitigate=reshape(Btgridmitigate,floor(N*mitigateFract),floor(N*mitigateFract));

                range=(ceil((1-mitigateFract)*N/2):(ceil((1-
```

```
mitigateFract)*N/2)+round(N*mitigateFract)-1))+1;
                Btgrid(range,range)=Btgridmitigate;
            end
        end

        Btgrid(Btsamplegrid==1)=1;
        Btgrid(refugesamplegrid==1)=2;

        if t==1
            % grid of initial densities
            X=10^-4*ones(N,N);
            X(Btgrid==2)=x0;
        end

        for i=1:9
            s=surv(i,:);
            Surv(:,:,i)=s(Btgrid);
        end

        %grid of male and female movement proportions
        RM=rm(Btgrid);
        RF=rf(Btgrid);

        % dispersal of male alleles
        for i=1:9
            Xs=.5*(1-RM).*W(:,:,i).*X;
            Xd=.5*RM.*W(:,:,i).*X;

            if disperseflagm==1
                %disperse males and alleles to 4 nearest cells
                Xd=(Mm*Xd+Xd*Mm);
            elseif disperseflagm==2
                %This is diamond dispersal
                Xd=reshape(Mm*Xd(:),N,N);
            else
                %global dispersal
                Xd=mean(mean(Xd))*ones(N,N);
            end
            Xm(:,:,i)=Xs+Xd;
        end
        Wm=Xm./repmat(sum(Xm,3),[1 1 9]);

        Wf=W;
        %mating
        for i1=1:N
            for i2=1:N
                for j=1:9
                    WWm=reshape(Wm(i1,i2,1:9),9,1);
                    WWf=reshape(Wf(i1,i2,1:9),9,1);
                    W(i1,i2,j)=sum(sum((WWf*WWm').*V(:,:,j)));
                end
            end
        end

        % dispersal of female alleles and females
        for i=1:9
            Xs=.5*(1-RF).*W(:,:,i).*X;
            Xd=.5*RF.*W(:,:,i).*X;

            if disperseflagf==1,
                %disperse males and alleles to 4 nearest cells
                Xd=(Mf*Xd+Xd*Mf);
            elseif disperseflagf==2,
                %This is diamond dispersal
```

```
            Xd=reshape(Mf*Xd(:),N,N);
        else
            %global dispersal
            Xd=mean(mean(Xd))*ones(N,N);
        end
        Xf(:,:,i)=Xs+Xd;
    end
    Wf=Xf./repmat(sum(Xf,3),[1 1 9]);
    Xf=sum(Xf,3);
    W=Wf;

    % pre-selection allele frequencies
    Wo=W;

    %selection
    for i=1:9
        Wn(:,:,i)=W(:,:,i).*Surv(:,:,i);
    end
    S=sum(Wn,3)./sum(W,3);

    W=Wn./repmat(sum(Wn,3),[1 1 9]);

    % compute new densities
    X=S.*Xf;
    X=F1*X;
    X=X*exp(s_envir*randn(1)-s_envir^2/2);

    % mitigation with insecticide
    if mitigateflag==1 & insecticideflag==1
        range=(ceil((1-mitigateFract)*N/2):(ceil((1-
mitigateFract)*N/2)+round(N*mitigateFract)-1))+1;
        XX=X(range,range);

        XX(Btgrid(range,range)==1)=Sinsecticide*XX(Btgrid(range,range)==1);
        X(range,range)=XX;
    end

    X=X./(1+a*X);

    % compute gene frequencies
    p1=0;
    p2=0;
    for i1=1:N
        for i2=1:N
            p1=p1+X(i1,i2)*(sum(W(i1,i2,[3 6 9]))+.5*sum(W(i1,i2,[2 5 8])));
            p2=p2+X(i1,i2)*(sum(W(i1,i2,[7 8 9]))+.5*sum(W(i1,i2,[4 5 6])));

            P1(i1,i2)=sum(Wo(i1,i2,[3 6 9]))+.5*sum(Wo(i1,i2,[2 5 8]));
            P2(i1,i2)=sum(Wo(i1,i2,[7 8 9]))+.5*sum(Wo(i1,i2,[4 5 6]));
        end
    end

    % eliminate resistance allele in mitigation area
    if mitigateflag==1 & eliminateflag==1
        range=(ceil((1-mitigateFract)*N/2):(ceil((1-
mitigateFract)*N/2)+round(N*mitigateFract)-1))+1;
        PP1=P1(range,range);

        PP1=0;
        P1(range,range)=PP1;
    end

    p1=p1/sum(X(:));
    p2=p2/sum(X(:));
```

```
    Btsample(t,:,1)=X(Btsamplegrid==1)';
    refugesample(t,:,1)=X(refugesamplegrid==1)';
    Btsample(t,:,2)=P1(Btsamplegrid==1)';
    refugesample(t,:,2)=P1(refugesamplegrid==1)';
    Btsample(t,:,3)=S(Btsamplegrid==1)';
    refugesample(t,:,3)=S(refugesamplegrid==1)';

    if 1
        figure(1)
        subplot(3,1,1)
        surf(Btgrid/2+Btsamplegrid)
        view(2)
        axis square
        axis([1 N 1 N])
        if mitigateflag==0
            title('Bt and refuge fields')
        else
            title('\fontsize{24}Mitigation','Color','red')
        end

        subplot(3,1,2)
        surf(P1)
        view(2)
        axis square
        axis([1 N 1 N])
        title('r allele frequency')

        subplot(3,1,3)
        surf(log(X))
        view(2)
        axis square
        axis([1 N 1 N])
        title('Insect density')

        pause(.01)
    end
end
GensToFailure=t-Tstart-1;
[Q GensToFailure]

Btsample=Btsample(Tstart:t,:,:);
refugesample=refugesample(Tstart:t,:,:);

figure(2)
subplot(3,1,1)
plot(Btsample(:,:,1))
ylim([0, 1.2])
xlabel('\fontsize{20}Generations')
ylabel('\fontsize{20}Density in Bt fields')
title('\fontsize{22}In-field injury')

subplot(3,1,2)
plot(refugesample(:,:,1))
ylim([0, 1.2])
xlabel('\fontsize{20}Generations')
ylabel('\fontsize{20}Density in refuge fields')

subplot(3,1,3)
plot(Btsample(:,:,1)./refugesample(:,:,1))
ylim([0, 1.2])
xlabel('\fontsize{20}Generations')
ylabel('\fontsize{20}Relative density Bt:refuge')
```

```
    figure(3)
    subplot(2,1,1)
    plot(Btsample(:,:,3)./refugesample(:,:,3))
    xlabel('\fontsize{20}Generations')
    ylabel('\fontsize{20}Relative survival Bt:refuge')
    title('\fontsize{22}Feeding assays')

    subplot(2,1,2)
    semilogy(Btsample(:,:,3)./refugesample(:,:,3))
    xlabel('\fontsize{20}Generations')
    ylabel('\fontsize{20}Relative survival Bt:refuge')

    figure(4)
    subplot(2,1,1)
    plot(refugesample(:,:,2),'-g')
    ylim([.5*p1start, 1])
    title('\fontsize{22}Genetic assays')
    xlabel('\fontsize{20}Generations')
    ylabel('\fontsize{20}r allele frequency')
    hold on
    plot(Btsample(:,:,2),'-k')
    hold off

    subplot(2,1,2)
    semilogy(refugesample(:,:,2),'-g')
    ylim([.5*min(min(refugesample(:,:,2))), 1])
    xlabel('\fontsize{20}Generations')
    ylabel('\fontsize{20}r allele frequency')
    hold on
    semilogy(Btsample(:,:,2),'-k')
    hold off

end
```

APPENDIX 2: Supplement to Figure 3.1- Rate of Resistance Evolution

Description of the model presented by Anthony R. Ives during the SAP meeting

The model computes the proportion of refuge in a seed blend (proportion of non-Bt seeds) that gives the same durability as a structured refuge as a function of the proportion of structured refuge in the landscape, *Q*. It uses the approximations published in Ives and Andow (2002) to give the rate of resistance evolution.

The model includes the following assumptions:

(i) The proportion of Bt pollen equals the proportion of Bt plants in RIB.

(ii) Bt is hemizygous within plants (50% pollen with Bt).

(iii) To obtain the survival of SS and rS larvae within corn ears as a function of the proportion of kernels that express Bt toxins, the model follows Caprio et al. (2016) in assuming that survival SS genotypes is the product of survivals from encountering Bt-expressing kernels. Thus, if the survival of SS susceptibles on single Bt kernels is z_{ss} and the proportion of Bt-expressing kernels is *p*, then the survival of larvae to adulthood is $((1\text{-}p)+p\ z_{ss})^n$, where n = 25, the number of kernels consumed by larvae in an ear. A similar equation is used for rS heterozygotes.

Model code (Matlab)

```
% This is for EPA use only and is copyrighted by Anthony R. Ives

R=25;
n=25;

pBtpollen=.5; %proportion of toxic kernals (assuming Bt is hemizygous)

for i=1:4

    %%%%%%%%%%%%%%%%%
    %Yang 2017 (S. frugiperda)
    if i==1
        Sss_refuge=1/5;

        q=1-pBtpollen*.8; %proportion of toxic kernals for case of 20% non-Bt
        zss=(Sss_refuge^(1/n)-q)/(1-q);

        Srs_Bt=.017; %from the study
        zsr=1;
    end

    %%%%%%%%%%%%%%%%%
    %Yang 2014
    if i==2
        Sss_refuge=1/2.7;

        q=1-pBtpollen*.95; %proportion of toxic kernals for case of 5% non-Bt
        zss=(Sss_refuge^(1/n)-q)/(1-q);

        Srs_Bt=.095; %assumed for h = 0.05
        zsr=1;
    end

    %%%%%%%%%%%%%%%%%
```

```
    %Onstad 2018 -- mean
    if i==3
        Sss_refuge=1/1.32;

        q=1-pBtpollen*.95; %proportion of toxic kernals for case of 5% non-Bt RIB (this
differs among studies)
        zss=(Sss_refuge^(1/n)-q)/(1-q);

        Srs_Bt=.095; %assumed for h = 0.05
        zsr=1;
    end

    %%%%%%%%%%%%%%%%%
    %Onstad 2018 -- low
    if i==4
        Sss_refuge=1/1.16;

        q=1-pBtpollen*.95; %proportion of toxic kernals for case of 5% non-Bt RIB (this
differs among studies)
        zss=(Sss_refuge^(1/n)-q)/(1-q);

        Srs_Bt=.095; %assumed for h = 0.05
        zsr=1;
    end

    Qp=.001*(1:1000);
    Pp=pBtpollen*(1-Qp);
    SS=((1-Pp)+Pp.*zss).^n;
    rS=((1-Pp)+Pp.*zsr).^n;

    Q=Qp-(rS./SS-1)./(R*Srs_Bt);
    Qp=Qp(Q>.02);
    Q=Q(Q>.02);

    figure(1)
    ch=['-r','-g','-k','-b'];
    plot(Q, Qp, ch(2*(i-1)+1:2*i),'LineWidth',2)
    xlim([0,.5])
    ylim([0,1])
    xlabel('\fontsize{24}Structured refuge')
    ylabel('\fontsize{24}RIB refuge')
    hold on
end
hold off
```