

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 US: *H. zea* collected from sweet corn fields in eastern US, *S. frugiperda* collected in southeastern US, and *S. albicosta* populations from northern lake states in the US and Canada (Section III. A of White Paper).

EPA asks the panel to discuss these reports and address the following:

- a. The degree of confidence that these reports represent cases of confirmed resistance to Bt PIPs.

EPA asks the panel to comment on:

- b. 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 US, given the cited reports.

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 (Section III. B. 6 of White Paper)

- a. EPA asks that the panel 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 Unexpected Injury (UXI) fields.

B. Diet Bioassays (Section III. B. 6 of White Paper)

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?

C. Unexpected Injury Threshold (UXI) in Bt Corn and Bt Cotton (Section III. B. 7 of White Paper)

As described in the white 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 white 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. EPA asks that the panel 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.

D. Definition of resistance (see Section III. B. 8 of White Paper)

EPA's definition of pest resistance is based on heritability of the resistant 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 white paper, however, this definition does not allow for proactive mitigation of resistance (i.e., 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. EPA asks the panel to discuss the criteria and additional considerations for defining resistance beyond those listed in the white paper that enhance the ability to detect resistance faster and implement effective mitigation to extend the durability of Bt trait(s).

3. Resistance Risk of Seed Blend Corn in the Southern US

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 US. 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 varieties 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 US. Solutions of these types would provide adequate refuge compliance and sound resistance management for ear-feeding pests of corn. (Section III. B. 4. of White Paper)

EPA asks the panel to discuss and evaluate:

- a. 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 (i.e., multiple generations per year and overwintering capacity);
- b. 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.

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 US, 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 Insect Resistance Management (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 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 (Sections III. B. 2, 9, 10 and III. A.).

- a. Given the temporal and spatial cropping patterns in the southern US, EPA asks the panel to 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.

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 US 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 US 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 White 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. (Sections III. B. 12 and IV. C. of White Paper)

- a. EPA asks the panel to 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*?

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 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 Mitigation Action Area (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. (see Section III. B. 12 of White Paper)

EPA asks that the panel discuss:

- a. The delineation of a Mitigation Action Area (MAA) 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. Population management approaches for cases of widespread field-resistance.

7. *Grower Non-Compliance with Refuges in the Southern US*

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 US, 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. (Section III. A. 5 of White Paper)

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

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 (see Section IV. of White Paper). Some of the available options to address the current limitations are:

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

EPA asks that the panel discuss:

- a. Advantages and limitations of the stewardship options discussed in the Agency white 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. 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.