Response to the EPA Draft Proposal to Address Resistance Risks to Lepidopteran Pests of Bt Following the July 2018 FIFRA Scientific Advisory Panel Recommendation Memorandum

From the members of the National Cotton States Arthropod Pest Management Working group and signatories from the multistate research project NC246: Ecology and Management of Arthropods in Corn

Prepared by: Dominic Reisig, Galen Dively, Jeff Gore, *Helicoverpa zea* section; Chris DiFonzo, Yasmine Farhan, Jocelyn Smith, *Striacosta albicosta* section

Executive summary: Our groups support changes to resistance management for non-high dose lepidopteran pests and have suggestions for improvement. We commend the EPA proposal for following the suggestions of the 2018 Scientific Advisory Panel regarding sentinel plots, but recommend that they follow the Panel's suggestion for other areas. Our comments in this letter are oriented toward *Helicoverpa zea* and *Striacosta albicosta*, but actions for these pests should cover other species of concern, such as *Spodoptera frugiperda*. We encourage the EPA to modify the proposal so that: 1) Vip3Aa should not be available in field corn hybrids sold in cotton-growing regions and selectively deployed in the Midwest and Great Lakes corn growing regions where it is the only available PIP option for *S. albicosta* management; 2) only single toxin corn products should be phased out; 3) further PIP pyramid releases should coincide with the immediate phase-out of the old pyramid; 4) refuge in a bag (RIB) should be prohibited in cotton growing regions; 5) a 20% block refuge for Vip3Aa hybrids should be required in the Midwest and Great Lakes corn growing regions; 6) implementation of sentinel monitoring program should be described in detail and made more prescriptive, including the use of sweet corn vs. field corn; 7) unexpected injury (UXI) thresholds should be set to reflect what was recommended by the SAP and published observations in the field; 8) UXI events should be reported to the Extension specialist from the Land Grant University in that region within 24 hours of a suspected UXI event occurring; 9) registrants should be required, as a condition of registration, to provide purified Bt protein or standardized lyophilized plant tissue to public sector researchers to establish baseline resistance allele frequencies; 10) growers should adopt the best management practices (BMP) and IPM strategies already developed by their respective Land Grant Universities; 11) programs like the NRCS Conservation Stewardship Program should be continued and expanded to encourage growers to plant non-Bt refuge corn.

Introduction

We applaud the EPA's effort to improve IRM for PIPs targeting non-high dose pests. One of the many functions of our organizations, the National Cotton States Arthropod Pest Management Working Group and the multistate research project NC246: Ecology and Management of Arthropods in Corn, is to seek ways to support these efforts — development of robust resistance management strategies that are compatible with and supportive of Integrated Pest

Management (IPM) — as Extension and research entomologists in the Land Grant Universities. While there are many positive aspects of the draft proposal, we wish to use this letter to comment on areas that can be improved.

We are extremely concerned about maintaining the susceptibility of pests targeted by Bt toxins in both cotton and corn. Our primary concern in corn is *Striacosta albicosta* (Western bean cutworm) which is resistant to Cry1F, but still susceptible to Vip3Aa. In cotton, our primary concern is *Helicoverpa zea* (bollworm, corn earworm, tomato fruitworm) which is resistant to both Cry1 and Cry2A proteins. *Helicoverpa zea* infests both corn and cotton annually and is not an economic pest of field corn in most regions, although it is exposed to Bt toxin in field corn. A further concern is *Spodoptera frugiperda* (fall armyworm), but many of the proposed action steps we recommend for *H. zea* should minimize the risk of resistance for this pest as well.

All new Bt cotton varieties being released express the Vip3Aa toxin. Initially these varieties will rarely be sprayed with insecticide targeting *H. zea*, since thresholds are based on live larvae or damaged bolls. Corn hybrids that express the Vip3Aa toxin are already commercially available, but so far do not make up the majority of acreage. However, the planting of these hybrids is increasing. Therefore, *S. albicosta* will be selected on Vip3Aa corn and *H. zea* will still be selected on Vip3Aa cotton and corn, with minimal opportunity for foliar insecticides to have an impact. Hence, we expect selection for resistance to Vip3Aa to be rapid and intense in this system. For *H.* zea, there are indications that resistance alleles in *H. zea* are already present at a frequency that cannot be considered low (Yang et al. 2019, 2020) and shifts are already taking place (Dively et al. in press). This is a rare case in biology, where we know exactly what will happen next. If the proposed guidelines are implemented, for *H. zea*, <u>we will sacrifice the enormous performance benefits of Vip3Aa in cotton, where it is a major economic pest, to protect field corn from an insect that is rarely an economic pest</u>.

There are many aspects of the proposal that differ from the recommendations of the 2018 SAP. Most alarmingly is the suggestion to phase out all corn pyramids without the Vip3Aa trait. One of the strongest recommendations of the panel was to prohibit the planting of Vip3Aa corn hybrids in cotton-growing regions, for reasons we will outline in detail below. A further recommendation was that development of the UXI threshold should be included in product registration materials. The panel also recommended that UXI thresholds should be crop and pest specific and that they should be higher than the economic thresholds, falling in the 1.5 to 2x economic threshold range. In these respects, and many others, the EPA proposal does not reflect the SAP recommendations.

The intended outcome of this letter is to encourage the EPA to modify the proposal to reflect the following key points:

1) Vip3Aa should not be available in field corn hybrids sold in cotton-growing regions and selectively deployed in the Midwest and Great Lakes corn growing regions where it is the only available PIP option for *S. albicosta* management

2) Only single toxin corn products should be phased out
3) Further PIP pyramid releases should coincide with the immediate phase-out of the old pyramid
4) Refuge in a bag (RIB) should be prohibited in cotton growing regions
5) A 20% block refuge for Vip3Aa hybrids should be required in the Midwest and Great Lakes corn growing regions
6) Implementation of sentinel monitoring program should be described in detail and made more prescriptive, including the use of sweet corn vs. field corn
7) Unexpected injury (UXI) thresholds should be set to reflect what was recommended by the SAP and published observations in the field
8) UXI events should be reported to the Extension specialist from the Land Grant University in that region within 24 hours of a suspected UXI event occurring
9) Registrants should be required, as a condition of registration, to provide purified Bt protein or standardized lyophilized plant tissue to public sector researchers to establish baseline resistance allele frequencies
10) Growers should adopt the best management practices (BMP) and IPM strategies already developed by their respective Land Grant Universities
11) Programs like the NRCS Conservation Stewardship Program should be continued and expanded to encourage growers to plant non-Bt refuge corn

This letter is broken into two sections, a section focused on *H. zea* and a section focused on *S. albicosta* to improve flow and readability. However, **the aims are united in the points listed above**.

Helicoverpa zea

Classification of a pest as non-high dose

The proposal indicates a "proposed new resistance definition for non-high dose pests", which are given as *H. zea*, *S. frugiperda*, *S. albicosta* in the framework. High dose PIP events have been defined by USEPA as producing a level of toxin 25 times greater than is needed to kill all susceptible insects. This definition was used, for example, by the USEPA (1998) Scientific Advisory Panel Subpanel in the registration of MIR162 (the transformation event to produce Vip3Aa). The SAP also outlined five techniques to determine high dose:

(1) Serial dilution bioassay with artificial diet containing lyophilized tissues of Bt plants (tissue from non-Bt plants serving as controls);

(2) Bioassays using plant lines with expression levels approximately 25-fold lower than the commercial cultivar (determined by quantitative enzyme-linked immunosorbent assay [ELISA] or some more reliable technique);

(3) Survey large numbers of commercial plants in the field to make sure that the cultivar is at the $LD_{99.99}$ or higher to assure that 95% of heterozygotes would probably be killed;

(4) Similar to #3 above, but would use controlled infestation with a laboratory strain of the pest that had an LD50 value similar to field strains;

(5) Determine if a later larval instar of the targeted pest could be found with an LD_{50} that was about 25-fold higher than that of the neonate larvae. If so, the later stage could be tested on the Bt crop plants to determine if 95% or more of the later stage larvae were killed."

However, we note that non-high dose is not clearly defined and that there is no differentiation among non-high dose events, or how changes in toxin expression during plant growth affect this definition. There is also no clear information stating how pyramided events should be considered for estimation of a non-high dose pest. If pyramided toxins are considered at the same time, for example by using plant material for bioassays as proposed above, then the redundant killing provided by the pyramid may occlude single toxin estimates of high dose.

In addition, we note that the evolution of pest resistance to non-high dose events, especially in pyramided products, may not always occur on a single allele or gene (e.g., Yang et al. 2020, Yang et al. unpublished data). Furthermore, we recognize that there isn't enough scientific information to accurately define what a non-high dose event is in all cases. However, we also suggest that the differences are not semantic, since the implications for resistance management of pests with different dose response susceptibility are great. For example, under low or moderate doses, more individuals heterozygous for a resistant allele will be selected (Tabashnik et al. 2004). Therefore, the refuge strategy may not be as effective.

One important consideration for classification of dose is the initial frequency of resistance alleles. Our knowledge of this for most pests with non-high dose events is scanty at best. Generally, resistance alleles are assessed after a PIP is released, making classification difficult. An additional consideration is geography. For example, one of the pests mentioned in the proposal is *H. zea*, which is panmictic. Because of this, if F_2 screens are used in one location, we might assume that these results could be applied across the Cotton Belt. However, we know in practice that current Bt resistance is incomplete and susceptibilities vary widely across geographies on local, regional, and national levels (*e.g.*, Bilbo et al. 2019a, Kaur et al. 2019, Arends 2020). In agreement with this observation, emerging evidence supports that resistance to Cry1F PIP in *S. frugiperda* develops locally, even though populations of this pest across the US are considered a panmictic population (Banerjee et al. 2017, Schlum et. al submitted manuscript). Therefore, how many populations should be tested and across how wide of a geography to categorize a product as non-high dose for a particular pest?

Knowing the initial frequency of resistance alleles is critical for later actions as well. For example, the proposal mentions that "If the estimated resistance allele frequency from the F2 screen is less than 2% then the pest population will no longer be considered at practical resistance and the mitigation actions will be withdrawn. Otherwise, the F2 screen will support the trigger of practical resistance and confirm the observed resistance is heritable." Without knowing the initial frequency of resistance alleles, there will be no way to measure changes over time. However, we do not have enough data to submit an alternative recommendation for frequency of resistance alleles to trigger actions on practical resistance.

Finally, we wish to bring the focus back to the first principles of resistance management: 1) limit use; 2) diversify use; and 3) segregate usage in space and time to produce refuges. While we may not have perfect knowledge concerning the dose required to delay resistance, we can employ these three simple principles of resistance management.

Sentinel plots

We agree in concept with EPA's proposal to implement sweet corn sentinel plots for monitoring field-evolved resistance in target pest populations, especially for the pests for which there is not a high dose product available. However, we have questions as to how these would be deployed. Will this be an obligatory charge or merely a suggestion to the seed company or Land Grant University Extension to use sentinel plots for resistance monitoring? Additionally, how would these be funded? How many in a given location and how many locations are adequate for resistance monitoring?

One potential consideration would be to use the existing Land Grant University Extension system to deploy a field corn sentinel monitoring program in cooperation with the Agricultural Biotechnology Stewardship Technology Committee (ABSTC). Some disadvantages to using hybrid field corn compared to sweet corn are that true isolines are not available, it is not as attractive for oviposition, and Bt expression in silks is not as high (Dively et al. in press). Therefore, we do not recommend replacing the sweet corn sentinel plot program proposal, but supplementing it with a field corn sentinel plot program. For example, some advantages are that field corn is easier to grow (in most of the southern US irrigation is not needed, for example, in contrast to sweet corn), standard planting configurations can be used, and it is potentially easier to deploy many more monitoring sites. For example, two hybrids that are from a similar genetic background (one with PIPs and one without) could be deployed in Official Variety Trials and other university variety trials for a very small fee. Since hybrids could potentially vary in their attractiveness to *H. zea* and response to feeding, equivalency tests could be performed to see if hybrids responded the same. Such an approach was used by Arends (2020) who deployed paired non-Bt and Bt corn plots across North and South Carolina from 2017 to 2019. During 2019, new seed for the Bt hybrid used during 2017 and 2018 was unavailable. Hence, both Bt hybrids were planted in 27 locations (in addition to the non-Bt hybrid) during 2019 to compare their performance to one another.

In addition to the BMPs proposed for sweet corn sentinel monitoring, we propose the following deployment and evaluation practices (Dively et al. in press): 1) sentinel plots should be planted on multiple dates at each location that includes at least one late planting so that silking occurs after *H. zea* has experienced a generation of selection in Bt field corn; 2) sentinel monitoring should also be conducted in northern locations, where European corn borer, *Ostrinia nubilalis*, is more prevalent and *H. zea* infestations result primarily from migrant moths coming from southern populations including those pre-selected for resistance in Bt corn—indeed many northern states in 2018 reported *H. zea* infestations in Bt corn (Unglesbee 2019); 3) Since *H. zea* has already developed resistance to Cry toxins, sweet corn sentinel trials at a minimum need only to include side-by-side plantings of Cry1Ab + Vip3A sweet corn and the non-Bt isogenic hybrid to monitor changes in susceptibility to the Vip3A toxin. The Cry1Ab + Vip3A sweet corn may be a better sentinel plant than sweet corn varieties expressing Cry1Ab or Cry1A.105 + Cry2Ab2 alone for detecting shifts in *O. nubilalis* susceptibility to Cry toxins because Vip3A has no effect on this pest and the absence of the more competitive *H. zea* larvae should allow more corn borers to survive and be exposed to Cry1Ab in the kernels. However, field corn sentinel trials in the south should also include Cry1A.105 + Cry2Ab2 (VT2Pro) and Cry1A.105 + Cry2Ab2 + Vip3A hybrids, since there is evidence of *H. zea* susceptibility to Cry2Ab2; 4) larger sample sizes of ears should be examined to increase the probability of detecting UXI and resistant larvae. A 30-ear sample is too small to detect early changes in *H. zea* susceptibility to the Vip3Aa toxin. We suggest a minimum sample of 500 ears from the Vip3Aa plots (note that as damaged kernel area decreases, the optimum sample size required for detection increases exponentially- see Figure 3 in Reay-Jones et al. (2018)); and 5) because toxin expression delays *H. zea* larval development, ear sampling during the milk stage should be conducted at different times to record the highest level of kernel damage and number of surviving larvae present, usually 5-6 days later for Bt hybrid compared to non-Bt.

Finally, Bt and isoline sweet corn (or near isoline field corn) paired side-by-side can lower the risk of UXI 'false positives' resulting from mismatches in pest pressure and allows for paired statistical analysis of differences between Bt and isoline plots of multiple trials. Yet, the EPA proposal does not mention any consideration of damage or larval development data from the non-Bt isoline plots. In addition to the EPA's UXI thresholds that trigger a "practical resistance" definition for *H. zea*, we propose that a significant decrease in control efficacy expressed as the difference in percentage of ears damaged between pairs of Bt and isoline sweet corn tracked over time is also evidence of resistance. Moreover, if sampled when the highest number of surviving larvae are present, the ratio of larvae per Bt ear to larvae per non-Bt ear provides an estimate of phenotypic frequency of resistance, and relative measure of changes related to the frequency of resistance alleles (*e.g.*, Venette et al. 2000). These data gathered from a sentinel network of multiple locations can be pooled by year in order to make inferences about the overall *H. zea* population based on differences in control efficacy and phenotypic frequencies of resistance.

UXI thresholds

Cotton

In general, we note that the UXI thresholds should be more specific. For instance, 6% injury could be calculated on per plant basis or a per fruiting form basis. We propose that injury should be specified as a single penetration event though the carpel wall of an individual fruiting form, which could be defined as squares, when bolls are not present, or bolls when they are present.

We also propose that the scouting method should be better defined. This is not an easy task, since most UXI events will be noticed after the insects have fed extensively, potentially pupating in the soil and causing abscission of damaged tissue. Therefore, we propose that each UXI event should present the opportunity, without obligation, to be verified by an Extension specialist from the Land Grant University in the respective state to avoid bias toward or against the UXI threshold. Moreover, the Extension specialist should be notified within 24 hours of a suspected UXI event occurring.

Corn

As in cotton, we note that the UXI thresholds should be more specific, but higher than those proposed for Vip and lower for Cry2A corn. We propose that the UXI thresholds should apply to measurements that include both unpollinated kernels at the tip and pollinated kernels on the cob. The reason for this is that Bt concentrations are the same, or greater, in the tip compared to kernels (Bilbo et al. 2019b). Furthermore, it is not uncommon for >50% of the area on a corn ear damaged by *H. zea* to occur on unpollinated kernels at the tip (Bilbo et al. 2019b, Bilbo et al. 2020). For instance, Bilbo et al. (2020) report injury to pollinated kernels (4.3 cm^2 in Cry2A corn and 4.7cm^2 in non-Bt corn) and to unpollinated kernels (3.6 in cm^2 in Cry2A corn and 2.5cm^2 in non-Bt corn). Some of our members have noted (unpublished observations) that *H. zea* can complete development by feeding on silks and cob tips only, without consuming unpollinated or pollinated kernels. While we note that this does not match the findings of Bilbo et al. (2019b) concerning toxin expression, we note that toxin expression may vary by PIP event, hybrid, and environment. Furthermore, *H. zea* exhibits behavioral feeding plasticity, with aversion to diets with Bt (Hoy et al. 1998, Gore et al. 2005, Carrière et al. 2010, Caprio et al. 2015, Zalucki et al. 2017). Therefore, using area or number of kernels consumed may underestimate UXI. One suggestion was to use percent infested ears with larvae that are at 2nd instar stage or older. However, we cannot agree on a specific percentage for a UXI threshold.

The UXI thresholds are clearly too high for Cry2A corn. If they were lowered, practical resistance could easily be demonstrated and UXI from *H. zea* to Cry2A corn is not unexpected. Although resistance is incomplete in time and space, it is widespread (e.g., Dively et al. 2016, Bilbo et al. 2019a, Kaur et al. 2019), and relatively high injury levels in corn expressing Cry2A toxins, although incomplete in time and space, are not unexpected. For example, Bilbo et al. (2019a) noted only 2-4 cm^2 was damaged from *H. zea* feeding on non-Bt corn. Furthermore, Reay-Jones et al. (2018) sampled both Bt and non-Bt corn from 2012 to 2017 and never exceeded 8 cm^2 of kernel damage from *H. zea* across those years. It is important to note that both of these studies reported damage from pollinated kernels only. Because injury to pollinated kernels, even in non-Bt corn, is frequently less than the UXI proposed for Cry2A corn, we propose that the Cry2A UXI threshold be lowered to that of Vip.

Moreover, the UXI thresholds should be different for field corn and sweet corn. Expression of Bt is thought to be higher in sweet corn than field corn (Dively, unpublished data, but referenced in Dively et al. submitted), but sweet corn is more attractive to *H. zea* for oviposition than field corn. Moreover, sweet corn thresholds for economic injury are much lower than those for field corn. Thus, we submit that the UXI thresholds be based on realistic levels observed in the field for sweet corn (Dively et al. 2016, Dively et al. submitted) and field corn (Reay-Jones and Reisig 2014, Bibb et al. 2018, Reay Jones et al. 2018, Bilbo et al. 2019a, Olivi et al. 2019). For example, we already have data from the ongoing sentinel monitoring network that Cry1A.105+Cry2Ab2 expressing sweet corn exceeded the threshold of 10% of ears with greater than 8 cm^2 of kernel consumption caused by late instars along with exit holes. Under high population pressure, it is common to find multiple ear infestations of late larvae and extensive kernel damage in Cry2A ears, whereas infestation levels and damage are usually less in field corn.

In addition, sampling time relative to the UXI is important. For example, sampling at R3 would be appropriate to note what species are present in the ear and to assess percent infestation. It would be too early, however, to assess the extent of feeding. In contrast, sampling at R5 is appropriate, in most situations, to assess the extent of feeding. However, many larvae will have completed development at this time and it might not be apparent what species were present. Moreover, sap beetles (Nitidulidae) are common secondary feeders that often move in behind ears damaged by ear-feeding lepidopteran pests. These pests add an additional potential confounding variable to assess ear feeding as time passes from when ear-feeding lepidopteran pests exited the ear.

Furthermore, both *H. zea* and *S. frugiperda* can develop on corn at even later stages than R5. In particular, *S. frugiperda* can commonly be found on ears up to harvest, especially in late planted corn. When Cry1F-resistant *S. frugiperda* were first detected in the mainland US (Huang et al. 2014), the only indication that they were present was from ears dropping in the field. These larvae were feeding on physiologically mature corn ears on the kernel, cob, and tunneling into the shank causing the ears to drop. This most certainly was a UXI event, but would not fit the current framework proposed by the EPA. Finally, since field corn is not routinely sampled, UXI events will likely be reported too late to tell what species were present and may not fit the proposed UXI thresholds.

In conclusion, our groups wish to covey to the EPA that the UXI thresholds should be changed. One suggestion was to use percent infested ears with larvae that are at 2nd instar stage or older. However, we cannot agree on what these UXI thresholds should be. A major reason for this is we are not comfortable with a standard number given the variability in insect pest pressure, Bt toxin expression, time during the growing season, etc. A good standard would be to set an UXI threshold relative to damage present in non-Bt refuge corn planted by the grower with the UXI event. Nonetheless, we realize that this may not be practical given variability in planting date, hybrid maturity, geographical location, etc. Furthermore, if the EPA's suggestion to allow RIB to be planted across the US is implemented, assessing UXI will be nearly impossible to identify, since many non-Bt ears will be infested, even allowing survival on Bt ears from cross pollination with non-Bt plants in the same field. Finally, we encourage the EPA to work with both of our groups on developing a robust UXI threshold. Recognizing this gap in knowledge, our groups are working on gathering data a method development for this purpose.

Both Cotton and Corn

The UXI thresholds should also apply to *S. frugiperda*. However, we also recognize that efforts to delay resistance for this pest may be less than optimal, since this pest likely does not overwinter in most of the mainland U.S. As a result, resistance that is detected in the mainland U.S. likely developed elsewhere, and resistance management actions in the mainland U.S. will have minimal impact.

F_2 screen methodology

We note that seed companies will be tasked with developing standardized methodology. However, we also note that commercially available diets should be modified to achieve the proper protein to carbohydrate ratio, to maximize larval survival when exposed to Bt (Deans et al. 2017).

Furthermore, we request the EPA to go beyond "strongly encouraging" seed companies to provide standard toxin stocks and traited seed to public sector researchers. If past experience is a guide, this must be mandated. Researchers require access to these proteins for independent confirmation and access has been routinely denied in the past, even after verbal and written assurances that toxin or seed would be provided. This has occurred as recently as 2020, the year of this letter. Providing protein and traited seed to the research and Extension scientists either free of charge, or for a reasonable fee, should be required contingent on registration of the product. While we are open to relaxed research agreements between the seed companies and public sector researchers, these have stalled and are still not in place. As a result, there are instances where independent research cannot be conducted because it is not approved by the seed company.

Another suggestion would be to require seed companies to allow comparison among their patented toxins and toxins with amino acid similarity that are publicly available and can serve as surrogates. Researchers could then compare the dose response curves (e.g., Welch et al. 2015) to make even more meaningful conclusions. However, without access to protein and traited seed, this will not be possible.

BMPs in Cotton and Corn following an UXI event

We would like to emphasize our strong belief that proactive measures to delay resistance to PIPs will be more effective than reactive measurements to contain resistance once it has evolved. Our shared past experiences across systems is that attempts to mitigate resistance after an UXI event are largely ineffective. This could be for many reasons, including lack of timeliness to implement the containment measure, lack of effective mitigation tools, high degree of pest mobility, and the parallel evolution of resistance in multiple areas outside the area of the UXI.

One of the proposed actions is to apply a foliar insecticide "only if economically viable in corn". This is problematic in sweet corn, as well as in field corn. In most cases, if the UXI is exceeded in sweet corn, it is too late to salvage that crop and it would likely not be marketable. As a result, that corn will sit in the field until the grower can get around to destroying it. At that point, the cost of an insecticide application is an added cost to the grower. Moreover, in field corn, *H. zea* is the most common ear-feeding pest across the cotton growing region, but typically does not limit yield in corn (Reay-Jones and Reisig 2014, Bibb et al. 2018, Olivi et al. 2019). An exception is the extreme southern-growing subtropical regions where *H. zea*, *S. frugiperda*, and weather can interact to decrease yield or increase aflatoxin levels (Dowd 2000, Bowen et al. 2014, Wahl et al. 2016, Weaver et al. 2017, Pruter et al. 2019, Pruter et al. 2020). Therefore, *H. zea* is rarely a target of insecticide applications. Moreover, foliar insecticides are generally ineffective once pests enter the ear, begging the question as to when a foliar spray will be economically viable in field corn once UXI has been observed. Crop destruction and tillage have been proven effective to kill nearly all *H. zea* pupae if "deep tillage" (*e.g.*, moldboard plowing) is used (Fife and Graham 1966, Hopkins et al. 1972, Young and Price 1977). However, if these practices are required following an UXI, how will growers be compensated for the cost associated with the tillage and potential degradation of soil? Additionally, very few growers possess the tillage implements needed to accomplish "deep tillage".

Another potential issue in corn is that, if RIB is permitted (which we do not recommend in cotton growing regions), then an UXI event in these fields will be very difficult to detect and always subject to extensive and protracted debate. A review of 14 studies on RIB showed that *H. zea* survival was only reduced by 16% in non-Bt plants compared to those in structured non-Bt refuge (Onstad et al. 2018). While these studies were from hybrids that did not express Vip3Aa, a recent study showed only a 27.5% reduction of *H. zea* survival in non-Bt plants in a RIB with a Vip3Aa-expressing hybrid compared to those in structured non-Bt refuge (Dimase et al. 2020). Therefore, a majority of non-Bt ears in a RIB field with a Vip3Aa-hybrid will likely contain a *H. zea* larva and artificially skew the detection of an UXI event.

For the UXI thresholds listed for varieties expressing both Vip3Aa and Cry2A in cotton, UXI events will occur frequently. In areas of the Midsouth (Arkansas, Louisiana, Mississippi) and Southeast (*i.e.*, North Carolina) where *H. zea* pressure in cotton is heavy, varieties expressing Vip3Aa annually exceed 6% damaged bolls, especially later in the season as toxin expression likely wanes and population sizes increase. If an insecticide application is required, will the grower be required to pay for this application? Who determines if follow up sprays are needed? Finally, what evidence is there that a foliar spray will have any impact on resistance alleles in the population? Because of these concerns, we propose that the UXI threshold should follow the recommendation of the SAP- "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. Vip3Aa cotton cultivars have not currently been widely adopted. *H. zea* remains more susceptible to Vip3Aa compared to the Cry proteins. The UXI recommended in Vip3Aa cotton varieties should be when a sample of fruiting forms are damaged at 9-12% and larvae (L3 stage or greater) are present."

BMPs for the next growing season are likely to change little in practice, since the recommended management options are only "recommended" and will be interpreted as optional. We note that there needs to be some definition of what constitutes a BMP. For instance, the endophytic entomopathogenic seed treatments that EPA mentions have not been properly tested. The National Cotton States Arthropod Pest Management Working Group has put together a BMP document for this specific purpose. Therefore, we recognize that it is our responsibility as Land Grant University scientists to help growers implement the BMPs and not the EPA's.

Finally, we also question the value of increased communication, since previous communication efforts have proven ineffective to increase the amount of non-Bt corn refuge growers intend to plant (Reisig 2017). That is to say, while we are supportive of communicating the value of refuge compliance to resistance management, past communication has been shown to be ineffective without addressing the larger problem of the evolution of resistance as a wicked problem (Gould et al. 2018). Other interventions will be necessary to increase refuge compliance beyond communication and could include things proven to increase refuge compliance such as moral suasion (Brown 2018). The proposal already encourages increasing mandatory on-farm visits. Later in this letter we touch on this subject, but we might assume that this will increase costs and time-burden for seed companies and for Land Grant University Extension. One possibility would be to ask seed companies to shift the resources required for increasing on-farm visits (the stick approach) to increasing efforts for moral suasion (the carrot approach). Another would be to partner with USDA-NIFA CPPM and develop dedicated resources there for Land Grant University Extension scientists to develop effective programs for changing behaviors in the farming community.

Annual reporting

The EPA proposes additional reports on incidences of insecticide sprays in Bt fields as an early warning indicator of UXI. Many in this group already publish such losses annually in the Cotton Insect Losses. Since UXI will be reported to Extension, we could add number or acreage of UXI events to these losses.

Phase down of single traits and non-functional pyramids

The EPA and SAP designated single toxin corn as a risk factor. Single Cry-toxin corn provides little to no selection pressure for resistance in *H. zea*. It could be argued that pyramids are contributing to increased resistance in this insect because they are more selective. For the long-term phase down (5 years), the levels of resistance to Cry proteins will likely be so high that corn with Vip3Aa will functionally be a single trait event. For example, Kerns et al. (unpublished data, supported by a USDA NIFA BRAG funding source) measured *H. zea* resistance levels across the Midsouth from 2016 to 2019 using diet overlay bioassays. They found that average resistance ratios (RR) were very high:

SUMMARY OF RESISTANCE MONITORING, INHERITANCE TESTS AND F2 SCREEN ALLELE FREQUENCIES FOR *H. zea* TO Bt PROTEINS

	Percentage of populations with RR > 10X				Inheritance characterization			2019 Allele frequency
***Bt* protein**	**2016**	**2017**	**2018**	**2019**	**Sex linkage**	**Genes**	**Dominance**	
Cry1Ac	40%*	100%	94%	96%	Autosomal	Monogenic/ polygenic	Incomplete-completely dominant	(0.42-0.49)
Cry2Ab2	80%	77%	73%	73%	Autosomal	Polygenic	Incompletely dominant/recessive	(0.07-0.10)
Cry1F	ND	100%*	100%	100%	ND	ND	ND	ND
Vip3Aa39	0%	0%	0%	0%	Autosomal	Monogenic	Completely recessive	0.0065

Moreover, we will likely be starting to detect resistance to Vip3Aa by the time the five-year phase out is complete. This amounts to sacrificing the performance of Vip3Aa in cotton in the southern US for an insect that is rarely an economic pest in field corn. It begs the question – what is the next step once this inevitability is reached? There are no indications that a new suite of Bt or other PIPs will be available commercially at that time.

A recent model showed that including Vip3Aa in corn will hasten resistance in Vip3Aa cotton (Caprio et al. 2019). Furthermore, the 2018 SAP recommended "the prohibition of the use of field corn cultivars which produce Vip3Aa insecticidal proteins in the South" (page 13 in the section entitled "The Panel Recommended the Following"). Work in resistant *Diabrotica virgifera virgifera* in the Midwestern US Corn Belt provides a useful cautionary tale, where widespread Cry3Bb1, mCry3A and eCry3.1Ab resistance meant that Cry34/35 toxins essentially stood alone where they were stacked in hybrids, resulting in high selection pressure and relatively rapid, and entirely predictable, resistance to Cry34/35 (Gassmann et al. 2016), leaving farmers with no reliable options for PIP hybrids in the region.

Furthermore, the Roush (1998) definition of a compromised Bt pyramid "where less than 95% control is provided by one or more of the toxins expressed therein" (based on modeling) is not feasible to measure in the field. In order to measure this, we would need to know what percentage of insects that successfully eclosed from eggs survived to contribute to the next generation. Therefore, how can we deem a pyramid as functional based on UXI alone? Our suggestion would be to eliminate the definition of a compromised pyramid proposed by Roush (1998). As mentioned earlier, we propose that a significant decrease in control efficacy expressed as the difference in percentage of ears damaged between pairs of Bt and isoline sweet corn tracked over time should be used as evidence of resistance.

The SAP did not make a specific recommendation to define a UXI event in corn. Furthermore, the suggestion to set the UXI in the 1.5 to 2x economic threshold range is not helpful since there is no economic threshold for *H. zea* in corn. The UXI event in Bt corn on a given farm could be tied to non-Bt corn on the same farm, but differences in relative maturity of the hybrids, placement of the refuge relative to the UXI event, planting date differences, and the assumption that the grower planted refuge complicates the issue. As mentioned previously, we cannot provide guidance on what the UXI threshold should be.

Finally, the overlap of pyramids with single toxin products in the US has likely hastened the evolution of resistance (Carrière et al. 2016). <u>We recommend a phase down of single Bt-trait corn products only</u>. This would allow the potential planting and development of pyramided corn hybrids without Vip3Aa, relaxing some of the selection pressure on this toxin. Furthermore, it would provide pyramided protection against other pests still susceptible to Cry toxins. Finally, we recommend that <u>further PIP pyramid releases coincide with the immediate phase-out of the old pyramid</u>, similar to the Australian example highlighted in Carrière et al. (2016).

Increasing Percent Refuge in Seed Blend Products

The proposal is unclear with the statement “increased refuge in all pyramided RIB products to 10% nationwide”. As mentioned in the proposal, the 2018 SAP recommended a prohibition of RIB in southern corn-growing regions. There should be no need for RIB in the cotton-growing regions with the following statement in the proposal to “maintain current requirements to plant a separate 20% block refuge in cotton growing states”. We take this to mean an increase in percent RIB corn in non-cotton growing regions and where RIB is planted without a structured refuge. Furthermore, the differential survival hypothesis (Mallet and Porter 1992, Gould 1994) could be the most important thing influencing the evolution of resistance, if individuals heterozygous for resistance alleles are selected in blended refuge ears (Brevault et al. 2015). A recent model for the cotton-growing regions suggested that RIB was inferior to delay *H. zea* resistance to Vip3Aa, even when block refuge compliance was only 25% (Caprio et al. 2019). Therefore, all RIB hybrids should be prohibited in these regions.

Refuge compliance

The EPA’s suggestion to require annual reporting for production of refuge seed will likely increase refuge planting. If the seed companies have inventory (supply), they will be incentivized to create a demand to exhaust that inventory. However, these hybrids must be advertised and supplied to distributors and retail sales locations. We commend the proposal for asking seed providers to ensure availability of elite non-Bt hybrids for refuge. In our previous conversations with seed providers, however, they have assured us that these hybrids are available. Moreover, they have mentioned that growers will not buy all these seeds, creating excess inventory for the distributors. Growers, however, often complain that they are not available, or that the hybrid doesn't have a characteristic that matches their farming operation (e.g., a non-Bt hybrid that is both Roundup Ready and LibertyLink may be available from Company A, but not Companies B, C, and D). Thus, asking for documentation of adequate seed production for block refuge may not have the desired effect.

We have recently become aware of an NRCS Conservation Stewardship Program that rewards growers who plant Bt refuge. We see this as one of the best ways to increase compliance. Perhaps the EPA, NRCS, Research/Extension, Industry (Cotton Incorporated, National Cotton Council, Cotton Trust Protocol, etc.), and seed companies can communicate to develop an integrated program that first increases compliance and second increases refuge size.

Finally, we have many questions about the increase in mandatory on-farm visits. However, the proposal is too general for us to generate comments. A simple way to obtain evidence would seem to be to require growers planting Bt corn in the cotton growing regions to show a receipt of sale as evidence that they purchased enough non-Bt seed according to their total corn acreage. However, we recognize growers could buy non-Bt seed up-front and then turn it in later saying they couldn't get it planted. Sometimes this is actually the case when weather turns bad.

In conclusion, we would like to simplify refuge compliance. Our group's unified consensus is that structured refuge in any form is a positive step toward resistance management. Although we do not have any unified recommendations relative to increasing refuge at this time, we encourage EPA to support proposals to integrate new ideas that can increase structured refuge by any amount.

Striacosta albicosta

This section specifically addresses the EPA proposed framework, point by point, for western bean cutworm (*S. albicosta)*. The comments below are from the perspective of Great Lakes region, the northeastern US, and eastern Canada.

(1) "The Agency supports the unanimous consensus of the SAP regarding documented cases of Bt resistance for ... *Striacosta albicosta* for specific Bt toxins."

- We are glad to see that EPA addresses *S. albicosta* throughout the framework document. *Striacosta albicosta* is a key pest some key corn production areas, and its status has only increased after the field failures of Cry1F hybrids in 2016/2017. Changes to the regulation and deployment of Vip3Aa toxin, based on the adoption of this framework, will greatly impact our efforts to manage WBC in the future, and not always in a positive way.

(2) "Proposed new resistance definition for non-high dose pests"
(given as *H. zea, S. frugiperda*, *S. albicosta* on pg. 10 in the framework)

- Similar to our comments in the *H. zea* section, the term 'non-high dose pest' isn't clear with regard to *S. albicosta*. Is the attempt to replace the older terms 'primary' and 'secondary' with 'high dose' and 'non-high dose'? If so, the terms aren't equivalent. Primary and secondary were used to indicate importance of a species as a pest or Bt corn target (range across the corn belt, number of acres infested, yield and quality lost). *Striacosta albicosta* has become a primary pest in parts of its expanded range, especially in the Great Lakes region. High versus non-high dose indicates the effect of Bt expression in the crop in relation to a given pest target. The Vip3Aa toxin is considered near-high dose in Vip3Aa-expressing corn (USEPA 2009) for *S. albicosta*, but this is true only during the early instars (Farhan et al 2019). Later instar larvae are much more tolerant to Vip3Aa toxin and this situation would not be considered high dose (Farhan et al. 2019).

(3) "Practical resistance will be defined as unexpected injury (UXI) that exceeds established levels in Bt corn. For *S. albicosta*, given as "a 30-ear sample, 10% of ears have second instar larvae or an exit hole and 60 damaged kernels (2 kernels/ear) or more than 75 cm^2 injury (~2.5 cm^2/ear) with second instar larvae present or exit holes" on pg 11.

- We do not know where this UXI level originated for *S. albicosta*, especially its focus on second instar larvae and on kernel feeding. First to third instar larvae initially feed in the tassel, on pollen in the leaf axils, or on silks. At 10 days after hatch, DiFonzo (2019) reported that 93% of larvae were still in axils or on silks. Third and fourth instars were recovered in the ear tip at 14 days after hatch. Only after 21 days were all larvae (by then, 4th and 5th instars) feeding directly on the ear. Later instar larvae often feed at the ear tip on moist cob tissue or undeveloped kernels, without damaging many whole kernels. Finally, a 30-ear sample is too small in our opinion to detect UXI in the field (see the discussion below about seed blends). *Striacosta albicosta* lay eggs preferentially on pre-tassel or tasseling corn. To account for variability in plant stage across a field (especially with varying soil types or topography common in our region), we recommend scouting multiple points in a field and checking many more ears to detect infestations. Thus, a more accurate UXI for *S. albicosta* should be developed to reflect how it typically infests and damages field corn.

(3) Proposed resistance monitoring: To monitor for UXI, sentinel plots will be implemented in high risk regions of the corn belt for *S. albicosta*.

- The entomologists in areas at risk for *S. albicosta* infestation support the idea of sentinel plots for *S. albicosta*. In fact, in 2020 several of researchers in the affected region recorded *S. albicosta* larvae and damage in the current sweet corn sentinel plot network for earworm. Many questions remain about EPA's vision of a sentinel plot program. The questions are similar to those spelled out in the *Helicoverpa zea* section of this letter and do not need to be repeated here. Since Vip3Aa is the only toxin effective against *S. albicosta*, a sentinel program could be targeted to just Vip3Aa and isoline hybrids but include several planting dates to overlap moth flight. As with *H. zea*, a sample size of 30 ears is too small to detect initial changes in *S. albicosta* susceptibility to Vip3Aa or future toxins, and thus many more ears will need to be evaluated.

- The proposal indicates that sentinel plots may be managed by industry or academic partnerships; however, for regions that may not be considered "high risk" or for smaller markets industry interest may be lower and these regions may be overlooked. Therefore, sentinel plots that may be implemented by public researchers should also be considered in this monitoring effort. The proposal states that EPA will accept registrant data submissions or reports from peer-reviewed literature; therefore, in accordance with the previous recommendation, we recommend that reports from annual academic research efforts should also be accepted for consideration.
- We also caution against efforts for resistance monitoring of "non-high dose pests" solely in "high risk areas" as there is no clear definition of these regions. In fact, the risk of resistance development may actually be elevated on the margins of the corn belt where pockets of production fields are geographically isolated (for example, in the Upper Peninsula of Michigan, central Pennsylvania, the Canadian Maritimes). The recent development of Cry1F resistance in *O. nubilalis*, which is considered a "high-dose pest", occurred in Nova Scotia (Smith et al. 2019). This province was not traditionally considered high risk due to small acreage/market share, diverse crop rotations, and a relatively short growing season.

(4) EPA recommends that F2 screens be used to monitor for arising resistance cases instead of UXI alone.

- F2 screening may provide a proactive approach to determining changes in resistance allele frequency, allowing for the detection of early warnings of resistance. Specimens may be collected from sentinel plots and screened annually; however, the target of 400 insect genomes from a UXI field or sentinel plot to establish a collection may not be attainable, particularly when targeting collections from sentinel plots or UXI fields with seed blends. As has been observed with resistance monitoring efforts for the high dose pest *O. nubilalis*, it is often very difficult to obtain even 50-100 individuals from seed blend fields until resistance has developed. Therefore, a lower target of 50-100 individuals should be acceptable for early detection of resistance. Furthermore, infestations of *S. albicosta* vary significantly in size and timing from year to year in the Midwest and Great Lakes regions creating challenges in making large collections each year.
- We note that registrants will be tasked with developing standardized methodology for F2 screening; however, we would encourage collaboration with public sector researchers. There are questions regarding bioassay methodology, including diet and rearing optimization that require further investigation. These are lessons that we can learn from the western corn rootworm resistance story and subsequent screening hurdles.
- We continue to see the value in baseline susceptibility determination for high and non-high dose pests against Bt toxins for resistance monitoring efforts; however, the greatest limitation of this work is the ability to relate laboratory-derived results with Bt protein expression *in planta*. We strongly encourage EPA to require registrants to allow evaluation of Bt protein expression *in planta* by public researchers as a condition of registration and to provide purified Bt protein or standardized lyophilized plant tissue. This is key information

that would better inform resistance monitoring efforts. We have not had access to this in the past, and it has been a "rate-limiting step" in addressing resistance development. Again, these are lessons that can be learned from recent past experiences.

(5) Proposed enhanced resistance mitigation / BMPs (Table 1 on page 15)

- In the current and next growing season, one proposed action is to apply an appropriate foliar insecticide. This is problematic in field corn for *S. albicosta* and for any ear-feeding pest, including *H. zea* and *Spodoptera frugiperda*. Foliar insecticides applied once caterpillars enter the ear zone are generally ineffective and therefore have almost no impact on resistance allele frequency. Furthermore, *S. albicosta* typically does not reduce yield in corn but instead impacts grain quality due to exacerbation of infection by mycotoxigenic fungi (Parker et al. 2017, Smith et al. 2018).
- Another proposed BMP is "...additional control tactics as appropriate (e.g., additional foliar insecticide applications, tillage practices, etc.) ". These practices are not effective control strategies for *S. albicosta*, nor is crop rotation. "Timely planting" to avoid *S. albicosta* is difficult (if not impossible) to implement since the overlap of moth flight with an attractive stage for egg laying typically covers weeks and is not easy to predict for an individual field. Furthermore, switching to a different Bt mode of action is not an option since the Vip3Aa toxin is the only effective trait against *S. albicosta*. Ultimately, if the Vip3Aa trait fails and there is no new transgenic option for *S. albicosta* control, growers will need to follow IPM recommendations to scout for egg masses and apply insecticide when thresholds based on egg mass infestation are exceeded. Currently, there are no economic thresholds based larval infestation or feeding.
- Finally, the BMPs encourage "assessment of fields adjacent to the affected field with similar trait(s)". *Striacosta albicosta* is a strong flyer, moving around the landscape to find pre-tassel corn fields for egg-laying, or moving into dry beans (where the two crops coexist) when local corn fields are past their prime for egg laying. When the Cry1F trait failed in the 2016 season, UXI fields in the Great Lakes region were not distributed as point sources of adjacent fields, but instead were spread widely across the landscape. If UXIs are identified for *S. albicosta,* communication efforts or BMP recommendations for Bt resistance mitigation should be deployed on a much broader regional basis.

(6) Proposed annual reporting: EPA will continue to require refuge compliance reports from ABSTC and UXI notification from individual registrants.

- As with other pest Lepidoptera, industry reports about *S. albicosta* should be shared by EPA so that public sector entomologists and extension personnel have timely notification of pending resistance issues. Ideally, notification should be done within 24 hours of a suspected UXI event, as proposed in the *H. zea* section. A regional communications platform with registrants and regular teleconferences with EPA will certainly help. We have not meaningfully done this with past resistance issues, to our detriment.

(7) EPA is proposing as part of this draft framework to receive records of "total acres treated reports for insecticide over-sprays targeted at pests in various registered Bt crops".

- Insecticide applications in field corn are not a good indication of UXI for *S. albicosta,* as foliar insecticides may be targeted for other corn pest. In the Great Lakes region, they are also increasingly applied in a routine manner with fungicides during the early reproductive stages of corn development. Furthermore, reporting will be impractical and difficult to do well. How will registrants know when a grower applies an insecticide and how can they compel an individual grower to provide this information?

(8) Short-term phase down of single trait corn products (Cry1F and Cry1Ab, Table 2, pg 18)

- Cry1Ab does not control *S. albicosta* and Cry1F no longer has *S. albicosta* on the label because of widespread resistance. Thus, eliminating single trait Cry1Ab and Cry1F hybrids has no impact on *S. albicosta* management or resistance development. However, phasing out single-trait hybrids may be important for other Lepidoptera species, especially *O. nubilalis*. For example, Cry1F resistance in Canada was linked to the widespread planting of single traits hybrids in isolated production areas (Smith et al 2019). Given the importance of maintaining Bt susceptibility in *O. nubilalis*, is three years 'short term' enough and can a quicker (by next season) phase out be accomplished?

(9) Long-term phase down of compromised pyramid corn and cotton products "to a minimal acreage cap". (pyramids listed in Table 3, page 18)

- None of the corn pyramids listed in Table 3 control *S. albicosta*; as with Cry1Ab and Cry1F alone, a phase out of these pyramids would have no impact on this pest. However, in our region, these 'non functional' pyramids are still functional for the second key pest, *O. nubilalis*. If the proposal to phase out the pyramids in Table 3 is being driven by industry to make hybrid development more efficient, then that is a market decision. But if it is purely an Agency proposal, it doesn't make sense for field corn in the northern corn belt.
- Furthermore, the idea of a minimal acreage cap is not explained. How minimal is 'minimal'? How would that level be set, and then acreage parsed out by pyramid or by company? Would the cap allow planting of pyramids only where they are still effective? It is difficult to comment further about this part of the proposal without more information

(10) Traits unaffected by phase down (Table 4, page 18)

- As proposed, only corn hybrids which include Vip3Aa will be sold five years from now; therefore, Vip3Aa will be in every Bt corn acre. For *S. albicosta*, Vip3Aa is currently the only effective Bt protein available; therefore, all hybrids expressing Vip3Aa are functionally single toxin products targeting this pest. It is easy to envision what happens next – resistance will likely develop in short order. If single toxin products are to be phased out (see Table 2), it is contradictory that Vip3Aa hybrids could still be deployed in regions where *S. albicosta* is a target pest (i.e. Midwest Corn Belt and Central Plains).
- Furthermore, it can be assumed that the proposed extensive deployment of Vip3Aa hybrids will compromise any future pyramid products containing Vip3Aa for *S. albicosta* control. In fact, these won't be pyramids at all. Models show that resistance evolution to Bt proteins deployed in a pyramid, whether high or non-high dose, is accelerated following long-term use of single Bt product (Onstad and Meinke 2010). For non-high dose pests, there are two

recent examples. As pointed out in the *H. zea* section, *D. virgifera virgifera* developed resistance to Cry3Bb1, mCry3A and eCry3.1Ab, leaving Cry34/35 as a functional single toxin in pyramided hybrids, predictably resulting in high selection pressure and relatively rapid resistance to that toxin (Gassmann et al. 2016). A more relevant case is that of *S. albicosta* resistance to Cry1F. Pyramids with Cry1F were true pyramids for most corn lepidopterans, but single trait for *S. albicosta.* Resistance evolved in a relatively short time, especially after the deployment of seed blend refuge (Smith et al. 2017, Coates et al. 2020).

(11) "Increasing Percent Refuge in Seed Blend Products: An increase from 5% refuge-in-a-bag in Bt corn pyramid products to 10% refuge nationwide."

- Increasing the percentage of non-Bt plants in Vip3Aa hybrid seed blend mixtures may increase and accelerate the risk of *S. albicosta* resistance to Vip3Aa. *Striacosta albicosta* larvae move from plant to plant in later instars (Hagen 1962, Pannuti et al. 2016). It is well documented that older larvae of many Lepidopteran species survive higher doses of Bt than younger larvae. Since older *S. albicosta* larvae have much greater (over 58-fold) tolerance to Vip3Aa than first instars (Farhan et al. 2017, Farhan et al. 2019), increasing the proportion of non-Bt plants will allow greater numbers of larvae to develop to older instars before moving onto Vip3Aa plants. The higher probability of older larvae surviving their first dose of Bt will drive resistance evolution. Additionally, refuge contamination due to cross-pollination between non-Bt and Bt plants may be worsened by increasing the refuge percentage to 10%. Cross-pollination between refuge and Bt plants results in the expression of Bt in refuge ears, which renders them non-functional as a refuge. Furthermore, cross-pollination results in reduced expression and doses of Bt toxins in Bt ears (Chilcutt and Tabashnik 2004, Burkness et al. 2011, Onstad et al. 2011). This is especially concerning for ear feeding insects like *S. albicosta*. Cross-pollination between the Bt and refuge may result in an increase in the number of survivors in the field on the Bt corn because of the reduced dose but may not translate in the laboratory when conducting diet bioassays.
- As indicated in the *H. zea* section, increasing the seed blend refuge to 10% as proposed further complicates the proposed UXI threshold of "10% of ears with second instar larvae or an exit hole". This level of injury may be dismissed by growers or scouts as larval feeding on refuge plants, resulting in delayed detection, reporting and mitigation.

In the Midwest and Great Lakes corn growing regions, we believe that a 20% block refuge should be required for Vip3Aa hybrids which, regardless of pyramid, only express one Bt protein targeting *S. albicosta*. A block refuge would reduce the risk of larval movement between Vip3Aa and non-Vip3Aa plants. Furthermore, the block could be treated with fungicide targeting ear mold pathogens exacerbated by the presence of *S. albicosta* damage, as well as harvested and handled separately if mycotoxins are a concern. In years with favorable environmental conditions for infection by mycotoxigenic pathogens, the block refuge may be sprayed with insecticide targeting *S. albicosta* if needed. The structured refuge may be a more manageable size for growers to scout for insect injury and provide a clear comparison when UXI develops. Furthermore, we recommend that Vip3Aa hybrids are not grown frequently in this region and that growers utilize IPM practices in non-Vip3Aa hybrids to scout for egg masses and apply insecticide in response to exceeded thresholds.

(12) Refuge Compliance Monitoring: Mandatory registrant on-farm refuge compliance visits, measures for non-compliant growers, increased seed dealer record keeping.

- We have no specific comments for *S. albicosta* that are different from those provided for *H. zea.*

Conclusions for both *Helicoverpa zea* and *Striacosta albicosta*

Again, the groups (NCSAPMWG and NC246) applaud EPA for addressing this important issue in U.S. agriculture. One major weakness in the entire system has been a breakdown in communication between all of the parties involved. We would encourage the EPA to seek involvement and advice from partners at Land Grant Universities on issues related to the performance of PIP's and IRM in the various regions of the country. Given the necessity of cooperation and coordination of industry, academic and federal partners across geographies (and nations) we would also request that EPA provide funding support for many of these activities. Corn hybrids and cotton varieties that express PIP's have revolutionized insect pest management across the U.S. and their continued success is vital to maintain a thriving agricultural industry. With the current challenges of resistance and potential future challenges, it will become more important for everyone involved to communicate and work together to solve these challenges. These groups include, but are not limited to, the EPA, Land Grant Universities, USDA, Seed and Chemical Companies, Commodity Groups, and most importantly Grower and Consultant Organizations.

We would like to reiterate that our main desired outcomes are that:

1) Vip3Aa should not be available in field corn hybrids sold in cotton-growing regions and selectively deployed in the Midwest and Great Lakes corn growing regions where it is the only available PIP option for *S. albicosta* management
2) Only single toxin corn products should be phased out
3) Further PIP pyramid releases should coincide with the immediate phase-out of the old pyramid
4) Refuge in a bag (RIB) should be prohibited in cotton growing regions
5) A 20% block refuge for Vip3Aa hybrids should be required in the Midwest and Great Lakes corn growing regions
6) Implementation of sentinel monitoring program should be described in detail and made more prescriptive, including the use of sweet corn vs. field corn
7) Unexpected injury (UXI) thresholds should be set to reflect what was recommended by the SAP and published observations in the field
8) UXI events should be reported to the Extension specialist from the Land Grant University in that region within 24 hours of a suspected UXI event occurring

9) Registrants should be required, as a condition of registration, to provide purified Bt protein or standardized lyophilized plant tissue to public sector researchers to establish baseline resistance allele frequencies
10) Growers should adopt the best management practices (BMP) and IPM strategies already developed by their respective Land Grant Universities
11) Programs like the NRCS Conservation Stewardship Program should be continued and expanded to encourage growers to plant non-Bt refuge corn

The members of the National Cotton States Arthropod Pest Management Working group agree to this letter (members with singular departures of opinion were encouraged to submit individual letters), as do the signatories from the multistate research project NC246: Ecology and Management of Arthropods in Corn including:

Tracey Baute, Ontario Ministry of Agriculture, Food and Rural Affairs

Holly Davis, Texas A&M University

Chris DiFonzo, Michigan State University

Galen Dively, University of Maryland

Aaron Gassmann, Iowa State University

Kelly Hamby, University of Maryland

Erin Hodgson, Iowa State University

Fangneng Huang, Louisiana State University

Thomas Hunt, University of Nebraska

Bill Hutchison, University of Minnesota

Bryan Jensen, University of Wisconsin

Juan Luis Jurat-Fuentes, University of Tennessee

Janet Knodel, North Dakota State University

Katelyn Kesheimer, Auburn University

Christian Krupke, Purdue University

Xianchun Li, University of Arizona

Charles Mason, University of Delaware

Brian McCornack, Kansas State University

Andy Michel, The Ohio State University

Nick Miller- Illinois Institute of Technology

David Mota-Sanchez, University of Michigan

Kenneth Ostlie, University of Minnesota

David Owens, University of Delaware

Julie Peterson, University of Nebraska

Patrick Porter, Texas A&M University

Francis Reay-Jones, Clemson University

Dominic Reisig, North Carolina State University

Art Schaafsma, University of Guelph

Nick Seiter, University of Illinois

Elson Shields, Cornell University

Jocelyn Smith, University of Guelph

Joseph Spencer, University of Illinois

Sally Taylor, Virginia Tech University

Kelley Tilmon, The Ohio State University

John Tooker, The Pennsylvania State University

Raul Villanueva, University of Kentucky

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