RE: Docket ID EPA-HQ-OPP-2017-0617

Dear SAP Panelists:

The National Cotton States Arthropod Pest Management Working Group (NCSAPMWG, https://southernpests.org/) is 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. Originally, the group convened for the first meeting in 1978 in Orlando, FL, and was called the "1978 National Cotton Pest Management Seminar." The NCSAPMWG has recently been officially recognized by the Southern and Western Region IPM Centers as a working group.

We would like to thank the U.S. Environmental Protection Agency (EPA) and the Scientific Advisory Panel (SAP) for the opportunity to comment and provide information on Docket EPA-HQ-OPP-2017-0617, "Resistance in Lepidopteran Pests to Bt PIPs in the United States". We are the public sector scientists who have responsibility for directly developing and delivering the science that supports practitioners of Integrated Pest Management (IPM) including resistance management. The SAP will advise and influence the decisions by the EPA that will have direct impact on the stakeholders of our programs in the agricultural community.

Two important facts should guide any discussion regarding the continued use and resistance management of existing Bt crops: 1) Bt crops have provided, and continue to provide today, tremendous value to growers in our regions even in areas where resistance has been detected. As host plant resistance traits, Bt PIPs remain integral components of successful IPM programs across the southern U.S. in corn and cotton. 2) The IRM strategies mandated by the EPA were meant to delay, not prevent resistance. No insect control technology is exempt from the basic principles of natural selection and resistance evolution. In this sense, resistances can and do constantly arise in insect populations, and EPAs original goal to help industry and stakeholders delay resistance was appropriate. In general terms, the current IRM strategy has been successful at delaying resistance, especially for the target pests where the Bt crops provide a high dose. More importantly, the current strategies, inclusive of other IPM elements, have helped maintain the utility of Bt crops against many different primary and secondary targets for over two decades, even where resistances have developed and altered control tactics locally.

1. Resistance Reports for Lepidopteran Pests of Bt PIPs

The NCSAPMWG 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. We applaud EPA for recognizing and stating in their White Paper that resistance in *Helicoverpa zea* (Boddie), a non-high dose pest, is occurring because of selection pressure in field corn. *H. zea* resistance to Cry1Ac and Cry2Ab is not complete nor geographically universal and cotton varieties with these two proteins still provide very good control of the pest when compared to non-Bt cotton. Pre-dating any broad-scale selection pressures with Bt PIPs, supplemental control with foliar insecticides had been required in Bt cotton since 1996, the first year of commercial production of Cry1Ac cotton (Bollgard®) and has continued since the introduction of dual-gene cottons (Bollgard II®, Widestrike™, and TwinLink®). However, supplemental control is often needed more frequently than in the past in many areas. Likewise, significant infestations have been observed in single-gene and dual-gene Bt corns since their respective commercialization. This further supports that neither Bt corn nor Bt cotton has ever provided a high dose for *H. zea*. 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.

2. Resistance Monitoring for Non-High Dose Pests:

The EPA pointed out in their white paper that random sampling for *H. zea* has not produced the intended results. Resistance monitoring, regardless of the method used (traditional, diet-based, field-based, or molecular) or the target (high dose pest, near high dose pest, or low dose pest) is not a panacea for resistance management. It is an approach that informs science, less so practice. Resistance monitoring remains a retrospective tool of resistance management. More research is needed to link resistance monitoring technologies and approaches to resistance management insights and outcomes. The science is not yet there to demand or require an exact or precise approach. In general, there has been insufficient coordination among all parties involved (grower groups, industry, University Extension/Experiment Station personnel, agricultural consultants, and EPA).

Furthermore, the resistance monitoring methods are highly biased toward diet-based bioassays, likely because these results are easily quantifiable. However, results from diet-based bioassays can vary without carefully coordinated methodology. For example, when a susceptible colony of *Heliothis virescens* was screened for Cry1Ac susceptibility, EC_{50} values varied from 4–10x, depending on the diet that was used in the assay. Furthermore, comparing a Cry1Ac susceptible and resistant *H. virescens* colony, resistance ratios could vary from 24 to 275, depending on the diet used in the assay (Blanco et al. 2009). Similar to *H. virescens*, LC_{50} bioassay results were 100-fold different between the same *H. zea* population when tested on optimal protein rich diet versus suboptimal carbohydrate rich diets (Deans et al. 2017).

It is also very difficult to link diet-based bioassay results to field performance of Bt traits. For example, *H. zea* populations were collected from non-Bt corn in 28 North Carolina and one Virginia locations during 2017. Survival of these populations in a diet-based bioassay on a 95% lethal dose of Cry1Ac ranged from 1% to 70%. Furthermore, feeding on a Bt corn hybrid (expressing Cry1A.105 + Cry2Ab2) compared to a genetically similar non-Bt corn hybrid ranged from a 72% reduction of feeding in the Bt hybrid to an increase of 334% of feeding in the Bt hybrid compared to the non-Bt hybrid. Rates of feeding reduction, proportion of injured ears and pupal exit holes in the Bt hybrid (corrected by dividing the non-Bt datum point at each location to account for pressure) were significantly correlated with each other by location. However, diet-based bioassay survival was not correlated to any of the field injury measurements taken. Likely, the field provides pest insects with environmental variability that can encourage or discourage survival that cannot be replicated in a tightly controlled laboratory assay.

Caution should be exercised when comparing diet-based bioassays to field injury data. We encourage the development of on-plant bioassays, like those for *Diabrotica virgifera virgifera* (Nowatski et al. 2008, Gausmann et al. 2011, Wen and Chen 2018), for lepidopteran pests of corn and cotton. This could help eliminate some of the uncertainty from results obtained from unexpected injury fields. Often, when unexpected injury is recognized, the population abundance is overwhelming. This can be problematic for pests that can survive on Bt cotton at moderate doses or pests that are cannibalistic. *Helicoverpa zea* is one such pest. On-plant bioassays could serve as a bridge between diet-based bioassays and unexpected damage in the field to provide the insects a more realistic feeding environment.

A small point about cotton fields with unexpected injury is that the indeterminate nature of the crop makes pest collection and post-mortem evaluation of the plant injury difficult. Lepidopteran feeding that penetrates the boll wall very often triggers ethylene production and abscission of the reproductive tissue. Visits to the field must be very timely to accurately capture the true extent of the injury, before most tissues abscise. Similarly, collections can be difficult for the same reason.

Additionally, simply switching to molecular techniques is not a complete solution. There are mutations that will be missed and without a priori knowledge of the resistance that is likely to develop, millions could be invested in monitoring the wrong resistance and exaggerating the actual problem or worse a different mechanism arises that nobody knew about. Waiting for field failures as is suggested is of great academic importance, but of little utility in resistance management.

Finally, we feel that resistance monitoring should not be considered an important component of resistance management. To date, we are not aware of compelling examples where resistance monitoring resulted in actionable efforts to effectively mitigate resistance. Resistance monitoring is however an important academic exercise that can change or shape IPM recommendations from Extension.

2. Resistance Risk of Seed Blend Corn in the Southern U.S.

There is considerable debate around the use of seed blends in corn as a refuge strategy in cotton growing regions of the U.S. It surrounds the risks of having a mosaic of kernels with expression of different proteins versus the guarantee of refuge compliance. Onstad et al. (2018) described two main arguments to support structured non-Bt refuge over refuges provided by seed blends. One argument claimed that refuges from seed blends would produce fewer susceptible insects than structured refuges. However, this hypothesis seems unlikely since a review of the available studies (n=14) showed that *H. zea* survival was reduced by only 16% in seed blends compared to structured refuge plants. A two-year study in the Texas Panhandle examined *H. zea* adult emergence from seed blend refuge ears in a 95:5% planting of Pioneer® brand Optimum® Leptra™ corn product (TC-1507 x MON810 x MIR162) (P1401VYHR) and Pioneer P1498R non-Bt. In the first year there was no reduction in *H. zea* emergence from seed blend refuge ears as compared to structured refuge ears. However, a 90.5% reduction in adult emergence from seed blend refuge ears was observed in year 2 (Porter, Bynum and Glass, unpublished). In both years there were significantly fewer fall armyworm adults emerging from seed blend ears than ears in an adjacent non-Bt block. In a similar two-year study in Mississippi, seed blends with different percentages of non-Bt seed in a Vip hybrid produced similar or greater percentages of *H. zea* moth emergence relative to moth emergence in non-Bt corn (Towles et al. unpublished). These recent studies demonstrate the high level of variability that can be expected with seed blends.

The second argument claims that seed blends in corn will negatively influence the survival of susceptible homozygous individuals and increase survival of resistant heterozygous individuals. The knowledge of this "will determine the success of seed blends for IRM for insecticidal crops" (Onstad et al. 2018), but remains untested for *H. zea*. One documented risk of seed blend refuge in corn is that toxin expression is lower in seed blend ears than in ears grown in pure stand (Carroll, et al. 2013.) This reduction in dose will probably lead to increased functional dominance of resistance alleles in the heterozygotes, and thus speed resistance evolution. In contrast, growers feel (either real or perceived) that refuges have a cost that severely limits their profitability in the current production system (see below). Until the immediate (short term) costs that growers experience with a refuge are offset, the long term goals of resistance management will suffer. Direct comparisons need to be made between seed blends with near complete compliance and structured refuges with low compliance based on real estimates of compliance.

4. Bt Traits Expressed in Corn and Cotton

The NCSAPMWG recognizes that *H. zea* provides a unique challenge for resistance management because of the sequence of host use by this pest. One to two generations are completed almost exclusively on corn in many regions of the country. The generations that emerge from corn disperse across the landscape and oviposit on multiple cultivated and non-cultivated host plants. As such, many of them end up in cotton where they are likely to be exposed to Bt PIPs. No other pest has this resistance risk. We agree with the EPA White Paper stating that resistance to the Cry proteins occurred because of their selection in corn and accept that numerous other cultivated and non-cultivated hosts provide a sufficient natural refuge for cotton. It is important to note that *H. zea* is not considered an economic pest of corn in most areas of the U.S. Bt corn is planted primarily for the management of corn borer species. In contrast, *H. zea* can be a significant pest of cotton in many states, but *Heliothis virescens* (F.) was the primary target of Bt cotton. As a result, we are asking corn growers, who may or may not grow cotton, to provide a refuge to preserve the susceptibility of an insect for which there is no immediate economic cost. However, the NCSAPMWG views the preservation of susceptibility to any pest species to Bt proteins as being in the public good as stated by EPA.

5. Resistance Management for *S. albicosta*

S. albicosta is not a pest in most areas of country represented by the NCSAPMWG.

6. Mitigation of Resistance

The EPA white paper discusses IPM in the context of a mitigation strategy, essentially suggesting IPM as a last resort. The introduction of Bt crops has been no different than the numerous introductions of new classes of insecticides, or more appropriately any new host plant resistance factor. As such, PIPs are tactics, not strategies. Until we stop viewing new control technologies as "the" management strategy and start recognizing them as tactical components of IPM, we will continue to miss opportunities to anticipate and mitigate resistances. There are two main reasons we do this and why refuge compliance has been poor. First, any new trait gets put into the elite germplasm that is coming. There may be few comparably high performing non-Bt corn hybrids available in many areas, but seed supplies are extremely limited (for example, the number of non-Bt hybrid maize hybrids published in local seed guide catalogs from three major seed companies, relative to Bt hybrids, decreased yearly from 2010 to 2018 by approximately one to five percent). The situation is more extreme in cotton. Second, margins for growers are very thin in all crops in many areas of the U.S. Every year, growers want to hit a home run with yields to dig out of the hole they are in; much of that pressure comes from lenders and land owners. This, combined with seed companies pushing their elite germplasm that has every trait available creates conditions where resistance is inevitable.

7. Grower Non-Compliance with Refuges in the Southern U.S.

Directed, concerted, and proactive efforts of Extension educators are necessary to support the socio-cultural changes that are needed for refuge compliance (Reisig 2017) because structured refuge is a common pool resource (Brown 2018). Experimental evidence from the social sciences could be helpful and suggests that community-based regional programs could be more effective to delay resistance than top-down mandated programs (Ostrom 1994, Gould 2018). Based on these ideas, Reisig (unpublished data) created a short "emotional appeal" Extension presentation during 2018 North Carolina grower meetings (similar methodology to Reisig 2017). Before the emotional appeal, 42% of growers indicated they would plant structured refuge (n = 238); however, after the emotional appeal, 58% of growers indicated they would plant structured refuge (n = 216; Z = 2.65, P = 0.004). These preliminary results suggest that refuge compliance can be achieved if we recognize that it is a common pool resource. This is not to suggest that EPA should mandate how Extension provides educational programming related to resistance management, but that there are viable options to improve refuge compliance in the context of the role of IPM practitioners. Additionally, incentive programs provide another method for improving refuge compliance among growers. It is highly likely that no one method will completely remedy poor refuge compliance, but a coordinated effort among all groups in the agricultural community (EPA, Industry, Growers, Extension, etc.) using different strategies will likely make a big difference.

8. New IRM Framework for Lepidopteran Pests of Bt

To address a new IRM framework, it is important to remember the principles of resistance management. 1) Limit the use of the control agent to the lowest possible (practical) degree, 2) diversify modes of action, and 3) partition modes of action through space and/or time to support refuges and to remind ourselves that these principles have not changed. We have no real fundamental change in our understanding of how resistance develops or what one does to manage it. As a result, resistance monitoring is often superfluous to resistance management and should be secondary to the discussion of resistance management. Any new IRM strategy should be founded on the principles of IPM and seek to reduce selection pressure. The principles of IPM can and will effectively reduce selection pressure to PIPs. Prior to the introduction of Bt crops, planting date was one of the primary foundations of IPM in both corn and cotton. For both of these crops, the target insect pests (Primarily Lepidoptera) increase throughout the year and population densities are usually greatest late in the growing season. For field corn in the southern U.S., we can often minimize infestations of both *H. zea* and corn borer species during the most susceptible stages of the crop by planting in a timely manner. Similarly, infestations of *H. zea* can be minimized by planting cotton as early as practical and using early maturing varieties. Since the introduction of Bt cotton, later season varieties have dominated current breeding programs. Utilizing other tactics of IPM such as scouting and use of foliar insecticide applications only when pest thresholds are reached can further reduce selection pressure from Bt PIPs. Minimizing foliar sprays for other pests and eliminating "automatic" applications to preserve beneficial insects in cotton can reduce *H. zea* populations, thereby reducing selection pressure. IPM should be the foundation of IRM for PIPs rather than part of a mitigation plan. Additionally, it is imperative that industry, EPA, and all other stakeholders consider a logical plan for incorporating new PIPs into corn and cotton cropping ecosystems where *H. zea* and/or *S. frujiperda* are important pests. Incorporating the same or similar PIPs into corn and cotton should be avoided in the future.

Finally, the NCSAPMWG thanks the EPA and SAP Panel members for considering these comments. The deployment of PIPs in cotton and field corn have revolutionized pest management in these crops. Despite a few cases of resistance to PIPs in the U.S., the IRM plans to this point have effectively delayed resistance and PIPs remain a valuable component of current IPM programs. They have effectively reduced the use of foliar insecticides while minimizing negative impacts to the environment and making crop production more profitable for growers.

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