

Bt Resistance Implications for *Helicoverpa zea* (Lepidoptera: Noctuidae) Insecticide Resistance Management in the United States

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Subject Editor: Steven Naranjo

Received 19 June 2018; Editorial decision 27 August 2018

Abstract

Both maize and cotton genetically engineered to express Bt toxins are widely planted and important pest management tools in the United States. Recently, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) has developed resistance to two toxin Bt maize and cotton (Cry1A and Cry2A). Hence, growers are transitioning to three toxin Bt cotton and maize that express both Cry toxins and the Vip3Aa toxin. *H. zea* susceptibility to Vip3Aa is threatened by 1) a lack of availability of non-Bt refuge crop hosts, including a 1–5% annual decline in the number of non-Bt maize hybrids being marketed; 2) the ineffectiveness of three toxin cultivars to function as pyramids in some regions, with resistance to two out of three toxins in the pyramid; and 3) the lack of a high dose Vip3Aa event in cotton and maize. We propose that data should be collected on current Cry-resistant *H. zea* in the field to inform future Bt resistance models and that the deployment of Bt toxins and non-Bt refuge crops should be adjusted to favor susceptibility of *H. zea* to Bt toxins such as Vip3Aa. Finally, maize growers should be incentivized to plant non-Bt structured refuge and have access to hybrids with high-yielding genetic potential at a reasonable price.

Key words: maize, cotton, Cry, Vip3Aa, non-Bt refuge

Crops with genetic engineering have been an important pest management tool in the United States, where cotton (*Gossypium hirsutum* L.) and maize (*Zea mays* L.) cultivars that express Bt toxins are widely planted (USDA ERS 2018). Although the relative costs and benefits provided from genetic engineering are varied, Bt varieties have generally preserved yield and decreased insecticide sprays compared to non-Bt varieties (The NAS 2016). However, the greatest perceived threat to Bt crops is the development of resistance by the target pests (Onstad 2008, Carrière et al. 2010). The Environmental Protection Agency (EPA) regulates the use of pesticides, including Bt crops, in the United States. This organization has stated that Insecticide Resistance Management (IRM) practices aimed at reducing the potential for insect pests to become resistant to Bt are ‘of great importance because of the threat insect resistance poses to the future use of Bt plant-incorporated protectants and Bt technology as a whole’ and deemed susceptibility of Bt to be ‘in the public good’ (US EPA 2001). Moreover, IRM strategies are recognized as critical to maintain the viability of Bt crops (Bates et al. 2005) and are the cornerstone of the development of risk management decisions made by the EPA for Bt crops (Carrière et al. 2010).

Helicoverpa zea (Boddie) (Lepidoptera: Noctuidae) has developed practical resistance to some Bt toxins expressed in transgenic cotton (Cry1Ac; Tabashnik et al. 2013, Tabashnik 2015) and Cry2A (Reisig et al. 2018) and sweet corn (*Zea mays* *convar. saccharata* *var. rugosa* L.; Cry1A.105, Cry1Ab, and Cry2Ab2; Dively et al. 2016) in the United States. In both crops, this has led to an increasing number of foliar insecticide sprays to manage this pest and limit economic crop damage (Dively et al. 2016, Reisig et al. 2018). This change in susceptibility has also driven a transition from maize and cotton varieties expressing Cry toxins alone, to those expressing multiple toxins, including the Vip3Aa toxin, despite the fact that maize yield is generally not increased from Bt (Reay-Jones and Reisig 2014, Reay-Jones et al. 2016, Bibb et al. 2018). The EPA prioritizes resistance management for Plant-Incorporated Protectants (PIPs), including several Bt toxins. Resistance in *H. zea* to multiple Bt toxins highlights the difficulty of maintaining susceptibility to challenging pests. Pests that have developed resistance to Bt crops, such as *H. zea*, can provide lessons and opportunities to preserve future PIPs that have no known resistance. Currently, the IRM strategy to delay resistance to Bt crops in the United States relies on three tactics: 1) availability of non-Bt refuge hosts; 2) high-dose Bt toxins; and

3) pyramiding toxins. The goal of this perspective is to identify both challenges and opportunities for improving IRM of Bt crops for Cry-resistant *H. zea*, as well as to identify which of these challenges can be overcome to delay future resistance.

Challenges for *H. zea* IRM

Challenges to the three tactics to delay resistance to Bt crops in the United States vary among crops and insect pests. The original primary targets for Bt cotton were *Heliothis virescens* (Fabricius), *H. zea*, and *Pectinophora gossypiella* (Saunders) (US EPA 1995), while the original primary targets of Bt maize were *Diatraea grandiosella* (Dyar) and *Ostrinia nubilalis* (Hübner) (Storer et al. 2001). Of the aforementioned target species, only *H. zea*, has developed practical resistance (field-evolved resistance that reduces pesticide efficacy to a level that has consequences for pest control (Tabashnik et al. 2014) to Bt in the United States. The diversity of feeding and reproductive hosts, the Bt dose required for mortality, and the impact of different Bt toxin combinations (pyramiding toxins) vary for each of these insect pests. Thus, a cogent IRM plan for each crop and associated Bt proteins should incorporate factors relevant to all target species. Additionally, the IRM plans need to be adopted to be effective. The lack of IRM plan adoption for *H. zea* in Bt crops in the United States has severely challenged the continued efficacy of these valuable pest management tools. These challenges are discussed in terms of 1) availability of non-Bt refuge hosts; 2) effectiveness of pyramiding toxins; and 3) dose of current insecticidal events.

Availability of Non-Bt Refuge Hosts

Planting non-Bt crop refuge remains one of the key components of durable IRM programs for PIPs and is one factor that growers can manipulate to favor susceptible insect populations on their farm. For example, in the United States, non-Bt structured maize refuge in the Cotton Belt is still considered an important component of IRM and is required by law. However, in 2011, non-Bt cotton refuge requirements were eliminated with the exclusive planting of pyramided varieties containing multiple Bt toxins in the eastern United States, and portions of Texas, where pink bollworm (*P. gossypiella* Saunders) was not present. When single toxin Bt cotton was eliminated from the United States, resistance management tactics for both *H. zea* and *H. virescens* were dependent on nonstructured refuge in a combination of non-Bt cultivated crops and noncultivated hosts. The logic behind this decision was based on studies demonstrating that cotton was a relatively unimportant host of both *H. zea* and *H. virescens*, and that maize, sorghum, soybean and noncultivated plant species produced vastly more susceptible individuals than the existing non-Bt cotton refuge (Head et al. 2010). Consequently, the non-Bt cotton refuge was considered an insignificant source of susceptible moths to delay Bt resistance for these two pest species.

Despite refuge changes in cotton, the prevalence of other *H. zea* host crops expressing Bt, such as maize, complicates the maintenance of susceptible individuals in populations. Moreover, the number of growers complying with maize structured refuge regulations has eroded over time. For example, only a minority of growers in North Carolina plant a non-Bt maize refuge that is in full compliance with these regulations (Reisig 2017), even though maize yields are rarely increased by Bt (Reay-Jones and Reisig 2014, Reay-Jones et al. 2016, Bibb et al. 2018). A 2015 phone survey across states in the U.S. Cotton Belt indicated that 36% of maize growers in this region did not plant non-Bt refuge, 38% met the refuge size requirement, and that 26% partially met the refuge size requirement (ABSTC 2016).

This was likely an underestimation, since smaller-sized maize growers are less likely to plant non-Bt refuge (Reisig 2017) and were not included in the phone survey.

Furthermore, non-Bt maize hybrid availability is decreasing in the U.S. Cotton Belt. Although non-Bt maize hybrids are available from major seed companies, local print and online catalogs available to growers reflect a decrease in marketing for non-Bt maize hybrids in the U.S. Cotton Belt. For example, the number of non-Bt hybrid maize hybrids published in local seed guide catalogs and online from three major companies, relative to Bt hybrids, decreased yearly from 2010 to 2018 by approximately 1–5% (Fig. 1). Two of three of these companies had zero non-Bt hybrids listed in their local seed catalog during 2018. Eight years previous, 33 to 56% of the hybrids listed in these catalogs or online were non-Bt hybrids. Maize hybrids with Bt traits are often more expensive for growers to purchase than non-Bt hybrids. Therefore, there is likely a higher profit potential for the seed industry to sell Bt hybrids compared to non-Bt hybrids and little incentive for the seed industry to sacrifice short-term profits. The decrease in relative availability of Bt to non-Bt hybrids is also reflected in a lack of publicly generated agronomic data for non-Bt hybrids. For example, many states conduct Official Variety Testing supported by university-based Extension personnel. These tests place replicated variety trials in different locations, providing unbiased results that are intended to assist grower selection of crop varieties. From 2013 to 2017, all of the 459 separate hybrid entries into the North Carolina maize grain Official Variety Testing Program were Bt hybrids and none were non-Bt (NCARS 2018). Without a supply of high yielding non-Bt maize hybrids that growers would want to plant and have agronomic information about, structured non-Bt maize refuge compliance will continue to languish in the U.S. Cotton Belt.

H. zea is the most common pest of maize in the U.S. Cotton Belt (Reisig et al. 2015, Reay-Jones et al. 2016). Studies conducted in this region confirmed that, prior to widespread resistance, many Bt maize hybrids could reduce injury from this insect (Buntin et al. 2001, Buntin et al. 2004, Buntin 2008, Reay-Jones and Wiatrak 2011, Bowen et al. 2014, Reay-Jones and Reisig 2014, Reisig and Reay-Jones 2015, Reisig et al. 2015, Reay-Jones et al. 2016, Bibb et al. 2018). Furthermore, *H. zea* alone does not limit yield in this region when maize is planted on time (Reay-Jones and Reisig 2014, Bibb et al. 2018). However, maize yields can be limited when *H. zea* is present with other pest species (e.g., *D. grandiosella* (Dyar), *Diatraea saccharalis* (F.), or *Spodoptera frugiperda* (J. E. Smith)), with yield losses more common in late-planted corn (Buntin et al. 2001, Buntin et al. 2004, Reay-Jones et al. 2016). Growers usually purchase maize seed during the fall in the year prior to spring planting. Because of this time lag, pest pressure and the ability to plant on time are not predictable. Paired with a lack of information on yield potential and with no shared burden in the event of significant yield loss, U.S. Cotton Belt maize growers are understandably hesitant to plant non-Bt hybrids from a risk management perspective.

The absence of the required amount of non-Bt refuge across the U.S. Cotton Belt could be one explanation for the widespread Cry toxin resistance in *H. zea* populations throughout the eastern United States. Because both maize and cotton express similar Bt toxins, exposure to both crops within and among growing seasons without an acceptable amount of refuge could undermine the efficacy of Bt traits and affect the rate of resistance selection. Cross-crop selection may play a previously underestimated role in Bt trait efficacy in the U.S. Cotton Belt because most Bt maize hybrids and Bt cotton varieties currently planted in this region express one of these toxins. In this region, *H. zea* develops almost exclusively on C₄ plants during at least one generation (Gould et al. 2002, Head et al.

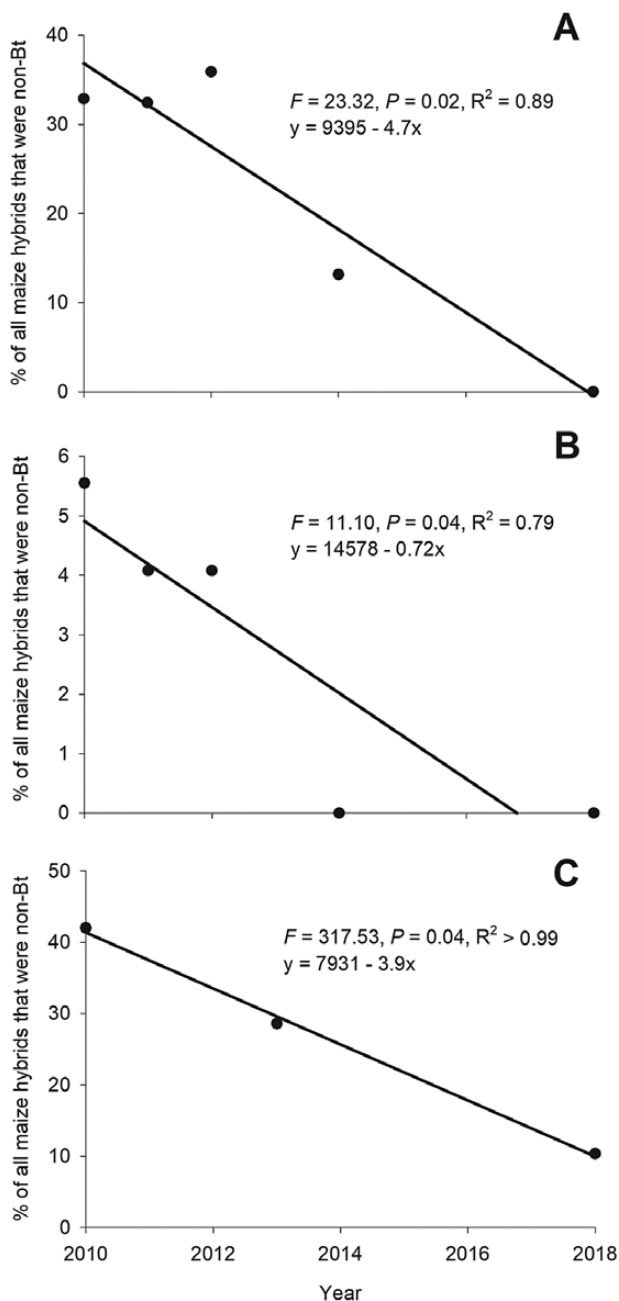


Fig. 1. Percentage of all maize hybrids that were non-Bt in local seed catalogs available in print and online from 2010 to 2018 for (A) DEKALB ($n = 274$ hybrids; Monsanto Company 2009, Monsanto Company 2010, Monsanto Company 2011, Heiniger 2014, Monsanto Company 2017), (B) NK ($n = 159$ hybrids; Syngenta Seeds Inc. 2009, Syngenta Seeds Inc. 2010, Syngenta Seeds Inc. 2011, Syngenta Seeds Inc. 2013, Syngenta Seeds Inc. 2017), and (C) Pioneer ($n = 107$ hybrids; Pioneer Hi-Bred International 2010, Pioneer Hi-Bred International 2012, Pioneer Hi-Bred International 2018). Data were not complete due to missing files. The percentage of non-Bt relative to Bt hybrids available in these catalogs was compared using an analysis of variance model (PROC GLM; SAS Institute 2011) to see if regression parameters were different among the companies. The dependent variable was the percentage of non-Bt relative to Bt hybrids, and the independent variables were the year and the interaction of year with seed company. The overall model was significant ($F = 15.57$, $df = 3,9$, $P = 0.0012$), as was the interaction of year with seed company ($F = 15.57$, $P = 0.0012$); therefore, regression parameters were different among the companies. Three separate regressions (PROC REG; SAS Institute 2011) were performed, one for each seed company, where the dependent variable was the percentage of non-Bt relative to Bt hybrids, and the independent variable was the year.

2010). Since *H. zea* prefers maize as a host during this generation, larvae are more abundant in maize compared with any other crop in the system (Stadelbacher et al. 1986), except sorghum in some specific cases. Furthermore, maize is a regionally dominant crop and is assumed to be the most important C_4 host plant during July and August for *H. zea* in this region (Jackson et al. 2008, Head et al. 2010). Therefore, Bt maize is thought to be a primary driver for *H. zea* resistance selection against Bt toxins in both maize and cotton (Head et al. 2010). Because maize is the key crop for resistance selection in this region, the use of structured non-Bt maize refuges is recommended to manage *H. zea* resistance in both maize and cotton (Head et al. 2010). Given the potential for cross-resistance among Cry1A proteins (Welch et al. 2015, Dively et al. 2016), the high adoption of Bt maize in the cotton-growing region of the United States, and the lack of an appropriate amount of structured refuge, the majority of *H. zea* have been pre-selected for resistance prior to moving into cotton; as a result pyramided Bt cotton is experiencing higher fruit damage than ever before (Reisig et al. 2018).

Pyramiding Toxins

Pyramiding, another tactic to delay resistance, incorporates multiple Bt toxins in the same plant that target a given pest species (Carrière et al. 2015). However, once an insect becomes resistant to one Bt toxin component in the pyramid, the effectiveness of the pyramid to delay resistance is reduced because the toxin combination no longer ‘redundantly’ kills target pests (Carrière et al. 2016). Bt cotton pyramids are now available that include the Vip3Aa19 toxin coupled with Cry1Ac + Cry1F, Cry1Ac + Cry2Ab2, or Cry1Ab + Cry2Ae. The situation is similar for maize, with Bt hybrids now available that include the Vip3Aa20 toxin coupled with Cry1Ab, Cry1Ab + Cry1F, and Cry1A.105 + Cry1Ab2 lepidopteran-specific toxins. While the addition of the Vip3Aa toxin to existing cotton and maize pyramids will improve control of *H. zea* in the near term (Reay-Jones et al. 2016, Little et al. 2017, Bilbo et al. 2018), the long-term effectiveness of the pyramid to delay resistance for Vip3Aa to *H. zea* will be reduced, because resistance to Cry1Ac + Cry1F, Cry1Ac + Cry2Ab2, Cry1Ab, Cry1Ab + Cry1F, and Cry1A.105 + Cry1Ab2 has already occurred (Dively et al. 2016, Reisig et al. 2018).

This issue is compounded by the overlap of two and three Bt toxin cotton varieties, as well as maize expressing Vip3Aa20. Pyramids function the best when pest populations are not resistant to the toxins in the pyramid and when the toxins in the pyramid do not overlap (Carrière et al. 2016). Two toxin cotton pyramided varieties overlapped with single toxin cotton varieties for 8 yr in the United States. Furthermore, before two toxin pyramids were released, *H. zea* had developed field-evolved resistance to one of the toxins (Cry1Ac) in the pyramid (Carrière et al. 2016). Therefore, the ability of two toxin cotton pyramids to maintain susceptibility in pest population was limited in the United States. Similarly, *H. zea* in the southern U.S. Cotton Belt is resistant to two out of the three toxins in cotton pyramided with Vip3Aa19, although there are populations that are still susceptible to Cry1Ac and Cry2Ab2 (Yang et al. 2017, Reisig et al. 2018). Furthermore, Cry1Ac + Cry1F + Vip3Aa19 cotton has been planted since 2015 and Cry1Ab + Vip3Aa20 maize has been planted since 2011. Hence, the overlap of both cotton and maize cultivars expressing Vip3Aa with those that express Cry toxins will likely precipitate resistance to all Bt toxins.

High dose Bt Toxins

Another tactic to delay resistance is to introduce the insecticide at a dose that kills most susceptible individuals. This is known as a high dose when toxin titers are high enough to kill 95% of individuals

heterozygous for a resistant allele (Gould 1998). Upon registration in cotton, the COT102 event, responsible for expressing Vip3Aa19, was determined to range from high dose to 'near high dose' (not well defined) when following several of EPAs five dose determination methods (US EPA 2008). Vip3Aa19 met EPAs high dose criteria when pyramided with the COT67B event expressing Cry1Ab toxin that has activity against *H. zea* (Carrière et al. 2015, Carrière et al. 2016). To determine Vip3Aa19 *H. zea* activity, lyophilized cotton terminal leaves and cotton bolls were diluted 25-fold in artificial diet. Results for a single *H. zea* population showed that mean corrected mortality ranged from 98.3 to 100% (US EPA 2008). In contrast, cotton leaf disks expressing Vip3Aa19 alone were 100% lethal to both first and fourth instars in a laboratory population of *H. zea* (Palekar et al. 2011). However, because *H. zea* populations have a wide range of susceptibility to Bt toxins (US EPA 2008), it is likely that COT102 cotton does not express a high dose of Vip3Aa19 sufficient to kill all populations of *H. zea*. In a Mississippi field experiment across five different sites, *H. zea* larvae were able to survive in the terminals and squares of Cry1Ac + Cry1F + Vip3Aa19 cotton and in the bolls of Cry1Ac + Cry1F + Vip3Aa19 (Little et al. 2017). In maize, the Cry1Ab + Vip3Aa20 pyramid is thought to be a near high dose for *H. zea* (Yang et al. 2015). This particular pyramid combination was determined to be an 'effective high dose', meaning that each toxin alone did not supply a high dose, but when used in combination they did (US EPA 2009). Although laboratory assays confirmed a baseline effective high dose, *H. zea* damage observations across a multi-year and state replicated experiment in a Cry1Ab + Cry1F + Vip3Aa20 maize hybrid found greater than expected damage (Reay-Jones et al. 2016). In a follow-up study, pupae were recovered that had developed off this same Bt maize pyramid (Cry1Ab + Cry1F + Vip3Aa20; Bilbo et al. 2018). Although contribution of viable offspring to the next generation has not been demonstrated at this time, the fact that larvae can survive long enough to cause measurable feeding injury in Vip3Aa maize and cotton and that pupae can develop from Vip3Aa20 maize is concerning. This is likely a step toward developing resistance to this toxin (Dively et al. 2016).

Opportunities for Improving IRM

The EPA has defined seven key elements that should be addressed in a Bt IRM strategy: 1) knowledge of pest biology and ecology; 2) appropriate dose expression strategy; 3) appropriate refuge; 4) resistance monitoring and a remedial action plan should resistance occur; 5) use of integrated pest management (IPM) to limit survival of resistant individuals; 6) communication and education strategies on use of the product; and 7) development of alternative modes of action to reduce overreliance on individual toxins (US EPA 2009). Fundamentally, IRM programs for Bt crops rely on simulation modeling results, which are dependent on a combination of evolutionary theory as well as empirical evidence. A US EPA Scientific Advisory Panel agreed that 'mathematical models were the only scientifically rigorous way to integrate all of the biological information available, and that without these models, the Agency would have little scientific basis for choosing among alternative resistance management options' (FIFRA SAP 2001). However, Panel members indicated that there was a need to more effectively compare the results of models when making policy decisions, and a need to judge the accuracy and applicability of specific models. They also agreed that 'mathematical models can only be as good as the data that are used to parameterize them' (FIFRA SAP 2001).

Various comparisons have been made between the outputs of resistance models and outcomes in the field to assess the IRM strategy

for Bt (e.g., Tabashnik et al. 2008, Huang et al. 2011, Tabashnik et al. 2013, Tabashnik and Carrière 2017). While the EPA takes these comparisons into consideration, the EPA logic for the IRM strategy still relies on models and theories laid out from the inception Bt crop commercialization (US EPA 2018). There is a general consensus among those evaluating resistance model outputs with successful outcomes in the field that the combination of high-dose Bt toxins with adequate non-Bt refuge has been successful to delay resistance. However, when either of these parameters is violated, the risk for a more rapid evolution of resistance increases. Deploying abundant non-Bt refuge can substantially delay resistance even when Bt toxins are not high dose (Tabashnik and Carrière 2017). Based on a review of the literature, Tabashnik and Carrière (2017) suggest that non-Bt refuges should consist of 50% or more of the landscape when only a single trait is effective, resistance is not rare, and toxins are not high dose. These parameters are likely violated for the *H. zea* Vip3Aa cotton and maize system. However, increasing non-Bt refuge in the landscape to 50% or greater would be very difficult in a system where refuge compliance is already low.

Collect Data on Current Resistance in the Field to Inform Future Resistance Models

Knowledge of resistance fitness costs, dominance and frequency of resistance genes are important for optimal resistance management, but are generally unknown in most cases (Gould et al. 2018). This is the current case for *H. zea* and Cry resistance. Hence, researchers can collect information on current Cry-resistant *H. zea*, to improve the accuracy of modeling and IRM efforts to delay resistance to Vip3Aa. To develop a more dynamic modeling approach, model results could be compared to newly collected field data. Integration of current data into near real-time modeling efforts may improve the efficacy of IRM programs through a transition from a descriptive to reactive approach that could further delay resistance development with timely interventions. For example, Australian IRM programs and resistance monitoring strategies were modified after the discovery of relatively high baseline levels of Cry2Ab resistance alleles in *Helicoverpa* species, the prevalence of dominant resistance alleles, and the prediction of spatio-temporal models predicting an increase in the rate of resistance evolution (Downes et al. 2016, Downes et al. 2017). Similar to the Australian IRM system, characterizing the spatio-temporal extent of Cry-resistance in U.S. *H. zea* populations, especially in relation to resistance allele frequency over time would be an effective means to inform IRM decisions and delay resistance to future toxins.

In addition, using field-relevant data for model parameterization, the underlying model assumptions must also be correct to design an IRM program that can successfully delay the onset of Bt resistance. Although models predicting the swift evolution of resistance agree with many of the field outcomes (Tabashnik and Carrière 2017), assumptions of models can have vast implications on outcomes. For example, when comparing whether blended refuge can delay resistance as quickly as structured non-Bt refuge, survival data are plentiful. A Onstad et al. (2018) review of numerous studies ($n = 14$) demonstrated that *H. zea* survival was reduced by only 16% in maize with blended refuge compared to structured refuge. Hence, a recent modeling study suggested that the development of *H. zea* resistance in the southern United States would be lower using blended maize refuge compared to structured non-Bt maize refuge, given the current rates of refuge compliance (Pan et al. 2016). However, Onstad et al. (2018) also noted that the blended refuge could be unsuccessful to delay resistance if survival of resistant heterozygous individuals relative to susceptible homozygous individuals is increased relative

to structured refuge. The impact of either refuge strategy on this for *H. zea* is unknown.

Often, variation in the genetic predisposition for resistance of a population does not agree with the assumed parameters used in mathematical models to document toxin durability. In Australia, resistance cases to Bt cotton have been both recessive and partially dominant (Downes et al. 2016). Similarly, in the United States, a dominant Cry1Ac resistance allele was found in a population of *H. zea* during the year 2000 (Burd et al. 2003). This variation is important because the dominance of a resistance trait influences the fitness of heterozygotes (Gould 1998). At the most basic level, the high dose/refuge strategy assumes that Bt crops should be deployed in such a way to produce toxin titers high enough to kill 95% of individuals heterozygous for a resistant allele (Gould 1998). This may be true for some pests targeted by Cry1Ab in maize (e.g., *O. nubilalis*), but not for a non-target pest (e.g., *H. zea*, for which Cry1Ab in maize is only moderately toxic) (Storer et al. 2001). Since both cotton and maize varieties producing Vip3Aa are being adopted in the United States, the relative toxicity of Vip3Aa expressed by both commercially planted cotton and maize events to *H. zea* will be important to understand the probability of non-high dose selection on populations that are exposed to both crops within and across growing seasons. Moreover, the frequency of *H. zea* resistance alleles to Vip3Aa, and the level of dominance of these alleles are salient to design predictive resistance models and to implement proactive IRM strategies specific to this pest.

Adjust the Way that Bt Toxins and Non-Bt Refuge are Deployed in the Field

Adjusting the way in which multiple toxins are deployed in major field crops could present an opportunity to minimize resistance development. Current IRM options for field crops are limited by the low diversity of Bt toxin pyramids (i.e., the major seed companies are all offering the same or very similar event pyramids) and the dose of individual toxins expressed within in any given pyramid. Since some populations of *H. zea* are resistant to the other two components of the Vip3Aa pyramid, managing Vip3Aa resistance by planting structured non-Bt maize refuge in the U.S. Cotton Belt is critical. A recent effort to increase structured non-Bt maize refuge in North Carolina was not successful (Reisig 2017). However, this effort ignored the fact that insecticide susceptibility 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 2011, Gould et al. 2018). For example, laboratory experiments, using games where individuals invested tokens into various simulated markets, including common pool resources, have demonstrated that outcomes are greater for the group if they can communicate, create a strategy for maximizing the group outcome, and establish a mechanism for sanctioning defectors from the strategy (Ostrom et al. 1994). These experiments also demonstrated that if defections from the strategy were too large (*viz.*, they were so large that they devalued the common pool resource for the other individuals in the experiment), or too numerous, often, but not always, other individuals would defect from the strategy that was designed to provide maximum benefit to the group. Although a coordinated IRM strategy is already in place in the United States that mandates planting non-Bt maize refuge to maintain susceptibility and a mechanism to sanction growers who plant Bt corn without the legally-required non-Bt refuge, few growers are invested in maintaining this resource and sanctions for violations are rare. The social science experimental results suggest that maize growers, the seed industry, and regulators could work together to establish a new strategy to increase and

incentivize non-Bt refuge compliance rather than continuing to rely on rare and ineffective punitive actions.

Finally, beyond the scope of non-Bt refuge compliance, future incentives could be created to encourage the crop-specific use of Bt toxins, rather than the use of the same, or similar, Bt toxin across all crops. While Vip3Aa is already being used in both cotton and maize, growers could be incentivized to restrict planting current and future PIPs in crops where they provide little benefit. In the case of Vip3Aa, growers in the U.S. Cotton Belt could be encouraged to only plant cotton expressing this toxin, and not maize. In contrast, in the Great Lakes region of the U.S. Corn Belt, *Striacosta albicosta* Smith, has emerged as a major maize pest. In parts of this region, Vip3Aa is the only Bt toxin available in maize that provides effective control for this pest (Smith et al. 2018). Moreover, in this region *H. zea* is uncommon and migratory. Therefore, Vip3Aa could be used in maize in this region with little interference on efforts to delay *H. zea* Vip3Aa resistance in the U.S. Cotton Belt, since migratory populations are likely less important to the overall genetic population structure compared to resident populations (Smith et al. 2018). In contrast, Vip3Aa could be restricted in maize outside the Great Lakes region of the U.S. Corn Belt where *S. albicosta* is less of a concern to reduce selection pressure for this toxin.

Current IRM plans are imposed because the EPA deems Bt susceptibility to be a public good, yet the risk of yield loss associated with IRM plan compliance is borne solely by each individual grower. Governmental funding has been recommended to improve resistance management strategies similarly to how investments are made in on farm erosion control through NRCS, the Conservation Reserve Program and other governmental cost-share programs (Gould et al. 2018). A similar program could be implemented to assist growers who do experience significant yield loss while complying with the mandated IRM plan. Since there are no similarly safe and effective plant-incorporated protectants to manage *H. zea* on the horizon beyond the Vip3Aa toxin, IRM plans need to be amended to more effectively incentivize plantings of non-Bt refuge for both growers and the seed industry to delay *H. zea* resistance evolution.

Finally, since Vip3Aa cotton and maize are not high dose and because non-Bt refuge compliance is low, blended refuge (mixing Bt and non-Bt plants within a single planting) has been suggested as a way to ensure compliance. However, numerous studies have documented that this has the potential to precipitate resistance. As stated earlier, blended refuge can be unsuccessful if heterozygous resistant individuals are increased relative to structured refuge. A greenhouse study estimated larval abundance and survival of Bt-susceptible and resistant *H. zea* in simulated arrangements of blended non-Bt and Bt cotton, as well as pure Bt cotton (Brévault et al. 2015). This study concluded that dominance of resistance was increased in the blended cotton since larvae that were likely to be heterozygous could move between non-Bt and Bt plants to survive. Furthermore, while similar data are unavailable for maize (Carrière et al. 2016), models and empirical studies have demonstrated that blended non-Bt maize refuge can precipitate resistance for *H. zea* compared to structured refuge, since Bt expression in kernels is variable due to cross-pollination between non-Bt and Bt plants (Mallet and Porter 1992, Chilcote and Tabashnik 2004, Burkness et al. 2011, Yang et al. 2014, Burkness et al. 2015). Therefore, structured non-Bt maize refuge, rather than blended refuge, should be the lynchpin in PIP IRM, especially for toxins such as Vip3Aa that are not high dose.

Meeting the Current IRM Challenges

The current resistance management strategy for Bt toxins active against *H. zea* in the United States needs to be improved given the

lack of structured refuge adoption in maize, the reduced efficacy of Bt toxin pyramids, and the ability of *H. zea* to feed on crops expressing Vip3Aa. Cotton and maize expressing Vip3Aa have only been planted on a limited basis to date; however, we expect adoption of varieties and hybrids expressing Vip3Aa to increase rapidly, especially in cotton, to replace varieties expressing ineffective toxin pyramids. Consequently, actions should be taken immediately to follow principles of IRM because selection for resistance to Vip3Aa will likely increase relative to the scale of adoption. One such action could be to reevaluate efforts to increase plantings of structured non-Bt maize refuge for Vip3Aa in the U.S. Cotton Belt. This could prove challenging given the lack of inclusion of non-Bt hybrids in official variety testing programs and consequently a lack of information on agronomics, including yield, for non-Bt maize hybrids. As mentioned earlier, breeding and marketing efforts track this trend, placing priority on Bt hybrids over non-Bt hybrids. A key step in delaying Bt resistance going forward is offering growers non-Bt hybrids that they want to or are even demanding to plant due to high yield potential and some mechanism to relieve the burden of significant refuge yield loss, when it occurs, being borne solely by the grower.

Incentivize Maize Growers to Plant Non-Bt Structured Refuge

Because Bt susceptibility is a common pool resource, enforcing sanctions is an option, but providing incentives to growers that do plant the required separate non-Bt maize refuge can also be an effective option. For example, putative enforcement measures as they are currently practiced have been ineffective, and refuge compliance has declined steadily over time in the United States (Jaffee 2009, Reisig 2017). However, a single study has demonstrated an intervention that increased non-Bt structured refuge compliance (Brown 2018). In this study, growers who purchased the required amount of non-Bt seed relative to their purchase of Bt seed from Monsanto were identified and permitted to vote for several donations to a local charity of their choice (the incentive). Monsanto also launched a short-term moral suasion campaign (persuasion as opposed to forced compliance), urging maize growers to plant separate non-Bt refuge (described in Brown 2018). The average maize grower exposed to the campaign in this region planted 2.6 to 5.8% more refuge than the average maize grower not exposed to this campaign in the region. Moreover, smaller-sized growers, which were likely the most noncompliant (Reisig 2017), were more responsive to this campaign than larger-sized growers. Hence, when data from larger-sized growers were included, planting of non-Bt refuge was not significantly influenced in the landscape. Furthermore, after this short-term campaign ceased, growers ceased to plant more non-Bt maize. Therefore, interventions to increase refuge compliance should focus on both smaller-sized growers, since they are most likely to be noncompliant, as well as larger-sized growers, to have the greatest impact on planting of non-Bt refuge in the landscape. Finally, because the effect diminished after the campaign ceased, behavioral campaigns should be ongoing.

The moral suasion campaign indirectly increased communication among growers about non-Bt structured maize refuge since it used a social comparison method. University Extension programming is another method of communication that could be used to influence plantings of non-Bt refuge, but with the lack of a meaningful incentive for growers to plant them, the traditional delivery format is ineffective (Reisig 2017). As a result, university Extension personnel could develop moral suasion campaigns in tandem with industry programming. Attention

should be given to more than just the message in these campaigns. Deeply engrained beliefs and practices are influenced only in part by the transfer of scientific information to the target audience. The importance of communicator credibility, the importance of group identity with the communicator, the importance of how the information is delivered to engender trust and membership of the community of the communicator and the target audience are demonstrated by many examples in the social sciences, including obesity reduction (Gray 1997, Gilson 2003, Bleich et al. 2007) and beliefs surrounding climate change and evolution (Kahn 2015). Thus, university Extension personnel could make use of the trust and credibility they have with growers and focus more on techniques designed to foster a group identity among maize growers to increase planting of non-Bt structured refuge. However, this job would be made much easier if high yielding non-Bt hybrids were available.

Offer Maize Growers Hybrids With High-Yielding Genetic Potential at a Reasonable Price

Cotton expressing Vip3Aa19 is being deployed as a pyramid with Cry toxins to improve resistance management for both *H. zea* and *H. virescens*, previously a very damaging insect pest in U.S. cotton prior to Bt cotton. Moreover, Vip3Aa is also useful to improve control of *H. zea*, especially now that this insect is resistant to Cry toxins in cotton. In contrast, the utility of Vip3Aa in southern U.S. maize is less clear. As mentioned previously, *H. zea* is the most common insect pest in southern U.S. maize, but does not impact yield in timely planted maize (Reay-Jones and Reisig 2014, Reay-Jones et al. 2016, Bibb et al. 2018). Other potentially yield-impacting pests in the southern United States, such as *D. grandiosella*, *D. saccharalis*, and *Elasmopalpus lignosellus* (Zeller), are sporadic secondary pests (Reisig et al. 2015, Reay-Jones et al. 2016). *Spodoptera frugiperda* can be a severely yield-limiting pest of maize in the southern United States, but is an outbreak pest that is most pronounced in late-planted corn. Consequently, structured refuges should not result in significant yield losses for southern U.S. maize growers. While maize expressing Vip3Aa can reduce overall injury from these pests, positive impacts on yield are not common and are limited to situations where outbreak pests are present or in late-planted maize (Reisig et al. 2015, Reay-Jones et al. 2016). Therefore, since cotton is not an important source of *H. zea* for resistance management, growers can delay resistance to Vip3Aa by increasing the maize area planted to structured refuge (non-Bt) with minimal impacts on yield. As mentioned previously, increasing area planted to refuge will not be straightforward. This is further confounded by the fact that, for structured refuges to be effective to delay resistance, they must increase in size as the dose required to kill resistant individuals increases, as is the case for Vip3Aa and *H. zea* (Tabashnik and Carrière 2017). Therefore, future studies identifying the optimum refuge size to delay resistance would be helpful. The results of these studies will only be useful if refuges are properly implemented both in size and configuration (e.g., not planted as blended refuge, which can accelerate resistance (Carrière et al. 2016)). Finally, growers will be more likely to plant structured refuges in maize if the hybrids that are offered are not cost prohibitive, have the genetic potential to yield as well as similar Bt maize hybrids and a mechanism to relieve the burden of significant refuge yield loss, when it occurs, being borne solely by the grower is implemented.

In conclusion, we predict that adoption of the cotton and maize expressing Vip3Aa will be rapid and urge the producers of these seeds and the end users to work together to steward this toxin to delay resistance by planting non-Bt maize refuge.

Acknowledgments

We are deeply indebted to Zach Brown for pointing out the issues of common pool resources. We also thank Anders Huseth for his manuscript edits.

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