Overview of Risk Factors and Strategies for Management of Insect-Derived Ear Injury and Aflatoxin Accumulation for Maize Grown in Subtropical Areas of North America

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Abstract

Pest and disease risk factors causing maize yield and kernel quality issues in subtropical areas of North America (between 35°N and 23.5°N latitude) are reviewed: preharvest Aspergillus flavus (Link) (Deuteromycetes: Moniliaceae) infection and propagation in maize ears and ear injury principally caused by Helicoverpa zeae (Boddie) and Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae). Risk is affected by potentially interacting factors of maize genetics, ear feeding with insects, fungal inoculum and growth, and weather (rainfall, temperature, and humidity). This review gives special attention to integration of the most efficient pest and disease management strategy combinations and the potential interaction of insect ear feeding and A. flavus occurrence. Management strategies reviewed include maximizing partial genetic resistance to A. flavus and selection of appropriate hybrids for the area, biological control of A. flavus using non-aflatoxigenic strains, avoiding pests and diseases using cultural practices like early planting, and reducing H. zeae and S. frugiperda ear injury using Bt transgenes. Understanding the combined influences and identifying combined management approaches may lead to reduced aflatoxin risk and maintaining yield. This review focuses on subtropical areas of North America because the amount of maize produced in warm environments within or similar to subtropical maize production areas is likely to increase due to projected increases in demand for maize and predicted increases in temperature may increase pest and disease risk.

Key words: corn earworm, fall armyworm, Bacillus thuringiensis, Aspergillus flavus, integrated pest management

Maize is the most produced grain by volume in the United States and Mexico (USDA-NASS 2013, USDA-ERS 2019). The United States is the number one producer of maize in the world, and Mexico is the seventh-largest by volume (USDA-FAS 2019). Ear feeding caused by Helicoverpa zeae (Boddie) and Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae) and infection of maize kernels by Aspergillus flavus (Link) (Deuteromycetes: Moniliaceae) put maize in subtropical growing areas at risk to preharvest losses (Wu et al. 2005, Porter et al. 2014, Wu 2014, Welikhe et al. 2016). Subtropical areas are defined as between 35°N and 23.5°N and S latitude (American Meteorological Society – Meteorology Glossary [AMS-MG] 2020). Subtropical areas commonly experience wide variation in weather patterns including periods of intense rainfall, heat, and drought which may intensify under global warming (Porter et al. 2014, Singha et al. 2017). These conditions can predispose maize to the risk of A. flavus infection (Abbas et al. 2007, Welikhe et al. 2016, Damianidis et al. 2018). Aflatoxin contamination levels are regulated in the United States beginning at >20 ppb (FDA-CVM 1994).

Weather variation associated with subtropical areas of North America is hard to predict and challenging to manage, particularly in areas with limited or no irrigation. Global temperatures are expected to rise approximately 2–4°C by 2100, which along with increasing demand for maize will likely expand the area of maize farmland grown under warm conditions similar to subtropical maize production areas (Wu et al. 2011, Marroquin-Cardona et al. 2014, Porter et al. 2014, Welikhe et al. 2016). Rising temperatures are predicted to have impacts on insect and aflatoxin dispersal (Bale et al. 2002, Marroquin-Cardona et al. 2014), which are projected to increase A. flavus infection and H. zeae and S. frugiperda injury in maize grown in North America (Wu et al. 2011). Risks are also exacerbated because only around 10% of the land devoted to maize production in subtropical areas of the United States and 8% in Mexico have access to irrigation (USDA-NASS 2013, USDA-ARS 2019).
The risks associated with maize production and *A. flavus* infection are affected by a suite of environmental factors including insect population levels, fungal inoculum potential, weather (rainfall, temperature, and humidity), and soil type (Smith and Riley 1992, Abbas et al. 2007, Welikhe et al. 2016, Damianidis et al. 2018). Aflatoxin accumulation in maize is often driven by plant stress associated with environmental factors. For instance, drought and high temperatures are the most common environmental factors associated with increased risk of *A. flavus* infection and yield losses in maize (Southworth et al. 2000, Abbas et al. 2007, Welikhe et al. 2016, Lenssen et al. 2018). Insect herbivory is also likely to increase during times of drought due to weakened plant defenses and has also been linked to increased risk of *A. flavus* infection and reduce yields in maize (Guo et al. 2005). To date, no maize hybrids have shown high resistance to *A. flavus* infection and growth, but appropriate hybrid selection for traits such as drought tolerance are crucial for maximizing benefits of genetic-based partial resistance (Bertran et al. 2005, Wahl et al. 2016). For instance, a maize plant with drought tolerance may be able to remobilize nutrients that would allow for development of a thicker pericarp to slow insect feeding. Insect-derived ear injury has been linked to increased plant stress and subsequent aflatoxin contamination in maize; however, the relationship is tenuous and subject to variation possibly due to confounding environmental and genetic factors (Windham et al. 1999, Wiatrak et al. 2005, Abbas et al. 2013, Pruter et al. 2019).

Maize does not require insect-derived ear injury to become infected with *A. flavus* (Dowd et al. 2005). *Aspergillus flavus* infection process may be passively aided by immature and adult *H. zea*, *S. frugiperda*, and other insects (Dowd et al. 2005, Abbas et al. 2007, Pruter et al. 2019). Ear feeding by *H. zea* and *S. frugiperda* larva causes mechanical wounds to the stems, husk, kernels, silks, and tips of the maize ear, allowing *A. flavus* spores to colonize the wounds (Dowd 2003, Dowd et al. 2005, Guo et al. 2005). Also, *A. flavus* spores have been shown to proliferate in the hindgut of *H. zea* larva that have consumed plant material already colonized by *A. flavus* (Dowd et al. 2005). Adult *H. zea* and *S. frugiperda* can also act as a passive source of inoculum by landing on maize ears already colonized with *A. flavus* and carrying the fungal spores on their bodies, appendages, and mouthparts (Dowd et al. 2005, Guo et al. 2005). Such potentially interacting factors may be affecting risk of aflatoxin contamination.

An understanding of potentially interacting biotic, weather, and maize genetic factors can help identify the integrated pest management (IPM) tactics that maximize their combined effects in reducing aflatoxin and ear feeding risk (Maikuku et al. 2019). The scope of risk factors reviewed includes factors influencing the *A. flavus* infection and propagation in maize ears, ear injury principally caused by *H. zea* and *S. frugiperda*, and the potential linkage of insect ear feeding injury and *A. flavus*. Selective other ear insects causing ear injury are introduced that have shown a relationship to *A. flavus*. Specifically, integrating maize breeding and hybrid selection with pest and disease management are reviewed: maximizing partial genetic resistance to *A. flavus* and selection of appropriate hybrid for the area, biological control of *A. flavus* using non-aflatoxigenic strains, avoiding pests and diseases using cultural practices like early planting, and reducing *H. zea* and *S. frugiperda* ear injury using Bt transgenes (Fig. 1).

**Overview of *Aspergillus flavus* and Aflatoxin Accumulation in Maize**

*Aspergillus flavus* is a thermo-tolerant fungus that prefers dry conditions accompanied by high temperatures up to 48°C (Guo et al. 2005). The combined tolerance to heat and drought conditions allows *A. flavus* to competitively exclude other fungi and dominate geographic areas that experience these weather patterns (Smith and Riley 1992, Guo et al. 2005). *Aspergillus flavus* poses a significant risk to maize produced by farmers in subtropical areas of North America (Abbas et al. 2009). Aflatoxins are carcinogenic, teratogenic, and immunosuppressive to mammals (FDA-CVM 1994). Maize with aflatoxin levels >300 ppb cannot be sold for animal feed and is usually destroyed, raising the cost of aflatoxin management in the United States to an estimated $250 million annually (FDA-CVM 1994). The underlying threat to human and animal health combined with the annual damage to agricultural commodities make *A. flavus* management one of the most intensively studied fungi (Scheidegger and Payne 2005).

While *A. flavus* is found in many environments and nearly all agricultural soils, the population density in various soils can vary by several log orders of magnitude (Horn 2006, Zablotowicz et al. 2007). The population diversity has been described through differences in sclerotia production (Cotty 1997), vegetative compatibility type (Leslie 1993), mating type (Ramirez-Camejo et al. 2012), visual phenotypes (Rodrigues et al. 2009), genetic markers (Grubisha and Cotty 2010), and differences in aflatoxin production capability (Bayman and Cotty 1993). There is an indication that some *A. flavus* genotypes may be adapted to certain niches as evidenced by the abundance of MAT 1-1 in clinical samples (Ramirez-Camejo et al. 2012) and MAT 1-2 in samples from maize (Sweany et al. 2011).

Insect-derived injury to the ear can serve as a source of *A. flavus* infection, but infection and subsequent aflatoxin accumulation can also occur in healthy kernels. Airborne spores can enter the ear by growth down the silk channel, typically colonizing from the ear tip.

![Fig. 1. Signs of *Aspergillus flavus* (green mold) around the insect-derived ear feeding injury that occurred toward the tip of a maize ear.](image-url)
down (Klich 2007). Infection of maize kernels may occur early or late in the grain maturation process and does not always cause aflatoxin production, but when aflatoxin accumulation does occur it generally does not begin until the maize is mature and dry due to changes in lipid and starch content inducing aflatoxin production (Fakhoury and Woloshuk 1997). Postharvest remediation of aflatoxin-contaminated grain is unfeasible because aflatoxin is not readily degraded or isolated (King and Prudente 2005). Consequently, disease and associated pest management strategies that can prevent or reduce preharvest yield losses attributed to A. flavus have been the primary avenues of research to identify management approaches to reduce risk (Payne and Widstrom 1992).

Insect Taxa Affiliated With A. flavus Contamination and Prominent Species

Many species of insects have been associated with enhanced A. flavus sporulation in coffee, cotton, figs, peanuts, small grains, and tree nuts (Dowd et al. 2005). Sixteen species across 11 families and five orders (Coleoptera, Hemiptera, Psocoptera, Thysanoptera, and Lepidoptera) have been associated with enhanced aflatoxin sporulation in maize (Dowd et al. 2005). Lepidopterous larvae cause most of the ear injury in subtropical North America associated with fungal infection of developing maize kernels (Smith and Riley 1992). Economically significant insect-derived ear injury and aflatoxin contamination are also reported from subtropical maize production areas of Mexico (Barry 1987, Rodríguez-del-Bosque et al. 2012). Among the Lepidoptera, there are eight species in four families documented to aid A. flavus infection of maize ears: H. zea (Boddie), S. frugiperda (J. E. Smith), Sesamia calamistis (Hampson) (Lepidoptera: Noctuidae), Sathrobrota rileyi (Walsingham) (Lepidoptera: Cosmopterigidae), Diatraea grandiosella (Dyar) (Lepidoptera: Crambidae), Massilia nigrirostris (Ragonot), Ostrinia nubilalis (Hubner), and Eldana saccharina (Walker) (Lepidoptera: Pyralidae) (Dowd et al. 2005). In subtropical North America, most insect-derived ear injury is attributed to S. frugiperda and H. zea which occur throughout the southern United States, most of Mexico, and the Caribbean islands (Dowd et al. 2005). Other species may be significant maize pests but are more regional, such as D. grandiosella attacking maize in the southeastern United States and sometimes the Corn Belt (Windham et al. 1999).

Spodoptera frugiperda is native to subtropical areas of North America (Sparks 1979). A female moth can lay 50–100 eggs in clusters covered by hairs from her body (Sparks 1979). A distinctive row of perforations in the leaves is characteristic when feeding occurs during the whorl stage (All et al. 1996). The larvae can chew a hole through the husk thereby gaining access to the ear as well as enter through the silk channel (Sparks 1979). In the ear, larger larvae will usually cannibalize smaller larvae, reducing the average larval density to one per ear (Sparks 1979). After feeding for 2–3 wk, larvae chew through the husk and burrow into the soil to pupate. Moths emerge approximately 2 wk later (Sparks 1979). The life cycle of S. frugiperda takes approximately 30 d during the summer and varies between 30 and 60 d in the spring and fall, depending on temperature and other environmental conditions (Sparks 1979). Multiple overlapping generations may occur in a single season, especially in subtropical areas of North America where populations build before migrating north (Sparks 1979, Westbrook et al. 2016).

Helicoverpa zea also successfully overwinters in subtropical areas of North America, particularly where maize is grown (Harding 1976). Oviposition begins about 3 d after emergence, and a female can oviposit up to 2,500 eggs (Harding 1976). Maize is the preferred host for H. zea where it is available, and newly emerging maize silks are attractive to female moths, with individual eggs commonly laid on silks (Harding 1976). The newly hatched larvae typically feed on the silk initially as they follow the silk channel inside the husk where it feeds on the developing kernels (Olmstead et al. 2016). Larvae become cannibalistic around the third instar, usually resulting in no greater than two larvae completing development in a single ear (Harding 1976). Larvae will remain within the ear until they are ready to pupate in the soil (Harding 1976). The H. zea life cycle takes approximately 30 d to complete (Harding 1976). Helicoverpa zea populations build in subtropical areas of North America during the spring, and northward long-distance movement coincides with annual maize cultivation in temperate maize growing areas such as the U.S. Midwest (Harding 1976) (Fig. 2).

Environmental Risk Factors Associated With Insect-Derived Ear Injury and Aflatoxin Accumulation in Maize

Drought stress in maize has been linked to increased insect herbivory, A. flavus infection, and aflatoxin accumulation (Smith and Riley 1992, Abbas et al. 2013). Drought stress in maize is caused by limited rainfall or irrigation. High temperatures can accelerate plant desiccation (Smith and Riley 1992). Plant desiccation may also cause nitrogen and potassium deficiencies due to the plant’s inability to remobilize nutrients (Rhoads and Bennett 1990). The stage of the maize life cycle in which drought stress occurs, the severity of the drought stress event, and the maize hybrid’s genetic tolerance to droughty conditions can affect the plant’s susceptibility to insect herbivory and A. flavus infection (Ludlow and Muchow 1990, Widstrom et al. 1990) (Fig. 3).

Optimal daily temperatures for maize growth occur between 25 and 32°C, depending on moisture levels, whereas A. flavus is well adapted to grow at up to 38°C (Schindler et al. 1967, Aldrich et al. 1975). When temperatures exceed 32°C, which is common in subtropical areas of North America during summer, risk of aflatoxin accumulation increases while the capacity for plant growth and defenses is reduced (Guo et al. 2005, Abbas et al. 2007). High temperatures also often coincide with infestations of H. zea and S. frugiperda populations (Harding 1976, Sparks 1979). Late plantings of maize may experience an increased risk of aflatoxin accumulation and insect-derived ear injury due to these associations with the weather (Abbas et al. 2007, 2009).

Strategies for Integrated Pest and Disease Management

Cultural Strategies

Cultural control is a component of IPM that includes reliance on farming practices to reduce the amount of plant stress caused by pests and diseases (Mahu ku et al. 2019). Early planting of maize in subtropical areas of North America can occur as early as February and is a common cultural practice for managing several sources of environmental stressors associated with yield losses in maize (Aldrich et al. 1975, Guo et al. 2005). Maize planted early can benefit from rains that typically occur in spring, which is particularly important in subtropical North America where there is limited irrigation. Rainfall that occurs during the flowering and grain fill stages of maize development can reduce canopy temperatures which is commonly considered the most important factor in reducing
A. flavus infection in maize (Guo et al. 2005). Alleviating drought stress has been linked to lower A. flavus inoculum levels and decreased pericarp damage caused by heat and insect feeding (Smith and Riley 1992, Guo et al. 2005). Early planting can also help avoid the timeframe later in the season in which H. zea and S. frugiperda population densities typically plateau and can cause maximum ear injury (Aldrich et al. 1975). It has been reported in the United States and Mexico that early plantings of maize experience less aflatoxin accumulation, and less severe temperature, drought, and insect pressure (Jones et al. 1981, Barry 1987, Wiatrak et al. 2005, Bruns and Abbas 2006, Rodríguez-del-Bosque et al. 2012).

In areas where H. zea populations develop early on wild weedy hosts, host removal through mowing or herbicide treatments can be effective at reducing H. zea populations (Capinera 2000). Proper weed removal can also alleviate plant stress by reducing competition for water and nutrients which have been directly linked to increased aflatoxin accumulation in maize (Zuber and Lillehoj 1979). Maximizing water utilization and avoiding high pest densities by planting early and reducing pest and disease hosts and weed competition are examples of how integrated pest and disease management strategies, as well as weed management, are interrelated and mutualistic.
Biocontrol Strategies for *Aspergillus flavus*

Biocontrol as a component of IPM includes predation and parasitism of pest arthropods, insect herbivory of weeds and noxious plants, and fungal competition with toxigenic diseases (Abrol and Shankar 2012). Throughout the 1990s, a novel approach to the biocontrol of aflatoxin was developed, utilizing large-scale production of non-aflatoxigenic strains of *A. flavus* and applying this inoculum to agricultural fields. This culminated in the U.S. Environmental Protection Agency (EPA) registration of strain AF36 by the Arizona Cotton Research and Pest Council and Aflaguard by Circle One Global (Sheppard, GA) (now Syngenta [Basel, Switzerland]) for cotton and peanuts, respectively. These registrations were subsequently expanded to protect maize from aflatoxin contamination (Cleveland et al. 2003, U.S.-EPA 2003, 2004, Bandyopadhyay et al. 2016). The approach has been validated in experiments around the world, with local strain selection, registration, and production in other countries (Bandyopadhyay et al. 2016). Despite the number and diversity of field trials validating this approach, the precise mechanism is not fully understood. Competitive exclusion (i.e., the numerical reduction of indigenous, toxigenic isolates of *A. flavus* from the soil or the plant infection court) is often cited and is almost certainly partly responsible for the reduction in aflatoxin contamination (Dorner 2004). Using non-aflatoxigenic *A. flavus* as part of an IPM strategy requires that competing non-aflatoxigenic species be present at high levels during the susceptible periods of the maize maturation to competitively exclude other aflatoxigenic species (Dorner 2005). Several recent experimental approaches have suggested other plausible mechanisms that may complement competitive exclusion (Sweany and Damann 2020). The biological control approach of using non-aflatoxigenic strains of *A. flavus* has been efficacious and cost-effective at reducing aflatoxin contamination in maize (Udomkun et al. 2017) and may be used in conjunction with Bt transgenes as noted in the next section.

Genetic-Based Resistance Strategies

Conventional breeding is widely considered the most reliable means of acquiring resistance to diseases and pests, especially for low-input crops like rain-fed maize in subtropical areas of North America (Dowd 2003, Guo et al. 2005, Robertson et al. 2005). Modern genetic modification technologies have had a more recent and large impact on genetic-based resistance strategies in maize, especially targeting Lepidopteran species with transgenic Bt maize (Buntin et al. 2004, Bruns and Abbas 2006, Bowen et al. 2014). In 1998, transgenic *Bacillus thuringiensis* (Cry)-Bt maize was released, primarily targeting the European corn borer, *O. nubilalis* (Bowen et al. 2014). Modern commercial maize hybrids combine multiple combinations of transgenes to target above-ground pests such as *H. zea, D. grandiosella,* and *S. frugiperda* as well as below ground pests such as *Diabrotica virgifera* (LeConte) (Coleoptera: Chrysomelidae) (All et al. 1996, Bowen et al. 2014). Genetic-based resistance for insect and disease management in maize may be separated into antibotic, antixenotic, and tolerant traits following the classification of Painter (1951). Antixenotic traits denote the use of morphological or chemical factors to dissuade insect herbivory, mating, or oviposition on the plant, or disease inoculation of the plant (Sadasivam and Thayumanavan 2003). Antibiotic resistance traits refer to adverse effects on the pest or disease feeding on the host plant (Painter 1951). Tolerance refers to the host plant’s ability to grow and reproduce despite insect and disease utilization of the plant (Sadasivam and Thayumanavan 2003). Conventional breeding and genetic modifications can both be used to confer genetic resistance in maize (Guo et al. 2005).

Husk coverage is an example of an antixenotic trait associated with insect and aflatoxin resistance. Husk length and tightness have been associated with reduced ear injury caused by insect feeding and improved aflatoxin resistance with or without the presence of ear injury (Lillehoj et al. 1978). A study conducted in Iowa, Missouri, and Georgia used five hybrids with increasing husk tightness to test for aflatoxin resistance. The degree of husk tightness was correlated with reductions in aflatoxin accumulation (Wiseman et al. 1977). Husk tightness has been associated with reduced insect-derived ear injury and aflatoxin accumulation, but it is more useful when combined with other traits that synergistically improve insect and aflatoxin resistance (Rector et al. 2002).

Bt maize is a good example of an antibiotic trait for which benefits may be increased when combined with cultural, biological, and other genetic-based pest and disease management strategies. Bt maize incorporates one or more genetic sequences from the bacterium *B. thuringiensis* into the genome that allows plant cells to produce δ-endotoxins or exotoxins depending on the type of Bt transgene (Chakroun et al. 2016). There are many different Bt transgenes in which toxicity can vary greatly depending on the type of Bt transgene incorporated into the plant and the susceptibility of the target insect species (Abbas et al. 2013, Brewer et al. 2014, Chakroun et al. 2016, Pruter et al. 2019).

Transgenic Bt maize was used to initially target *O. nubilalis,* but it also exhibited control of *H. zea, S. frugiperda,* and *D. grandiosella* to varying degrees (Bowen et al. 2014). Maize hybrids with Cry-Bt transgenes produce δ-endotoxins that are retained within the plant cell until the lepidopteran larvae feed upon Cry-Bt plant tissue. Once the plant cells are ingested by the larvae, the δ-endotoxins are released and activate proteases that bind to midgut epithelial cells. This process disrupts transmembrane potential and eventually kills the larvae (Chakroun et al. 2016).

Vip-Bt exotoxins have recently become commercially available in maize (Ruiz de Escudero et al. 2014). Vip-Bt exotoxins are secreted and are also expressed in higher levels than Cry-Bt δ-endotoxins in the silks and other reproductive structures, further protecting these plant structures from *H. zea and S. frugiperda* herbivory (Estruch et al. 1996). Cry-Bt and Vip-Bt also have different toxicities and may interact, depending on the insect studied. For example, the LC50 was almost 10-fold lower for Vip-Bt than a Cry-Bt on *S. frugiperda,* and the two toxins had a synergistic interaction (Bergamasco et al. 2013).

In concept, higher mortality of early instar larvae and reduced silk feeding damage may be enough to also negatively affect *A. flavus* infection. A two-factor experiment conducted in subtropical areas of Mississippi and Texas investigated the use of non-aflatoxigenic treatments in conjunction with maize hybrids expressing several Bt transgenes as a management strategy for reducing the risk of aflatoxin accumulation and insect-derived ear injury (Weaver et al. 2017). The study found that in this case there were no synergies between insect ear feeding reduction using Bt maize and aflatoxin reduction using non-aflatoxigenic strains of *A. flavus,* but the overall reduction of aflatoxin accumulation by both strategies individually support their use in tandem as cost permits (Weaver et al. 2017).
Challenges and Moving Forward in Integrated Insect and Aflatoxin Management

Past studies have had mixed results correlating insect ear injury aflatoxin in maize (Abbas et al. 2013). Multiple studies in the subtropical United States have shown reductions in aflatoxin concentrations among Cry-Bt isolines compared with their non-Bt counterparts (Windham et al. 1999, Wiatrak et al. 2005, Abbas et al. 2013), while others have had mixed results (Williams et al. 2002, 2010, Bruns and Abbas 2006, Bowen et al. 2014). Experiments in Illinois and Iowa reported no significant reduction in aflatoxin when comparing Cry-Bt and non-Bt isolines (Masoero et al. 1999, Dowd 2000, Munkvold 2003). The mixed results describing the association of Bt maize to insect ear injury and aflatoxin accumulation may have reflected the genetic variation of hybrids being tested and the environmental conditions, such as insect pressure and drought years, in which the experiments were conducted (Xinzhi et al. 2011, Brewer et al. 2014, Wahl et al. 2016, Pruter et al. 2019).

The current body of literature describing the association of Bt maize, insect ear injury, and aflatoxin accumulation primarily focuses on hybrids containing Cry-Bt transgenes (Dowd et al. 2005). Silk damage has been shown to affect aflatoxin accumulation (Dowd 2000), and hybrids with Cry-Bt transgenes do not offer the same protection to the maize silks as the hybrids with the expression of Vip-Bt transgenes (Lee et al. 2003). Therefore, hybrids with Vip-Bt transgenes may have indirect benefits of reducing aflatoxin accumulation by decreasing insect injury to the silk and kernels. Also, little is known about the use of hybrids with Vip-Bt transgenes in conjunction with cultural, biocontrol, and host plant resistance strategies beyond recent experimentation noted above by Weaver et al. (2017), in which A. flavus biocontrol was combined with hybrids containing Cry-Bt and Vip-Bt transgenes. Furthermore, in subtropical Texas, Pruter et al. (2019) observed that maize isolines with Cry-Bt genes typically had less ear injury than non-Bt isolines but more ear injury than Cry/Vip-Bt isolines. The maize isolines expressing both Cry/ Vip-Bt proteins had little to no ear injury in all cases, and aflatoxin accumulation was also lower in most cases. In a correspondence analysis of multiple maize hybrids, less ear injury corresponded with less aflatoxin accumulation for Cry-Bt and Cry/Vip-Bt isolines, but the relationship was inconsistent for non-Bt isolines (Pruter et al. 2019) (Fig. 4).

Maize research is currently benefiting from the development of hybrids adapted to subtropical areas, with hybrids containing some combination of drought and heat tolerance and reduced susceptibility to A. flavus (Guo et al. 2005). Increasing the number of hybrids developed for adaptation to subtropical areas is justified in part by the expected increase in global temperatures along with the area of farmland considered subtropical (Payne and Widstrom 1992, Betrán et al. 2005, Wahl et al. 2016). Typically, environmental conditions that favor aflatoxin production include drought stress, high temperatures, high humidity, and sizeable lepidopteran insect populations (Payne and Windstrom 1992, Buntin et al. 2004, Abbas et al. 2007). These environmental conditions are challenging to manipulate and reproduce in an experimental setting suggesting that research with maize hybrids should be concentrated in subtropical areas that experience high levels of environmental stress. In such environments, experimental manipulation of stressors, such as contrasts in irrigated and non-irrigated plots, may be possible and help measure the susceptibility of maize hybrids to insect-derived ear injury and A. flavus colonization. Expanded research should include experiments that incorporate pest and disease management approaches companion to maize hybrids that resist or tolerate these multiple stressors.

Fig. 4. Maize hybrids of similar genetic background containing no Bt transgenes (bottom), Cry-Bt transgenes (middle), and both Cry-Bt and Vip-Bt transgenes (top).

Many IPM tactics are available and are in development that addresses insect-derived ear injury and aflatoxin management. Knowledge of risk factors helps in understanding potential interactions and environmental and farming conditions that can modify risk. The continued and expanding research focused on the integration of insect and aflatoxin management strategies may help identify combined management approaches that optimize minimize yield losses in maize attributed to insect-derived ear injury and aflatoxin accumulation.

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