EVALUATION OF BT TRAITS FOR MANAGEMENT OF FALL ARMYWORM AND CORN EARWORM (LEPIDOPTERA: NOCTUIDAE) AND IMPROVING GRAIN YIELD IN FIELD CORN PRODUCTION

By

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(Under the Direction of G. David Buntin)

ABSTRACT

Corn expressing specific *Bacillus thuringiensis* (Bt) traits provides effective control of lepidopteran pest infestations in the southeastern United States region. Varying levels of control and resistance development among local populations call for regular monitoring of Bt technology in the field. Plantings done over two years in Georgia found both fall armyworm, *Spodoptera frugiperda* (J. E. Smith), and corn earworm, *Helicoverpa zea* (Boddie), infestations were significantly reduced by Bt hybrids expressing the Vip3Aa20 protein. Grain yield loss and increased fumonisin contamination directly associated with corn earworm damage were also prevented. Bt hybrids expressing only *Cry* proteins provided good control against fall armyworm and reduced grain yield loss yet were mostly ineffective against corn earworm. These findings present the current efficacy rates of Bt corn hybrids towards lepidopteran pest control in Georgia and the significance of using Vip3A proteins for insecticidal use while also providing a degree of fumonisin control.

INDEX WORDS: *Spodoptera frugiperda*, *Helicoverpa zea*, Noctuoidea, *Bacillus thuringiensis*, transgenic crops, *Zea mays*, agriculture, IPM, plant-insect interactions, entomology, Vip3A

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DEDICATION

To my Mom and Dad as well as my brother and sister for always being around and providing constant support and wisdom. Biggest much.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Fall armyworm and corn earworm management. Corn, *Zea mays* (L.), is a cereal grain commonly grown across the United States with 14.2 billion bushels produced at a rate of 172.0 bu/a in 2020 alone (USDA-NASS 2021). Field corn is the most common variety of the crop, intended for livestock feed, ethanol production, and processed goods. Corn production in the United States is most prominent in the Midwest region, otherwise known as the Corn Belt, but moderate production also occurs in other parts of the country, including the southeastern region. However, agricultural production in this region is frequently targeted by a variety of lepidopteran pest species, including the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), and corn earworm, *Helicoverpa zea* (Boddie). Both of these species use field corn as a host along with a variety of other crops for larval development.

Fall armyworm outbreaks historically only occurred in North and South America but had primarily been recorded within the United States in irregular intervals where they can cause significant monetary loss (Sparks 1979). The pest would later become recognized as an invasive species beginning in 2016 when it was first identified infesting corn fields across Africa (Goergen et al. 2016) before spreading into Asia (Ganiger et al. 2018, IPPC 2019, USDA-FAS 2019) and Australia (Spafford 2021). The inability for it to diapause in cold weather makes this pest species only active in warm climates as it cannot survive in areas where temperatures reach freezing. The moths migrate to other temperate regions during the summer months (Luginbill

1928, Sparks 1979). Fall armyworm from the southern Florida region migrate across the eastern United States, mostly east of the Appalachian Mountains, and moths from the southern Texas and Mexico regions migrate north across the Midwest (Nagoshi et al. 2008, Sparks 1979). Fall armyworm have two different host strains, the rice strain and the corn strain. Fall armyworm with the rice strain feed on rice, forages, and turfgrass, whereas those with the corn strain feed on corn, sorghum, and cotton (Pashley 1986, Pashley and Martin 1987). Corn plants become infested with fall armyworm expressing the corn strain when the female moths lay their egg masses on the underside of the leaves, each mass consisting several hundred eggs (Luginbill 1928). Larvae hatch from the eggs and feed primarily on the whorl foliage during the vegetative stages prior to corn silking, but they will also feed on kernels if ears are present. Very few studies evaluated the corn yield response to foliar damage by fall armyworm, though significant infestations can result in reduced ear and kernel development (Bilbo et al. 2020, Cruz and Turpin 1983, Marenco et al. 1992). The larvae feed through about six instars for roughly three weeks then burrow into the soil to pupate for up to two weeks before emerging as moths to migrate and reproduce (Steffey et al. 1999). Few effective control tactics implemented against fall armyworm infestations involve insecticidal sprays restricted to only when the egg masses are present or introducing the parasitoid wasp *Cotesia marginiyentris* (Cresson) on larvae (Steffey et al. 1999).

Corn earworm larvae are also present in North and South America and can overwinter as far north as 40°N (Dicke and Guthrie 1988, Sandstrom et al. 2007, Steffey et al. 1999). Females lay eggs individually mostly on the corn silks. The larvae proceed to feed on the silks and ears during the corn's reproductive stages and the whorl leaves on rare occasions. Depending on the temperature, larvae go through about six instars, and development takes about three weeks (Steffey et al. 1999). Corn ears with significant injury from larval feeding may be undesired for sweet corn production (Steffey et al. 1999), but overall grain yield for field corn production is hardly affected (Bibb et al. 2018). Most larval activity occurs at the tip region of the ear where unpollinated kernels are located (Dicke and Guthrie 1988). Larval feeding can spread across the rest of the ear containing viable kernels but would still require a large portion of the ear to be infested for grain yield to be reduced (Olivi et al. 2019, Reay-Jones and Reisig 2014). Additionally, the corn earworm larvae's cannibalistic behavior typically results in only one larva completing its development per infested ear (Dicke and Guthrie 1988). The larvae descend from the infested ears to pupate in the soil for two weeks then emerge as moths, surviving up to four weeks to reproduce and migrate (Steffey et al. 1999). Chemical control with insecticides is available by targeting oviposited moth eggs and larvae prior to entering corn ears, yet this control method is only cost effective in high value sweet corn (Steffey et al. 1999). A selection of predator, parasitoid, and pathogen species are identified as potential biological control agents against corn earworm at specific life stages (Steffey et al. 1999). Dicke and Guthrie (1988) also mention cultivation through plowing initially provided increased pupae mortality.

Despite their differences in feeding behavior and impacts on grain yield, the combination of fall armyworm and corn earworm infestations in field corn is the most significant and economically damaging lepidopteran pest complex in the southeastern United States region. Since the biological development of both fall armyworm and corn earworm is greatly affected by the temperature and climate (Steffey et al. 1999), susceptibility to larger infestations is higher when crops are planted much later in the growing season of temperate and tropical regions in North and South America (Ayala et al. 2013, Bilbo et al. 2020, Buntin 2008, Buntin et al. 2001, Reay-Jones et al. 2020). Planting field corn at the optimal time of the growing season, during the spring season when the soil temperature remains between 10° and 30° Celsius (Sassenrath et al.

2019), is highly recommended to avoid massive fall armyworm and corn earworm infestations. However, intentions for an optimal planting date can easily be hindered at any location due to unsuitable weather conditions for the crop. Corn may be planted in part of a double cropping practice that could also affect its vulnerability to pest exposure (Buntin 2008, Buntin et al. 2004a-b).

Successful insecticidal control against both fall armyworm and corn earworm infestations is achievable in field corn through genetically modified grain seed with proteins from the *Bacillus thuringiensis* (Bt) bacterium. Originally used as an insecticidal genetically modified organism (GMO) against the European corn borer, *Ostrinia nubilalis* (Hübner) (Koziel et al. 1993), these transgenic hybrids comprise genetic events that commonly express specific Bt strain crystal-like (*Cry*) proteins which activate within the target pest's midgut after ingested and destroys the cell lining (Ostlie et al. 1997). The first transgenic Bt hybrids were introduced to the southeastern United States region in 1998, including YieldGard® products which contain the genetic events Bt11 and MON810 that both express the Cry1Ab protein (Buntin et al. 2001). Plantings across Georgia and Alabama found these products provided partial fall armyworm and corn earworm control with significantly less whorl defoliation and ear damage compared to their untreated counterparts (Buntin et al. 2001, 2004a-b, DeLamar et al. 1999a-c).

Additional Bt toxins have been discovered and commercially available since these first introductions. One type of protein frequently used in lepidopteran pest control is Cry1Fa2 expressed from the TC1507 event, considered very effective against fall armyworm infestations in later plantings (Ayala et al. 2013, Buntin 2008, Hardke et al. 2011). The MON89034 event expresses the linked proteins Cry1A.105 and Cry2Ab2 in products including DeKalb brand VT Double PRO and VT Triple PRO that has provided enhanced pest control as a second-generation

Bt product (Drury et al. 2008, Rule et al. 2014). The second generation of Bt technology would also introduce a new variety of protein known as vegetative insecticidal proteins (*Vip*), including Vip3Aa20 expressed from the MIR162 event. This protein, compared to all other commercially available Bt proteins, currently provides the most effective control against both fall armyworm and corn earworm infestations (Burkness et al. 2010, Burtet et al. 2017, Cook et al. 2020, 2021a-c, Niu et al. 2021, Yang et al. 2019a). Most transgenic Bt hybrids currently available for commercial use are single crop varieties expressing multiple Bt toxins, a concept known as gene pyramiding. Implementing this tactic has generally provided better crop protection against multiple target pest species with enhanced insecticidal efficacy and less crop injury compared to single-gene treatments (Buntin et al. 2004b, Moscardini et al. 2020, Rule et al. 2014, Siebert et al. 2012).

The planting of transgenic corn Bt hybrids in the United States grew significantly in the past couple decades due its continuing success towards pest control, increasing from 19% of all corn acreage in 2000 to 82% in 2020 (USDA-ERS 2020). Implementing Bt technology in corn also saw reductions in grain yield loss, but such results have been inconsistent. Early reports of Bt corn in Georgia and Alabama found significant prevention of grain yield loss was associated with greater levels of lepidopteran pest infestations, especially in late plantings (Buntin 2008, Buntin et al. 2001, 2004a, DeLamar et al. 1999a-c). However, this association most likely was linked with increased whorl infestations from fall armyworm since insignificant yield differences between Bt and non-Bt hybrids occurred when fall armyworm infestations were low. Bilbo et al. (2020) found significant reductions in fall armyworm infestation greatly improved grain yields but only in Bt hybrids expressing Cry1A.105 and Cry2Ab2. Since average corn earworm infestations alone have not resulted in significant grain loss, implementing Bt technology made little to no difference in total yield (Bibb et al. 2018, Reay-Jones and Reisig 2014). Bowen et al. (2014) did find Bt corn hybrids frequently produced higher grain yields than non-Bt hybrids over a three-year study, but these yields were not associated with ear damage as a result of lepidopteran pest feeding. Reports by Cook et al. (2020, 2021a-c) found hybrids expressing a Vip3A protein for corn earworm control frequently produced higher yields than all other hybrids, but these results were never consistent among planting dates.

Over time, the repeated use of specific Bt toxins against fall armyworm and corn earworm infestations eventually caused reductions in their insecticidal potency as a result of Bt resistance development in local pest populations. Resistance development occurred most frequently among *Cry* proteins with cross-resistance emergence in pyramided Bt hybrids from similar single-gene products being a significant contributor and remaining a high-risk factor for other proteins (Carrière et al. 2016, Huang et al. 2014, Niu et al. 2013, 2016a, Welch et al. 2015). First documented incidents of field resistance development in fall armyworm occurred in Puerto Rico in late 2006 against specific Cry1F proteins (Storer et al. 2010). Similar results in the southeastern United States were first observed around 2011 and documented by Huang et al. (2014), alluding the possibility of Cry1F-resistant pest populations from Puerto Rico migrating to neighboring countries. Bt resistance continued to spread throughout regions of the continental United States with heavy pest activity, as well as other countries including Brazil that confirmed fall armyworm populations resistant to the Cry1Fa2 and Cry1Ab proteins (J. R. Farias et al. 2014, Omoto et al. 2015). Protocols for detecting resistance development alongside field monitoring include F_2 screenings that would identify Bt toxin resistant alleles and its frequency within offspring of collected pest samples. Niu et al. (2016b) and Yang et al. (2019b) performed this protocol with collected fall armyworm populations from the southeast region and respectively confirmed small numbers of F_2 families carrying Cry2Ab2 and Vip3Aa51-resistant alleles. While resistance development in corn earworm populations is not as widespread compared to fall armyworm, it still resulted in rendering specific Bt toxins ineffective towards their control in the United States. Resistance development has been extensive against Cry1A proteins but variable and local against Cry2 proteins as reported in corn earworm populations across several states including both North and South Carolina (Reay-Jones et al. 2020), Maryland (Dively et al. 2016), Louisiana (Kaur et al. 2019), and Texas (Yang et al. 2019a). Vip3A proteins remain a reliable and effective toxin source against corn earworm (Burkness et al. 2010, Niu et al. 2021, Reay-Jones et al. 2020) as well as fall armyworm (Burtet et al. 2017). The emergence of resistant alleles against Vip3A proteins has slowly been emerging in local populations (Yang et al. 2019b, 2020), but no confirmed cases of field resistance have yet been reported with the exception of early warning signs being acknowledged in corn earworm populations across the southeast region (Yang et al. 2021).

The impact of resistance development in fall armyworm and corn earworm populations has called into question the continued efficacy of Bt technology and its future use in the United States and other countries. Such phenomena has not only occurred within fall armyworm and corn earworm populations but also is present in other lepidopteran pest species across North America including the sugarcane borer, *Diatraea saccharalis* (Fabricius), (Huang et al. 2015) and western bean cutworm, *Striacosta albicosta* (Smith) (J. L. Smith et al. 2017). Continual resistance development could force many growers to revert back to insecticidal sprays as their primary source of pest control (Burtet et al. 2017). Buntin and Flanders (2019) provide a list of transgenic Bt hybrids and their relative efficacy towards controlling the most common above-ground pests of corn in the southeastern United States. A number of Bt hybrids are still effective against these lepidopteran pest species according to this list, including hybrids expressing the Vip3Aa20 protein, but insecticide resistance management (IRM) for Bt products requires additional efforts to slow down the evolution of Bt resistance. Planting non-Bt refuge corn is the most commonly performed IRM tactic that helps in producing susceptible moths that will mate with resistant moths, with single-gene hybrids requiring a higher proportion than pyramided hybrids (Buntin and Flanders 2019, Carrière et al. 2016, Reisig and Kurtz 2018). Reisig and Kurtz (2018) proposes IRM against corn earworm can be improved with increased available hybrid varieties, careful selections of pyramided Bt proteins, and implementing high-dose Vip3A proteins which Burkness et al. (2010) also recommended. Similar ideas could be utilized for fall armyworm control in regions where continuous generations occur. Applications of alternate Bt hybrids can also be performed on existing resistant populations (Niu et al. 2016a). Additional research should be considered for other areas of IRM, including evaluations for what negative impacts sublethal Bt exposure has on surviving lepidopteran pest populations (Guedes et al. 2017). Regular field monitoring and scouting for pest activity in Bt-treated plots must be a routine task in the meantime for keeping up to date on the efficacy of commercially available Bt products in the southeastern United States region.

Mycotoxin management. Mycotoxins are secondary metabolites produced by fungal organisms and are capable of causing lethal effects on humans and other animals. Of all different types of mycotoxin discovered so far, aflatoxin and fumonisin, accumulated from *Aspergillus* spp. (Micheli) and *Fusarium* spp. (Link) infections, respectively, are the most significant fungal threats to field corn production in the southeastern United States region. Contamination can potentially render grain yield to be unconsumable without causing ill effects or death which in turn can be an economical threat to corn industries (Mitchell et al. 2016, Wu 2007). Aflatoxin contamination was the cause of several hepatitis outbreaks in humans and animals since the 1970s in regions where corn is commonly consumed (Krishnamachari et al. 1975, Ngindu et al. 1982). A significant outbreak that occurred across rural Kenya in 2004 was the result of increased aflatoxin contamination levels in locally grown corn due to wet storage conditions from unseasonable rain during harvest, causing over 300 human cases and over one hundred confirmed deaths (CDC 2004, Lewis et al. 2005). Fumonisin grain contamination has been linked with variations of localized organ damage among tested mammalian species (Kriek et al. 1981) as well as the diagnosis of neural tube defects and embryotoxicity in pregnant women (Missmer et al. 2006, Sadler et al. 2002).

In the United States, the maximum allowed aflatoxin level in corn grain intended for human consumption is 20 ppb (FDA 2000). The maximum allowed level for grain intended for animal feed depends on the type of livestock it will be given to. Examples of these levels include 300 ppb for beef cattle, 200 ppb for large swine, 100 ppb for poultry, and 20 ppb for dairy or immature animals (FDA 2000). Fumonisin has a maximum allowed level of 2-4 ppm for corn grain intended for human consumption, the exact level depending on the grain’s intention as a product and how well degerminated it is (FDA 2001). Maximum allowed fumonisin contamination levels for grain intended for animal feed also depends on the type of animals. Livestock intended to be slaughtered have a maximum level around 60-100 ppm, whereas smaller animals and pets have a level between 5-10 ppm (FDA 2001). Provisional maximum tolerable daily intakes are available for mycotoxins but vary based on global regions, ethnicity, and how much corn makes up a local population’s diet (Burger et al. 2014).

Aflatoxin and fumonisin accumulations respectively due to *Aspergillus* spp. and *Fusarium* spp. infections in field corn are associated with increased plant stress from a combination of environmental conditions including high temperatures and relative humidity levels, water stress as a result of drought conditions, and plant genetics (McMillian et al. 1985, Pruter et al. 2019, 2020, M. S. Smith and Riley 1992, Wiatrak et al. 2005). Therefore, planting dates play a significant role towards fungal susceptibility in field corn production (Abbas et al. 2007, M. S. Smith and Riley 1992, Wiatrak et al. 2005). Early reports by Lillehoj et al. (1975) and McMillian et al. (1985) found increased aflatoxin contamination levels were linked with significant ear injury caused by lepidopteran pest species, including corn earworm. Increased larval feeding could promote mycotoxin contamination through induced plant stress (Pruter et al. 2019, 2020, M. S. Smith and Riley 1992) or the larvae vector these fungal spores within their gut that are transferred into host plants upon feeding (Abel et al. 2002).

Attempts to reduce mycotoxin contamination in corn production through fungicide applications have provided inconsistent results across multiple years (Meyers et al. 2015, Molo et al. 2018, Parker et al. 2016). Fungicides did effectivity reduce fumonisin contamination for other cereal grains in respect to application timing (Yoshida et al. 2008, 2012). However, reductions in fungal contamination of corn grain were observed in select transgenic hybrids expressing specific Bt toxins that provide effective lepidopteran pest control. Studies from NC State Extension found the Bt hybrid Viptera significantly reduced both aflatoxin and fumonisin contamination of grain to levels safe for human consumption compared to a YieldGard hybrid and non-Bt hybrid while retaining adequate yield results (Meyers et al. 2015, Molo et al. 2018). This Syngenta brand product expresses the Bt proteins Cry1Ab and Vip3Aa20, the latter considered an excellent source against corn earworm infestations in the southeastern United States region (Buntin and Flanders 2019).

Implementing Bt technology for reducing grain aflatoxin contamination in corn production has provided inconsistent results. A list of older and Bt-related studies report associations between aflatoxin accumulation produced by *Aspergillus flavus* (Link) and ear damage caused by corn earworm and/or fall armyworm (Lillehoj et al. 1975, McMillian et al. 1985, Pruter et al. 2019, 2020, M. S. Smith and Riley 1992) as well as the southwestern corn borer, *Diatraea grandiosella* (Dyar) (Williams et al. 2002, Windham et al. 1999). Other studies that made similar comparisons found less significance and no consistent results (Bibb et al. 2018, Bowen et al. 2014, Buntin et al. 2001, 2004b, C. A. Farias et al. 2014). The results from these studies would additionally reflect what implementing Bt technology would do towards reducing grain aflatoxin contamination. Pruter et al. (2019) and Wiatrak et al. (2005) noted hybrids expressing Bt toxins significantly reduced grain aflatoxin contamination, whereas Buntin et al. (2001, 2004b) and C. A. Farias et al. (2014) had highly variable contamination levels throughout their tested hybrids with no obvious trends. The effectiveness of planting transgenic Bt corn hybrids for reducing grain aflatoxin contamination, therefore, remains inconclusive.

In contrast, fumonisin accumulation from *Fusarium* spp. infection has a strong association with ear injury from lepidopteran pest feeding. The western bean cutworm, *Striacosta albicosta* (Smith), is associated with deoxynivalenol and Gibberella ear rot emergence from accumulated *Fusarium graminearum* of corn in the Midwestern United States and Ontario, Canada (Parker et al. 2016, J. L. Smith et al. 2018). In Iowa, Munkvold et al. (1999) found increased European corn borer injury induced Fusarium ear rot, and Bowers et al. (2013) found positive associations in fumonisin accumulations with increased ear injury from European corn borer, western bean cutworm, and corn earworm. Incorporating Bt technology can significantly reduce grain fumonisin contamination (Abbas et al. 2007, Munkvold et al. 1999), especially pyramided hybrids that express the Vip3Aa20 protein (Bowers et al. 2013, J. L. Smith et al. 2018).

Evaluating Bt trait efficacy for mycotoxin contamination reduction is expected to continue in regions where environmental conditions favor fungal development. The southeastern United States region is especially susceptible to mycotoxin contamination in grain production due to significant lepidopteran pest activity and experiencing a humid subtropical climate with only a small proportion of its total farm acreage actively irrigated (USDA-NASS 2018). Plant stress management should be considered for such regions to prevent excessive fungal development that involves increased irrigation, retaining soil quality, and potentially incorporating Bt technology in corn hybrids.

Research objectives: The objectives of this thesis were to evaluate the efficacy of Bt corn hybrids for managing fall armyworm and corn earworm infestations in field corn production and see if their significant control associates with reductions in grain yield loss and mycotoxin contamination. Additionally, the impact of fall armyworm whorl defoliation on grain production was further analyzed by comparing yields from corn plants with and without larval feeding damage. Field experiments were conducted across central and south Georgia in 2019 and 2020. Data collection observed levels of fall armyworm whorl defoliation during the mid-vegetative growth stages and corn earworm ear damage during the reproductive growth stages among treated and untreated plots. Harvested samples from each plot were evaluated for their grain yield and quality as well as their grain aflatoxin and fumonisin contamination levels and if these results were in any way associated with fall armyworm or corn earworm infestations.

We hypothesized all Bt corn hybrids tested will significantly reduce the presence of fall armyworm, but only those hybrids expressing a Vip3A protein will effectively reduce corn earworm infestations. Reductions in grain yield is only to be speculated with fall armyworm or severe outbreaks of corn earworm. Substantial reductions in aflatoxin and fumonisin contamination would only occur in areas of significant control of ear damage due to larval feeding from those including corn earworm.

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CHAPTER 2

BT TRAIT EFFICACY AGAINST CORN EARWORM (*HELICOVERPA ZEA*) FOR IMPROVING GRAIN YIELD AND REDUCING MYCOTOXIN CONTAMINATION OF FIELD CORN

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Abstract

The corn earworm, *Helicoverpa zea* (Boddie), causes persistent ear damage to corn grown in the southeastern United States region. Greater levels of ear damage have been associated with increased mycotoxin contamination in addition to grain yield loss. Corn hybrids expressing Bt traits have provided substantial earworm control. A selection of these hybrids were tested in field experiments across the state of Georgia over two years to monitor their efficacy towards corn earworm control and protecting grain quality. Ear damage was significantly reduced only by Bt hybrids expressing the Vip3Aa20 protein. All other Bt hybrids provided only marginal control. Ear damage had mixed results with grain yield and was not correlated with grain aflatoxin contamination. Grain fumonisin contamination was significantly associated with earworm damage. These results indicate Bt hybrids that effectively reduce corn earworm ear damage may also assist in reducing fumonisin contamination and possibly grain yield loss.

Key Words: *Zea mays*, transgenic crop, *Bacillus thuringiensis*, fumonisin, aflatoxin, IPM, plant-insect interactions, Vip3Aa20

Introduction

The introduction of corn, *Zea mays* (L.), genetically modified with proteins from the *Bacillus thuringiensis* (Bt) bacterium was initially used in North America as an insecticidal method against stalk-boring pests including the European corn borer, *Ostrinia nubilalis* (Hübner) (Koziel et al. 1993, Ostlie et al. 1997). This method ultimately provided mixed results when tested against corn earworm, *Helicoverpa zea* (Boddie), a lepidopteran pest species that hosts on an array of crops, including field corn grown in the southeastern United States region. Corn earworm larvae feed on the silks and corn ears for about six instars prior to pupating in the soil, resulting in significant damage to the upper ear region. The impact of larval feeding alone, however, is not generally an economical threat for field corn production (Bibb et al. 2018, Olivi et al. 2019, Reay-Jones and Reisig 2014) since most corn earworm activity occurs at the ear tip region where unpollinated kernels are located (Dicke and Guthrie 1988). However, significant ear damage with some level of grain yield reduction has been reported, especially in late plantings when larval activity is higher (Buntin et al. 2001, 2004a, Cook et al. 2021a-c). Furthermore, ear damage may result in corn ears becoming vulnerable to toxic secondary metabolites known as mycotoxins. Aflatoxin and fumonisin contamination accumulated from *Aspergillus* spp. (Micheli) and *Fusarium* spp. (Link) infections, respectively, are the most common-occurring mycotoxins in field corn production in the southeastern United States region.

Grain that is highly contaminated with aflatoxin or fumonisin would cause ill effects or death among populations if used for human consumption or animal feed. A major mycotoxicosis outbreak that occurred across rural Kenya in 2004 was the result of harvested corn stored under damp conditions which promoted severe grain aflatoxin contamination, resulting in numerous sick cases and death (CDC 2004). In addition to excessive moisture, heightened mycotoxin contamination can occur from other in-field factors including high temperatures, drought conditions, and plant genetics (Abbas et al. 2007, Pruter et al. 2019, 2020, M. S. Smith and Riley 1992, Wiatrak et al. 2005). The federal standard limit in grain intended for human consumption is 20 ppb for aflatoxin and 2-4 ppm for fumonisin, whereas if intended for animal feed they depend on the target animals (FDA 2000, 2001). Early studies observed significant grain aflatoxin contamination was present in ears with greater amounts of corn earworm feeding damage (Lillehoj et al. 1975, McMillian et al. 1985). The larvae vector fungal spores within their gut which may be transferred into plants upon feeding (Abel et al. 2002). The result of this ear damage can also promote mycotoxin contamination of grain through induced plant stress conditions as a result of warmer temperatures, high humidity levels, drought conditions, and plant genetics (McMillian et al. 1985, Pruter et al. 2019, 2020, M. S. Smith and Riley 1992, Wiatrak et al. 2005). Therefore, planting dates play a significant role towards fungal susceptibility for field corn production in temperate and tropical regions (Abbas et al. 2007, M. S. Smith and Riley 1992, Wiatrak et al. 2005).

Preliminary studies that evaluated the efficacy of transgenic Bt corn hybrids after commercially introduced to the southeastern United States region in 1998 found it provided partial control against corn earworm infestations, but significant control and improved grain yield was only observed against fall armyworm, *Spodoptera frugiperda* (J. E. Smith), whorl infestations (Buntin et al. 2001, 2004a-b, DeLamar et al. 1999a-c). These first tested hybrids consisted of the genetic events BT11 and MON810 which express Cry1Ab, a specific Bt strain crystal-like (*Cry*) protein with a mode of action that destroys the cell lining of the target pest's midgut where it activates after ingested (Ostlie et al. 1997). Additional Bt toxins that were discovered and commercially available in the United States had varying levels of efficacy towards corn earworm control. The TC1507 event expresses the Cry1Fa2 protein that is less effective than Cry1Ab against corn earworm (Buntin 2008). The MON89034 event expresses the pyramided proteins Cry1A.105 and Cry2Ab2 which, unlike Cry1Fa2, provided better control against corn earworm than Cry1Ab (Drury et al. 2008, Rule et al. 2014). Vegetative insecticidal proteins (*Vip*), including the Vip3Aa20 protein expressed from the MIR162 event, currently provide the most effective control in reducing corn earworm numbers using a different mode of action (Burkness et al. 2010, Cook et al. 2020, 2021a-c, Niu et al. 2021, Yang et al. 2019). Cook et al. (2020, 2021a-c) also found Bt hybrids expressing a Vip3A protein frequently produced higher yields than other hybrids, but these results were never consistent among planting dates.

The overall effect of transgenic Bt hybrids for reducing aflatoxin contamination has been inconsistent. In addition to the early studies by Lillehoj et al. (1975) and McMillian et al. (1985), a list of mostly Bt-related studies found significant associations between lepidopteran pest ear damage and aflatoxin contamination levels (Pruter et al. 2019, 2020, M. S. Smith and Riley 1992, Williams et al. 2002, Windham et al. 1999). However, other studies would find greater inconsistency and no association despite making similar comparisons (Bibb et al. 2018, Bowen et al. 2014, Buntin et al. 2001, 2004b, Farias et al. 2014). The results from these studies would also reflect what incorporating Bt technology has on aflatoxin contamination with some seeing a significant reduction (Pruter et al. 2019, M. S. Smith and Riley 1992, Wiatrak et al. 2005) and other studies finding no significant change (Bibb et al. 2018, Bowen et al. 2014, Buntin et al. 2001, 2004b, Farias et al. 2014). It remains inconclusive if Bt technology is considered an effective tool for aflatoxin reduction in field corn.

On the other hand, fumonisin contamination has a strong association with increased ear injury from lepidopteran pest feeding. The western bean cutworm, *Striacosta albicosta* (Smith), is associated with deoxynivalenol and Gibberella ear rot emergence from accumulated *Fusarium graminearium* of corn in the Midwestern United States and Ontario, Canada (Parker et al. 2016, J. L. Smith et al. 2018). In Iowa, Munkvold et al. (1999) found increased European corn borer injury induced Fusarium ear rot, and Bowers et al. (2013) found positive associations in fumonisin accumulations with increased ear injury from European corn borer, western bean cutworm, and corn earworm. Incorporating Bt technology could significantly reduce grain fumonisin contamination (Abbas et al. 2007, Munkvold et al. 1999), especially pyramided hybrids that express the Vip3Aa20 protein (Bowers et al. 2013, J. L. Smith et al. 2018).

The use of Bt traits as an insecticidal source in corn is widely utilized throughout the United States with 82% of all domestically planted corn in 2020 having expressed single or pyramided Bt toxins (USDA-ERS 2020). However, repeated applications of Bt technology eventually resulted in resistance development to occur across local lepidopteran pest populations. Fall armyworm control is one of the most affected areas with widespread resistance occurring to the Cry1Fa2 toxin (Storer et al. 2010, Huang et al. 2014). Bt resistance development also has emerged in other lepidopteran species including the sugarcane borer, *Diatraea saccharalis* (Fabricius), (Huang et al. 2015) and western bean cutworm (J. L. Smith et al. 2017). For corn earworm, resistance to Cry1A proteins is widespread and on local levels for Cry2 proteins as development was reported across several states including North and South Carolina (Reay-Jones et al. 2020), Maryland (Dively et al. 2016), Louisiana (Kaur et al. 2019), and Texas (Yang et al. 2019). The presence of resistant alleles to Vip3A proteins has been confirmed in at least local populations from Texas and the Mid-South, and currently field resistance to Vip3A may be increasing (Yang et al. 2020, 2021). Cross-resistance between single-gene and pyramided Bt toxins has contributed to the evolution of Bt resistance and remains a major risk factor for other toxins (Huang et al. 2014, Niu et al. 2013, Welch et al. 2015), including *Cry* proteins that provide only partial infestation control and leave behind a significant number of surviving larvae in treated plots (Guedes et al. 2017, Tabashnik et al. 2009).

Continual resistance development in lepidopteran pest species will place further pressure on Bt technology and its future intentions as an insecticide. Its progression will not only make ear protection difficult from larval feeding but may also require additional tactics for mycotoxin contamination control. A list of Bt products still remain effective in controlling corn earworm infestations in the southeastern United States, specifically those expressing *Vip* proteins (Buntin and Flanders 2019, Niu et al. 2021, Reay-Jones et al. 2020). Insecticide resistance management (IRM) requires growers to manage the types and timing of tactics to slow Bt resistance development. This should include increased plantings of non-Bt refuge crops (Buntin and Flanders 2019, Carrière et al. 2016). Additional tactics include alternate Bt hybrid applications, increased Vip3A treatments, and sentinel plot networks recommended by recent research publications based on their findings (Burkness et al. 2010, Dively et al. 2021, Niu et al. 2021, Reisig and Kurtz 2018). However, regular field monitoring of Bt-treated plots remains essential for detecting any changes in Bt trait efficacy in areas with high lepidopteran pest activity. We evaluated a selection of commercially available field corn hybrids expressing pyramided Bt traits to see how well they prevented corn earworm ear damage in the state of Georgia. Larval infestations and ear damage were measured and established if they were significantly associated with increases in grain yield loss or mycotoxin contamination levels and if Bt technology could reduce these factors. We hypothesize only those Bt hybrids expressing a Vip3A protein will effectively reduce corn earworm infestations. Reductions in grain yield loss are not expected to occur unless there is a severe outbreak of corn earworm. Substantial reductions in aflatoxin and fumonisin contamination down to their respective federal standard limits are expected only where there is significant control of corn earworm ear damage.

Materials and Methods

Field experiments: Field experiments were conducted at two locations per year in central and south Georgia in 2019 and 2020. Locations were the University of Georgia Bledsoe Research farm near Griffin (N 33.175964 W -84.409210), the Southwest Georgia Research and Education Center near Plains (N 32.046602 W -84.370610), and the University of Georgia Lang-Rigdon farm near Tifton (N 31.516910 W-83.548479). Corn seed was planted at a rate of 79,040 seed per ha in 91 cm wide rows at the Plains and Tifton locations and 76 cm wide rows at the Bledsoe location using a two-row Monosem® pneumatic planter. Plots were eight rows wide and 12.2 m long except in the 2020 Tifton planting which were eight rows wide and 10.7 m long.

Soil was an Appling sandy loam in Griffin, a Greenville sandy loam in Plains, and Tifton sandy loam in Tifton. Weed control and fertility practices followed the Georgia Extension Service recommendations for each location. Tillage was conventional at all locations with chisel plowing followed by disk harrowing. Before disking, 440 kg/ha of a 5-10-15 (N-P-K) granular fertilizer was applied and an additional 112 kg of nitrogen as ammonium nitrate was applied beside the rows and incorporated about 20 days after planting. Atrazine (Aatrex 4L, Syngenta Crop protection, Greensboro, North Carolina) with pendimethalin (Prowl 3.3EC, BASF, RTP, North Carolina) were applied at planting at all locations except in Griffin where Atrazine with acetochlor (Warrant, Bayer CropScience LP, St. Louis, Missouri) was applied. Plots were sprayed with glyphosate (Roundup WeatherMax, Monsanto Company, St. Louis, Missouri, or

Glyphos brands) about 20 days after planting for weed control. All corn seed was received from the seed companies and pretreated with two or three fungicides and either clothianidin at 0.5 mg per kernel (Poncho 250 or 500, Bayer CropSciences RTP, Greensboro, North Carolina) or thiamethoxam at 0.5 mg per kernel (Syngenta Crop Protection, Greensboro, North Carolina). No other insecticides were applied. Natural rainfall was supplement by irrigation weekly with 6 cm of water as needed.

A selection of available hybrids with various pyramided Bt proteins for above-ground pests were evaluated and compared with comparable non-Bt hybrids (Table 2.1). Hybrids were provided by DeKalb Seeds (Bayer CropSciences, St. Louis, Missouri) and DuPont Pioneer Hi-bred International Inc. (Corteva AgriScience, Johnston, Iowa). Planting dates in 2019 were April 24 in Plains and April 25 in Tifton. Planting dates in 2020 were April 3 in Tifton and May 16 in Griffin. Experimental design for all plantings was a randomized complete block design with four replicates. Several rows of a non-Bt corn hybrid, DeKalb DKC 6694, were planted on either ends of every experiment acting as border rows.

Data Collection: Observations began with measuring for whorl damage caused by lepidopteran defoliators, including fall armyworm larvae. Plant stand counts were made on the center two rows per plot while inspecting for whorl damage on the corn plants around the five to eight-leaf vegetative growth stages. Plant defoliation was rated using the Davis et al. (1992) 0-9 scale, where 0 represents no damage and 9 represents near total destruction of the whorl.

Evaluation for corn earworm infestations within these plots began when crops reached the milk stage (R3) (Adendroth et al. 2011). Fifteen random ears from each plot were opened from their husks and examined for the presence of corn earworm larvae. The total number of larvae was counted per plot with each larva categorized as either small (first and second instars), medium (third and fourth instars), or large (fifth and sixth instars) in size. Small larvae were identified as no larger than 7 mm, whereas large larvae were at least 24 mm in length. Medium-sized larvae, therefore, would fall in between 7 and 24 mm in length. Exit holes with underlying ear damage also counted towards corn earworm presence as they indicate a larva had completed development and exited the ear to pupate in the soil.

Plots were evaluated for total corn earworm damage at the late dent stage (R5) to early physiological maturity stage (R6) (Adendroth et al. 2011). Another fifteen random ears were inspected in each plot and the total area damage from larval feeding was measured in cm^2. Damage measurements for every ear were divided between the tip portion with unpollinated kernels and the rest of the ear containing viable kernels.

At full maturity, grain was harvested from the center two rows not used for earworm sampling of each plot using a two-row Wintersteiger Delta combine (Wintersteiger Inc., Salt Lake City, Utah) with an automated weighing system that measured plot grain weight, percent moisture content, and test weight (kg/hL). Grain yields (kg/ha) were calculated and adjusted to a standard 15.5% moisture content. One-kilogram grain subsamples from each plot were collected and delivered to Waters Agricultural Laboratories, Inc. in Camilla, Georgia where mycotoxin contamination levels were measured. The NEOGEN Veratox® method, a competitive direct ELISA, provided quantitative analyses of total aflatoxin (B_1, B_2, G_1, G_2) (ppb) and total fumonisin (B_1, B_2, B_3) (ppm) contamination levels of corn grain (NEOGENa-b). Both methods are approved by the AOAC Research Institute (Latimer 2019, NEOGEN 2009). Samples that read higher levels than the standard provided in each kit were diluted then re-analyzed with the additional dilution factor applied to the quantitative value.

Statistical Analysis: The statistical software SAS version 9.4 and JMP Pro version 15.0.0 were used for data analysis (SAS Institute 2013, 2019). Results were combined among location but were analyzed by year because tested hybrids differed between 2019 and 2020. Results were statistically analyzed through an analysis of variance (ANOVA) mixed model with PROC MIXED and appropriate statistical procedures for a randomized completed block design. Hybrids were treated as a fixed effect and replicates were treated as a random effect for this mixed model. Factorial arrangements of hybrid treatments, location, and the treatment x location interaction examined for any potential influences on corn earworm, yield, and mycotoxin results by year ($\alpha = 0.05$). Single degree-of-freedom contrasts of selecting hybrids compared the effects of expressed Bt proteins among non-Bt hybrids, Bt hybrids expressing only *Cry* proteins, and Bt hybrids expressing Vip3Aa20. Degrees of freedom were estimated using the Kenward-Roger method. Treatment means were separated using pairwise T-test groupings in PROC PLM when a significant difference was indicated among *F*-values ($\alpha = 0.05$). Linear regression through a bivariate fit of two continuous data type variables using PROC CORR (SAS Institute 2013) examined the associations of corn earworm ear damage with grain yield and contamination levels of aflatoxin and fumonisin.

Results

Infestation Rates and Ear Damage. No significant whorl defoliation was observed during the vegetative growth stages in all four plantings. Overall corn earworm infestations and damage ratings were lower than normal for field corn in southern Georgia for both 2019 and 2020. Infestation levels were significantly higher in those experiments planted from late April into May than planted in early April. Corn earworm infestations were significantly different among treatments (Table 2.2). Overall Bt hybrids significantly reduced corn earworm infestations during the R3 growth stage by an average of 65.2% in 2019 (Non-Bt: 73.12 ± 3.53% infested plants; Bt: 25.42 ± 4.53% infested plants) and 53.6% in 2020 (Non-Bt: 53.33 ± 5.08% infested plants; Bt: 24.76 ± 4.35% infested plants).

The Bt products Genuity Trecepta and Optimum Leptra had the lowest levels of larval infestation throughout every planting. These two products express the Vip3Aa20 protein in addition to *Cry* proteins (Table 2.1). Most plots that contained either product had very little to no infestation based on the R3 growth stage data collected both years (Tables 2.3 and 2.4). Instances where Bt hybrids expressing Vip3Aa20 did have recorded larval infestations were very low in number and only occurred in the late 2019 and early 2020 plantings (Tables A.1 and A.2). Bt hybrids expressing only *Cry* proteins provided intermediate infestation control, but these results varied in statistical significance from non-Bt hybrids. Genuity VT Double PRO (Cry1A.105, Cry2Ab2) and SmartStax (Cry1A.105, Cry2Ab2, Cry1Fa2) reduced corn earworm infestations to an extent, whereas Optimum Intrasect (Cry1Ab, Cry1Fa2) was not significantly different from non-Bt hybrids (Tables 2.3 and 2.4). In the early planting in 2020, Bt hybrids expressing only *Cry* proteins reduced corn earworm infestation about as effectively as Bt hybrids expressing Vip3Aa20. However, the later plantings overall had greater levels of infestation, and Bt hybrids expressing only *Cry* proteins had just as much corn earworm infestations as non-Bt hybrids (Table A.1). Such phenomena resulted in a significant treatment x location interaction for percent infestation (Table 2.2).

The results for average corn earworm larvae per ear were similar to that of infestation levels aside from a few discrepancies. Hybrids with higher levels of infestation tend to have more larvae larger in size or have already exited the ears they were feeding on. A combined total of five larvae and three exit holes were observed in all hybrids expressing Vip3Aa20 both years. This total larvae presence occurred almost exclusively during the early planting in 2020 and comprised of mostly large larvae (Tables 2.3 and 2.4). Bt hybrids expressing only *Cry* proteins had significantly less larvae than non-Bt hybrids both years despite the two groups having very similar infestation rates. Larvae from hybrids expressing only *Cry* proteins were mostly small or medium in size, as compared to non-Bt hybrid larvae that were mostly large or had already exited the ear (Tables 2.3 and 2.4, Figures 2.1 and 2.2). Total larvae counts were about equal across all Bt hybrids expressing only *Cry* proteins except for the Optimum Intrasect hybrids both years and DeKalb DKC 6826, a Genuity VT Double PRO product, in 2020 which had total larvae counts similar to that of non-Bt hybrids (Tables 2.3 and 2.4). Infestation rates were significant by treatment, location, and their interaction both years. Larvae per ear averages were not significant in 2019 but were significant in 2020 by location and the treatment x location interaction (Table 2.2).

Results for R6 growth stage ear damage were also significantly different by treatment as they were for R3 growth stage infestation rates and larval counts (Table 2.2). Overall Bt hybrids significantly reduced total corn earworm ear damage by an average of 75.0% in 2019 (Non-Bt: 4.16 ± 0.32 cm^2; Bt: 1.04 ± 0.26 cm^2) and 66.7% in 2020 (Non-Bt: 3.18 ± 0.30 cm^2; Bt: 1.06 ± 0.25 cm^2). Bt hybrids expressing Vip3Aa20 were undamaged in 2019 and had only minimal ear damage during the early planting in 2020 that was located mostly within the ear tip region (Tables 2.5, 2.6, and A.3). Bt hybrids expressing only *Cry* proteins significantly provided moderate reductions in both tip and kernel damage compared to non-Bt hybrids but not as effective as hybrids expressing Vip3Aa20 (Tables 2.5 and 2.6). Only in the early planting in 2020 did these two types of Bt hybrids perform equally in reducing ear damage (Table A.3). The proportion of damage in the tip and kernel regions was evenly the same in 2019, whereas in 2020, much more ear damage took place in the viable kernels (Figures 2.3 and 2.4). Ear damage was also significant by location and the treatment x location interaction both years (Table 2.2).

Corn Yield: Grain yields were different among hybrids in 2019 (Table 2.2), but these differences were not associated with Bt traits and instead reflected hybrid agronomics (Tables 2.7 and A.4). Linear regression analysis found no association between 2019 grain yield results with corn earworm ear damage dealt that year (Figure 2.5). In 2020, all Bt hybrids significantly produced on average about 5.5% more grain yield than non-Bt hybrids (Non-Bt: 12,642 ± 2,270 kg/ha; Bt: 13,380 ± 1,934 kg/ha) (Table 2.8). The expression of Vip3Aa20 among Bt hybrids provided a marginally significant improvement in grain yield compared to those Bt hybrids expressing only *Cry* proteins (Table 2.7). Grain yield in 2020 had a significantly weak negative association with corn earworm damage based on linear regression analysis, unlike the results from the year prior (Figure 2.5).

Test weights, which are a measure of grain quality, were significantly different among hybrids both years but partially due to hybrid agronomics. In 2019, overall Bt hybrids had significantly higher test weights compared to non-Bt hybrids with a significant difference found between non-Bt hybrids and Bt hybrids expressing Vip3Aa20 (Table 2.7). However, test weight results in 2020 were not significantly associated with Bt traits (Table 2.8).

Grain yields and test weights were significant by planting location both years (Table 2.2). Plantings at the Tifton location in 2019 and 2020 produced the highest grain yields while the late 2020 planting at the Griffin location had the lowest yields (Table A.4). The treatment x location interaction was only significant in the 2019 grain yield results and not at all for test weight results (Table 2.2).

Mycotoxin contamination levels: Grain aflatoxin contamination in 2019 and 2020 was highly variable among plot samples. Contamination levels were significantly different by treatment as a result of their genetic backgrounds (Tables 2.9, 2.10, and A.5). While most concentrations were around the federal standard limit of aflatoxin in grain intended for human consumption, 20 ppb, a small number of samples exceeded 100 ppb with a few exceeding 500 ppb. Variability in concentrations for a select few points resulted in skewing of statistical significance to their respective hybrids. The Bt hybrid Pioneer 1637YHR, an Optimum Intrasect product, was an example of this occurrence having an average aflatoxin contamination level in the triple digits for three of the four total plantings. Such phenomenon rendered results to be statistically different in the contrasting statements (Tables 2.9 and 2.10) as well as the treatment and location influence evaluations (Table 2.2). Aflatoxin contamination levels were not significantly different among Bt and non-Bt hybrids and were also not significantly associated with corn earworm damage based on linear regression analysis (Tables 2.9 and 2.10, Figure 2.6). The treatment x location interaction was also not significant either year (Table 2.2).

Grain fumonisin contamination in 2019 had variability among plots but was significantly different among hybrids (Tables 2.9 and A.6). Only Bt hybrids expressing the Vip3Aa20 protein had significantly lower contamination levels compared to non-Bt hybrids. However, grain fumonisin contamination levels for all hybrids in 2019 exceeded the federal standard limit of 2-4 ppm for grain intended for human consumption (FDA 2001). Bt hybrids expressing only *Cry* proteins did not significantly reduce fumonisin contamination as compared to non-Bt hybrids but also were not statistically different from Bt hybrids expressing Vip3Aa20 (Tables 2.10 and A.6). Grain fumonisin contamination levels in 2019 had a significant weak positive association with ear damage (Figure 2.7). Overall contamination levels in 2020 were much lower than what they were in 2019 but were not significantly different among hybrids. Grain fumonisin contamination levels were also not significantly different among Bt traits in 2020 (Table 2.10). However, linear regression analysis still found fumonisin contamination levels that year had a significant weak positive association with ear damage (Figure 2.7). Contamination levels were significant by planting location both years as well as by the treatment x location interaction in 2019 but not in 2020 (Table 2.2).

Discussion

Planting transgenic corn hybrids that express multiple Bt traits through gene pyramiding has been a continuing protocol enacted in temperate and tropical regions where lepidopteran pest species, including corn earworm, are common (Bowen et al. 2014, Buntin et al. 2004b, Rule et al. 2014, Siebert et al. 2012). Resistance development as well as cross-resistance to specific Bt traits has been growing more apparent in local corn earworm populations across the southeastern United States region, making them more difficult to control (Dively et al. 2016, Huang et al. 2014, Kaur et al. 2019, Niu et al. 2013, 2021, Reay-Jones et al. 2020, Welch et al. 2015, Yang et al. 2019, 2021). In this study, we evaluated the efficacy of commercially available field corn hybrids expressing pyramided Bt proteins against corn earworm infestations in Georgia as well as its performance towards reducing grain yield loss and mycotoxin contamination.

Bt hybrids expressing the Vip3Aa20 protein were the most effective in reducing corn earworm infestations and ear damage throughout all four plantings. Our results are consistent with other studies in performance of the protein as an insecticidal source where its expression in the crop leaves no more than a minimal presence of corn earworm (Bilbo et al. 2018, Burkness et al. 2010, Niu et al. 2021, Reay-Jones et al. 2020). Not only have these Bt products expressing Vip3Aa20 had minimal or no earworm infestations, but their efficacy has remained consistent throughout later plantings when corn earworm infestations are greater. Bt hybrids expressing only *Cry* proteins provided moderate reductions in earworm infestation and damage in optimal plantings but performed poorly in later plantings. We conducted a different study in 2019 but with a similar protocol from this study evaluating Bt hybrid performance against fall armyworm infestations of late-planted field corn while also checking the ears for corn earworm infestations. Results found both non-Bt hybrids and Bt hybrids expressing only *Cry* proteins averaged at least 90% infestation at the R3 and R6 growth stages, whereas hybrids expressing Vip3Aa20 proteins had no larvae or ear damage (Barton and Buntin, unpublished data). Future applications of *Cry* proteins in regions with notable corn earworm activity must be carefully considered in order to extend the lifespan of available Bt products, especially in double cropping practices.

Despite having corn earworm susceptibility similar to that of non-Bt hybrids, larval development in Bt hybrid plots expressing only *Cry* proteins had diminished with most recorded larvae measured between their first and fourth instars. Recorded larvae from non-Bt hybrids, while also higher in number, matured more frequently to the later instars and exited the ears in preparation to pupate in the soil. This indicates while the *Cry* protein did not cause much direct mortality, the toxins did delay the development of corn earworm larvae. It is unknown if any corn earworm from these Bt hybrid plots that survived into adulthood had reduced fecundity (Bilbo et al. 2018). As for ear damage, pyramided *Cry* proteins alone also caused partial reductions in both tip and kernel damage, partly due to decreased larval development. Bt hybrids that expressed only the Cry1Ab and Cry1Fa2 proteins failed to prevent any ear infestation or damage caused by corn earworm larvae. Cry1Fa2 has not been considered an effective insecticidal source against corn earworm (Buntin 2008), whereas Cry1Ab's potency has declined due to widespread resistance against the toxin in corn earworm populations across the southeastern United States region (Bilbo et al. 2018, Niu et al. 2021, Reay-Jones et al. 2020, Yang et al. 2019). Partial earworm control at optimally timed plantings was most notable for Bt products like Genuity VT Double PRO and SmartStax that express Cry1A.105 and Cry2Ab2, yet these products still remain vulnerable to further resistance development based on growing larval development and ear damage in its treated plots (Dively et al. 2016).

While corn earworm are notorious for infesting ears and feeding on the kernels, the result of their damage normally does not consider them an economically significant pest of field corn (Bibb et al. 2018, Reay-Jones 2019, Reay-Jones and Reisig 2014). Simulated corn earworm damage performed by Olivi et al. (2019) only resulted in significant yield loss in weight when at least 60 kernels, or 15 cm^2 based on the conversion 0.25 cm^2 = 1 kernel, were damaged per ear. Measured average kernel damage in both 2019 and 2020 was much lower than 60 kernels per ear even in the late plantings, not even considering the proportion of ear damage located the unpollinated ear tip. However, all Bt hybrids produced significantly more grain yield than non-Bt hybrids in 2020 when a higher proportion of viable kernel damage was also observed that year among untreated plots. While it currently may not be a primary issue in Georgia agriculture, Bt hybrids show promise for reducing minimal kernel loss as a result of larval ear feeding. Determining whether Bt hybrids could also improve grain quality based on test weight would require further evaluation and additional grain measurements. Bt hybrids will likely not be used solely for preventing grain yield loss caused by corn earworm in the southeastern United States region, but they will continue to provide effective control of other lepidopteran pest species including the European corn borer (Bowers et al. 2013, Ostlie et al. 1993) and fall armyworm (Buntin 2008, Buntin et al. 2001, 2004a).

Aflatoxin contamination accumulates from *Aspergillus* spp. infection and is notorious for emerging in an array of crops, including corn, whether transferred from pests (Abel et al. 2002) or develops through induced plant stress conditions in addition to feeding damage (Pruter et al. 2019, 2020, M. S. Smith and Riley 1992, Williams et al. 2002). Early studies suggest higher grain aflatoxin contamination levels occur in plants that have greater amounts of lepidopteran pest ear damage, especially corn earworm (Lillehoj et al. 1975, McMillian et al. 1985). Based on our results, we found grain aflatoxin contamination was not associated with corn earworm ear damage nor was it reduced by the presence of any Bt toxins. Varying levels of fungal contamination were observed in similar studies that also found insignificant results, some experiencing variability greater than others (Bibb et al. 2018, Bowen et al. 2014, Buntin et al. 2001, Farias et al. 2014). Relative differences in aflatoxin contamination levels may instead be influenced by temperature, humidity, kernel moisture content, and rainfall across planting locations and dates (Abbas et al. 2007, M. S. Smith and Riley 1992, Wiatrak et al. 2005). The state of Georgia experiences a humid subtropical climate alongside other states in the southeastern United States region which promotes fungal infection and growth. Larger spikes in contamination of select Bt and non-Bt hybrids may indicate induced stress conditions of localized grain in plots, though further analysis should investigate this phenomenon. Cultivation practices towards minimizing plant stress conditions may assist with reducing grain aflatoxin contamination in highly susceptible regions.

We also found Bt hybrids that effectively reduced corn earworm infestations also reduced grain fumonisin contamination accumulated from *Fusarium* spp. infection based on linear regression analysis (Figure 2.7). Past reports have found associations between fumonisin contamination and other lepidopteran pest species including the European corn borer (Munkvold et al. 1999) and western bean cutworm (Parker et al. 2016, J. L. Smith et al. 2018) but not as much attention towards corn earworm. Bowers et al. (2013) observed strong associations between increased fumonisin contamination and kernel injury by these three lepidopteran pest species that all were significantly reduced in plants expressing Cry1Ab and Vip3Aa20. However, no additional Bt toxins were involved in their study, similar to how Munkvold et al. (1999) only used Cry1Ab and Cry9c, and J. L. Smith et al. (2018) used Cry1Ab, Cry1Fa2, and Vip3Aa20. Here we provide additional data on fumonisin contamination levels in corn hybrids expressing a wider selection of pyramided Bt proteins intended for lepidopteran pest control. Despite Bt hybrids expressing Vip3Aa20 significantly reduced fumonisin contamination levels in 2019, overall contamination was still over the federal standard for grain intended for direct human consumption and only partially within standard for certain animal feeds (FDA 2001). It is highly suggested for future evaluations of Bt traits for reducing fumonisin contamination to focus on contamination levels in respect to federal standard limits of grain intended for human consumption or animal feed.

The recurring significance in results by location suggest overall better growing conditions in the Tifton region across both years. Data analysis by planting location in 2019 and 2020 showed the Tifton plantings experienced the least amount of corn earworm infestation and mycotoxin contamination in addition to the highest corn yields (Tables A.2 – A.6). The coastal plain region of Georgia experiences a relatively warmer climate and is where the Plains and Tifton plantings took place. The Tifton planting was the southernmost location in this study and was planted only a day apart from the Plains planting in 2019. Combinations of optimal planting dates and steadier plant conditions in that region might have reduced corn earworm infestation and stress conditions associated with higher mycotoxin contamination (Abbas et al. 2007, Pruter et al. 2019, 2020, Wiatrak et al. 2005).

In conclusion, this study demonstrates the importance of Vip3A proteins in Bt corn hybrids for corn earworm control in the southeastern United States region. Planting transgenic Bt corn hybrids that express *Vip* proteins such as Vip3Aa20 not only provides very effective control against corn earworm infestations but also could assist in reducing grain fumonisin contamination and marginal grain yield loss. While it also performs consistently in larger outbreaks and late plantings, the absence of *Vip*-resistant populations make these particular Bt hybrids useful towards controlling corn earworm populations with significant *Cry* protein resistance. High-dose Vip3A treatments are suggested towards these *Cry*-resistant populations (Burkness et al. 2010, Reisig and Kurtz 2018). However, Reay-Jones (2019) mentions increased usage of the protein across more than one crop for controlling resistant populations could reduce its durability and emphasizes the importance of IRM tactics such as planting non-Bt refuges (Buntin and Flanders 2019) and careful usage of pyramided Bt products. Hybrids expressing only Cry1Ab and Cry1Fa2 could act as refuge products for Bt hybrids expressing Vip3A proteins. Niu et al. (2021) proposes using Vip3A for future monitoring of larval susceptibility at concentration rates of 5 -10 µg/ cm^2, whereas Dively et al. (2021) recommends the use of sentinel plot networks to monitor for shifts in susceptibility of corn earworm and other target pests of Bt traits. Hybrids expressing only Cry1A.105 and Cry2Ab2 could act as an alternative for corn earworm control when Vip3A is unavailable, but they would not be as effective. Their usage also depends on the distribution of Cry1A and Cry2 resistance in the region.

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Table 2.1. Characteristics of all Bt and non-Bt field corn hybrids used in the 2019 and 2020 plantings.

Brand & Hybrid	**Product Name**	**Bt Traits**	**Year**
DeKalb DKC 6694	Non-Bt	None	2019, 2020
DeKalb DKC 6697	Genuity VT Double PRO	Cry1A.105, Cry2Ab2	2019, 2020
DeKalb DKC 6629	Genuity Trecepta	Cry1A.105, Cry2Ab2, Vip3Aa20	2019, 2020
DeKalb DKC 6205	Non-Bt	None	2019, 2020
DeKalb DKC 6208	SmartStax	Cry1A.105, Cry2Ab2, Cry1Fa2	2019, 2020
DeKalb DKC 6824	Non-Bt	None	2020
DeKalb DKC 6826	Genuity VT Double PRO	Cry1A.105, Cry2Ab2	2020
DeKalb DKC 6799	Genuity Trecepta	Cry1A.105, Cry2Ab2, Vip3Aa20	2020
Pioneer 1637R	None	None	2019, 2020
Pioneer 1637YHR	Optimum Intrasect	Cry1Ab, Cry1Fa2	2019, 2020
Pioneer 1637VYHR	Optimum Leptra	Cry1Ab, Cry1Fa2, Vip3Aa20	2019
Pioneer 1870R	None	None	2020
Pioneer 1870YHR	Optimum Intrasect	Cry1Ab, Cry1Fa2	2020
Pioneer 2088R	None	None	2019, 2020
Pioneer 2089VYHR	Optimum Leptra	Cry1Ab, Cry1Fa2, Vip3Aa20	2019, 2020

Table 2.2. Analysis of variance comparing corn earworm infestation, larvae counts, ear damage, corn yield, and mycotoxin contamination results across corn hybrid treatments, location, and their interaction for potential influence on Bt field corn by year.

Measurement	Source	2019			2020		
		df	*F*	*P*	df	*F*	*P*
Infestation	Treatment	9, 54	96.98	<0.0001	11, 66	25.85	<0.0001
	Location	1, 6	19.11	0.0047	1, 3	91.95	0.0024
	Interaction	9, 54	2.12	0.0437	11, 66	10.44	<0.0001
Larvae/Ear	Treatment	9, 54	48.82	<0.0001	11, 66	17.39	<0.0001
	Location	1, 6	1.60	0.2536	1, 3	81.29	0.0029
	Interaction	9, 54	1.26	0.2797	11, 66	7.58	<0.0001
Damage/Ear	Treatment	9, 54	55.84	<0.0001	11, 69	34.44	<0.0001
	Location	1, 6	13.34	0.0107	1, 69	202.90	<0.0001
	Interaction	9, 54	3.20	0.0036	11, 69	8.73	<0.0001
Total Yield	Treatment	9, 60	2.33	0.0255	11, 66	4.57	<0.0001
	Location	1, 6	87.27	<0.0001	1, 6	204.76	<0.0001
	Interaction	9, 60	1.19	0.3180	11, 66	3.49	0.0007
Test Weight	Treatment	9, 60	7.10	<0.0001	11, 72	2.01	0.0392
	Location	1, 60	311.88	<0.0001	1, 72	7.18	0.0091
	Interaction	9, 60	1.69	0.1118	11, 72	0.57	0.8438
Aflatoxin	Treatment	9, 60	3.15	0.0036	11, 66	2.60	0.0083
	Location	1, 6	10.09	0.0024	1, 6	1.37	0.2868
	Interaction	9, 60	1.15	0.3419	11, 66	1.75	0.0820
Fumonisin	Treatment	9, 54	8.80	<0.0001	11, 69	1.14	0.3438
	Location	1, 6	31.15	0.0014	1, 69	71.23	<0.0001
	Interaction	9, 54	2.28	0.0303	11, 69	1.44	0.1749

Table 2.3. Effect of Bt traits on LS means ± SEM of percentage corn earworm infested ears and number of corn earworm larvae per ear by size and exit holes in R3 growth stage 2019 field corn with contrasting statements.

Brand & Hybrid	**Bt Traits**	**Infested R3 ears (%)**	**Larvae by Size and Exit Holes per Ear**				
			Small	**Medium**	**Large**	**Exit**	**Total**
DKC 6694	None (RR2)	55.0 ± 5.2 c	0.15 ± 0.06 cd	0.18 ± 0.09 b	0.15 ± 0.05 bc	0.22 ± 0.06 a	0.70 ± 0.10 bc
DKC 6697	Genuity VT Double PRO[+]	28.3 ± 4.7 e	0.16 ± 0.04 cd	0.10 ± 0.03 bc	0.04 ± 0.02 cd	0.02 ± 0.01 cd	0.32 ± 0.04 d
DKC 6629	Genuity Trecepta[++]	0 f	0 d	0 c	0 d	0 d	0 e
DKC 6205	None (RR2)	65.8 ± 6.1 b	0.15 ± 0.03 cd	0.19 ± 0.04 b	0.20 ± 0.04 b	0.22 ± 0.05 a	0.76 ± 0.06 b
DKC 6208	SmartStax[+]	41.7 ± 6.0 d	0.23 ± 0.07 c	0.11 ± 0.05 bc	0.10 ± 0.03 bcd	0.05 ± 0.02 bcd	0.49 ± 0.06 cd
P 1637R	None (RR2)	84.2 ± 5.2 a	0.57 ± 0.15 b	0.36 ± 0.05 a	0.45 ± 0.16 a	0.08 ± 0.05 b	1.46 ± 0.15 a
P 1637YHR	Optimum Intrasect[+]	80.8 ± 4.8 a	0.79 ± 0.15 a	0.33 ± 0.11 a	0.10 ± 0.05 bcd	0.07 ± 0.03 bc	1.30 ± 0.16 a
P 1637VYHR	Optimum Leptra[++]	0 f	0 d	0 c	0 d	0 d	0 e
P 2088R	None (RR2)	87.5 ± 5.4 a	0.57 ± 0.10 b	0.42 ± 0.08 a	0.38 ± 0.11 a	0.16 ± 0.06 a	1.52 ± 0.17 a
P 2089VYHR	Optimum Leptra[++]	1.7 ± 1.7 f	0.02 ± 0.02 d	0 c	0 d	0 d	0.02 ± 0.02 e
F > (*P*)							
Hybrid		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Bt vs non-Bt		<0.0001	0.0009	<0.0001	<0.0001	<0.0001	<0.0001
Cry Bt vs non-Bt		<0.0001	0.0714	0.0829	<0.0001	<0.0001	0.0003
Vip Bt vs non-Bt		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cry Bt vs Vip Bt		<0.0001	<0.0001	<0.0001	0.0242	0.0116	<0.0001

LS means ± SEM within columns followed by the same letter are not significantly different (PROC MIXED, pair-wise t-tests of LSM, α = 0.05).

Hybrid: (df = 9, 54); Contrasting Statements: (df = 1, 54).

[+] Bt-hybrid expressing only pyramided *Cry* proteins.

[++] Bt-hybrid expressing pyramided *Cry* proteins with Vip3Aa20.

Table 2.4. Effect of Bt traits on LS means ± SEM of percentage corn earworm infested ears and number of corn earworm larvae per ear by size and exit holes in R3 growth stage 2020 field corn with contrasting statements.

Brand & Hybrid	**Bt Traits**	**Infested R3 ears (%)**	**Larvae by Size and Exit Holes per Ear**				
			Small	**Medium**	**Large**	**Exit**	**Total**
DKC 6694	None (RR2)	42.5 ± 14.3 cd	0.06 ± 0.03 ef	0.13 ± 0.07 bc	0.15 ± 0.05 ab	0.14 ± 0.06 b	0.48 ± 0.17 bcd
DKC 6697	Genuity VT Double PRO[+]	39.2 ± 13.6 d	0.18 ± 0.08 bcde	0.18 ± 0.07 abc	0.07 ± 0.04 bc	0 c	0.44 ± 0.16 cd
DKC 6629	Genuity Trecepta[++]	2.5 ± 2.5 e	0 f	0.01 ± 0.01 de	0.02 ± 0.02 c	0 c	0.02 ± 0.02 e
DKC 6205	None (RR2)	56.7 ± 10.8 ab	0.08 ± 0.03 def	0.11 ± 0.02 cde	0.16 ± 0.06 ab	0.22 ± 0.06 a	0.57 ± 0.11 abc
DKC 6208	SmartStax[+]	36.8 ± 13.4 d	0.20 ± 0.08 bcd	0.12 ± 0.07 cd	0.05 ± 0.03 c	0.02 ± 0.02 c	0.39 ± 0.15 d
DKC 6824	None (RR2)	55.0 ± 10.6 abc	0.12 ± 0.03 cdef	0.08 ± 0.02 cde	0.22 ± 0.08 a	0.17 ± 0.06 ab	0.60 ± 0.12 abc
DKC 6826	Genuity VT Double PRO[+]	37.5 ± 10.9 d	0.23 ± 0.09 bc	0.24 ± 0.09 ab	0.06 ± 0.03 c	0 c	0.53 ± 0.18 bcd
DKC 6799	Genuity Trecepta[++]	3.3 ± 3.3 e	0 f	0 e	0.01 ± 0.01 c	0.02 ± 0.02 c	0.03 ± 0.03 e
P 2088R	None (RR2)	49.2 ± 11.2 bcd	0.42 ± 0.11 a	0.16 ± 0.06 abc	0.06 ± 0.03 c	0.02 ± 0.01 c	0.65 ± 0.18 ab
P 2089VYHR	Optimum Leptra[++]	0 e	0 f	0 e	0 c	0 c	0 e
P 1637/1870R	None (RR2)	63.3 ± 11.1 a	0.22 ± 0.10 bc	0.19 ± 0.05 abc	0.20 ± 0.09 a	0.12 ± 0.03 b	0.73 ± 0.14 a
P 1637/1870YHR	Optimum Intrasect[+]	54.2 ± 10.9 abc	0.30 ± 0.08 ab	0.25 ± 0.12 a	0.04 ± 0.02 c	0.04 ± 0.01 c	0.63 ± 0.14 ab
F > (*P*)							
Hybrid		<0.0001	0.0002	0.0295	0.0058	<0.0001	<0.0001
Bt vs non-Bt		<0.0001	0.0630	0.3788	<0.0001	<0.0001	<0.0001
Cry Bt vs non-Bt		0.0003	0.1269	0.0218	<0.0001	<0.0001	0.0123
Vip Bt vs non-Bt		<0.0001	<0.0001	0.0003	<0.0001	<0.0001	<0.0001
Cry Bt vs Vip Bt		<0.0001	<0.0001	<0.0001	0.0492	0.6653	<0.0001

LS means ± SEM within columns followed by the same letter are not significantly different (PROC MIXED, pair-wise t-tests of LSM, α = 0.05).

Hybrid: (df = 11, 66); Contrasting Statements: (df = 1, 66).

[+] Bt-hybrid expressing only pyramided *Cry* proteins.

[++] Bt-hybrid expressing pyramided *Cry* proteins with Vip3Aa20.

Table 2.5. Effect of Bt traits on LS means ± SEM of percentage corn earworm infested ears and damaged area by ear region per ear in R6 growth stage 2019 field corn with contrasting statements.

Brand & Hybrid	**Bt Traits**	**Infested R6 ears (%)**	**Damage (cm^2) by Ear Region**		
			Ear Tip	**Kernels**	**Total**
DKC 6694	None (RR2)	57.5 ± 5.3 c	0.92 ± 0.14 e	1.15 ± 0.28 cd	2.07 ± 0.38 d
DKC 6697	Genuity VT Double PRO+	35.8 ± 6.7 d	0.47 ± 0.10 f	0.48 ± 0.15 ef	0.96 ± 0.22 e
DKC 6629	Genuity Trecepta++	0 e	0 g	0 f	0 f
DKC 6205	None (RR2)	61.7 ± 5.6 c	1.39 ± 0.16 cd	1.32 ± 0.26 cd	2.72 ± 0.35 cd
DKC 6208	SmartStax+	50.8 ± 4.2 c	1.15 ± 0.16 de	0.93 ± 0.19 de	2.08 ± 0.18 d
P 1637R	None (RR2)	90.8 ± 3.3 a	2.37 ± 0.19 b	2.75 ± 0.57 b	5.11 ± 0.66 b
P 1637YHR	Optimum Intrasect+	77.5 ± 7.0 b	1.59 ± 0.30 c	1.60 ± 0.33 c	3.19 ± 0.57 c
P 1637VYHR	Optimum Leptra++	0 e	0 g	0 f	0 f
P 2088R	None (RR2)	96.7 ± 2.2 a	3.25 ± 0.16 a	3.47 ± 0.51 a	6.72 ± 0.57 a
P 2089VYHR	Optimum Leptra++	0 e	0 g	0 f	0 f
F > (*P*)					
Hybrid		<0.0001	<0.0001	<0.0001	<0.0001
Bt vs non-Bt		<0.0001	<0.0001	<0.0001	<0.0001
Cry Bt vs non-Bt		<0.0001	<0.0001	<0.0001	<0.0001
Vip Bt vs non-Bt		<0.0001	<0.0001	<0.0001	<0.0001
Cry Bt vs Vip Bt		<0.0001	<0.0001	<0.0001	<0.0001

LS means ± SEM within columns followed by the same letter are not significantly different (PROC MIXED, pair-wise t-tests of LSM, $\alpha = 0.05$).
Hybrid: (df = 9, 54); Contrasting Statements: (df = 1, 54).
+ Bt-hybrid expressing only pyramided *Cry* proteins.
++ Bt-hybrid expressing pyramided *Cry* proteins with Vip3Aa20.

Table 2.6. Effect of Bt traits on LS means ± SEM of percentage corn earworm infested ears and damaged area by ear region per ear in R6 growth stage 2020 field corn with contrasting statements.

Brand & Hybrid	**Bt Traits**	**Infested R6 ears (%)**	**Damage (cm^2) by Ear Region**		
			Ear tip	**Kernels**	**Total**
DKC 6694	None (RR2)	38.3 ± 14.3 c	0.67 ± 0.26 d	0.74 ± 0.28 de	1.42 ± 0.54 de
DKC 6697	Genuity VT Double PRO$^+$	28.3 ± 10.5 c	0.37 ± 0.14 e	0.42 ± 0.17 ef	0.78 ± 0.31 ef
DKC 6629	Genuity Trecepta^{++}	0.8 ± 0.8 d	0.02 ± 0.02 f	0.01 ± 0.01 f	0.02 ± 0.02 f
DKC 6205	None (RR2)	55.8 ± 15.8 b	1.01 ± 0.32 c	1.48 ± 0.49 c	2.49 ± 0.81 c
DKC 6208	SmartStax$^+$	35.0 ± 9.9 c	0.64 ± 0.20 d	0.83 ± 0.28 de	1.47 ± 0.48 de
DKC 6824	None (RR2)	60.8 ± 10.6 ab	1.32 ± 0.28 ab	2.32 ± 0.44 b	3.64 ± 0.72 b
DKC 6826	Genuity VT Double PRO$^+$	35.8 ± 12.3 c	0.65 ± 0.25 d	1.02 ± 0.45 cd	1.67 ± 0.69 d
DKC 6799	Genuity Trecepta^{++}	0.8 ± 0.8 d	0.01 ± 0.01 f	0.02 ± 0.02 f	0.02 ± 0.02 f
P 2088R	None (RR2)	65.0 ± 8.1 ab	1.25 ± 0.21 abc	3.20 ± 0.62 a	4.45 ± 0.81 a
P 2089VYHR	Optimum Leptra^{++}	4.2 ± 2.5 d	0.11 ± 0.07 f	0.05 ± 0.05 f	0.16 ± 0.12 f
P 1637/1870R	None (RR2)	71.7 ± 9.2 a	1.48 ± 0.27 a	2.42 ± 0.45 b	3.90 ± 0.70 ab
P 1637/1870YHR	Optimum Intrasect$^+$	54.2 ± 10.4 b	1.12 ± 0.22 bc	2.19 ± 0.50 b	3.31 ± 0.70 b
F > (*P*)					
Hybrid		<0.0001	<0.0001	<0.0001	<0.0001
Bt vs non-Bt		<0.0001	<0.0001	<0.0001	<0.0001
Cry Bt vs non-Bt		<0.0001	<0.0001	<0.0001	<0.0001
Vip Bt vs non-Bt		<0.0001	<0.0001	<0.0001	<0.0001
Cry Bt vs Vip Bt		<0.0001	<0.0001	<0.0001	<0.0001

LS means ± SEM within columns followed by the same letter are not significantly different (PROC MIXED, pair-wise t-tests of LSM, α = 0.05).
Hybrid: (df = 11, 66); Contrasting Statements: (df = 1, 66).

$^+$ Bt-hybrid expressing only pyramided *Cry* proteins.

$^{++}$ Bt-hybrid expressing pyramided *Cry* proteins with Vip3Aa20.

Table 2.7. Effect of Bt traits on LS means ± SEM of corn yield based on grain and test weights from harvested 2019 field corn with contrasting statements.

Brand & Hybrid	**Bt Traits**	**Grain yield (kg/ha)**	**Test Weight (kg/hL)**
DKC 6694	None (RR2)	14732 ± 273 abcd	69.91 ± 1.67 a
DKC 6697	Genuity VT Double PRO^{+}	13954 ± 594 d	70.71 ± 1.62 a
DKC 6629	Genuity Trecepta^{++}	15313 ± 651 abc	69.69 ± 1.74 a
DKC 6205	None (RR2)	15658 ± 805 ab	70.11 ± 1.59 a
DKC 6208	SmartStax^{+}	15456 ± 779 ab	70.17 ± 1.57 a
P 1637R	None (RR2)	15140 ± 404 abc	68.63 ± 2.70 ab
P 1637YHR	Optimum Intrasect^{+}	15302 ± 477 abc	68.84 ± 2.06 ab
P 1637VYHR	Optimum Leptra^{++}	14201 ± 593 cd	70.28 ± 1.77 a
P 2088R	None (RR2)	14672 ± 682 bcd	63.31 ± 2.30 c
P 2089VYHR	Optimum Leptra^{++}	15830 ± 632 a	66.68 ± 2.55 b
F > (*P*)			
Hybrid		0.0255	<0.0001
Bt vs non-Bt		0.8764	0.0130
Cry Bt vs non-Bt		0.4139	0.6106
Vip Bt vs non-Bt		0.4232	0.0246
Cry Bt vs Vip Bt		0.5264	0.1464

LS means ± SEM within columns followed by the same letter are not significantly different (PROC MIXED, pair-wise t-tests of LSM, α = 0.05).
Hybrid: (df = 9, 54); Contrasting Statements: (df = 1, 54).

$^{+}$ Bt-hybrid expressing only pyramided *Cry* proteins.

$^{++}$ Bt-hybrid expressing pyramided *Cry* proteins with Vip3Aa20.

Table 2.8. Effect of Bt traits on LS means ± SEM of corn yield based on grain and test weights from harvested 2020 field corn with contrasting statements.

Brand & Hybrid	**Bt Traits**	**Grain yield (kg/ha)**	**Test Weight (kg/hL)**
DKC 6694	None (RR2)	12029 ± 412 ef	75.04 ± 4.00 a
DKC 6697	Genuity VT Double PRO[+]	13770 ± 686 a	70.29 ± 0.40 abc
DKC 6629	Genuity Trecepta[++]	13478 ± 678 abc	70.00 ± 0.79 abc
DKC 6205	None (RR2)	12498 ± 851 def	70.87 ± 1.78 abc
DKC 6208	SmartStax[+]	13272 ± 932 abcd	72.45 ± 4.12 ab
DKC 6824	None (RR2)	13049 ± 751 abcd	68.81 ± 1.05 bcd
DKC 6826	Genuity VT Double PRO[+]	12853 ± 652 bcde	68.91 ± 0.44 bcd
DKC 6799	Genuity Trecepta[++]	13706 ± 797 ab	69.54 ± 0.89 bcd
P 2088R	None (RR2)	13739 ± 556 a	64.59 ± 0.82 d
P 2089VYHR	Optimum Leptra[++]	13755 ± 727 a	66.24 ± 0.64 cd
P 1637/1870R	None (RR2)	11897 ± 1220 f	68.78 ± 1.47 bcd
P 1637/1870YHR	Optimum Intrasect[+]	12822 ± 376 cde	70.19 ± 1.00 abc
F > (*P*)			
Hybrid		<0.0001	0.0392
Bt vs non-Bt		<0.0001	0.9715
Cry Bt vs non-Bt		0.0128	0.5078
Vip Bt vs non-Bt		0.0011	0.3918
Cry Bt vs Vip Bt		0.0529	0.1978

LS means ± SEM within columns followed by the same letter are not significantly different (PROC MIXED, pair-wise t-tests of LSM, α = 0.05).

Hybrid: (df = 11, 66); Contrasting Statements: (df = 1, 66).

[+] Bt-hybrid expressing only pyramided *Cry* proteins.

[++] Bt-hybrid expressing pyramided *Cry* proteins with Vip3Aa20.

Table 2.9. Effect of Bt traits on LS means ± SEM of grain aflatoxin and fumonisin contamination levels from harvested 2019 field corn with contrasting statements.

Brand & Hybrid	**Bt Traits**	**Aflatoxin (ppb)**	**Fumonisin (ppm)**
DKC 6694	None (RR2)	57.00 ± 50.89 bc	32.00 ± 10.44 cde
DKC 6697	Genuity VT Double PRO^{+}	39.81 ± 25.08 bc	31.75 ± 10.47 def
DKC 6629	Genuity Trecepta^{++}	3.31 ± 1.26 c	14.88 ± 6.07 f
DKC 6205	None (RR2)	15.19 ± 10.96 bc	17.44 ± 6.44 ef
DKC 6208	SmartStax^{+}	29.19 ± 16.58 bc	17.25 ± 5.66 ef
P 1637R	None (RR2)	13.19 ± 8.89 bc	39.50 ± 9.75 bcd
P 1637YHR	Optimum Intrasect^{+}	184.63 ± 56.43 a	57.13 ± 8.21 ab
P 1637VYHR	Optimum Leptra^{++}	16.56 ± 12.53 bc	25.44 ± 9.52 ef
P 2088R	None (RR2)	120.38 ± 75.79 b	75.13 ± 11.26 a
P 2089VYHR	Optimum Leptra^{++}	116.98 ± 73.09 b	43.62 ± 9.15 abc
F > (*P*)			
Hybrid		0.0036	<0.0001
Bt vs non-Bt		0.4524	0.0002
Cry Bt vs non-Bt		0.0107	0.2962
Vip Bt vs non-Bt		0.2557	0.0001
Cry Bt vs Vip Bt		0.0075	0.1707

LS means ± SEM within columns followed by the same letter are not significantly different (PROC MIXED, pair-wise t-tests of LSM, α = 0.05).
Hybrid: (df = 9, 54); Contrasting Statements: (df = 1, 54).

$^{+}$ Bt-hybrid expressing only pyramided *Cry* proteins.

$^{++}$ Bt-hybrid expressing pyramided *Cry* proteins with Vip3Aa20.

Table 2.10. Effect of Bt traits on LS means ± SEM of grain aflatoxin and fumonisin contamination levels from harvested 2020 field corn with contrasting statements.

Brand & Hybrid	**Bt Traits**	**Aflatoxin (ppb)**	**Fumonisin (ppm)**
DKC 6694	None (RR2)	15.10 ± 14.99 bcd	3.39 ± 0.87 a
DKC 6697	Genuity VT Double PRO^{+}	8.73 ± 7.77 bcd	4.15 ± 0.91 a
DKC 6629	Genuity Trecepta^{++}	0.01 ± 0.01 d	4.36 ± 0.76 a
DKC 6205	None (RR2)	1.91 ± 1.59 bcd	6.40 ± 2.65 a
DKC 6208	SmartStax^{+}	25.46 ± 18.91 bcd	6.99 ± 3.40 a
DKC 6824	None (RR2)	1.37 ± 0.94 bcd	3.14 ± 1.15 a
DKC 6826	Genuity VT Double PRO^{+}	12.60 ± 12.49 bcd	5.19 ± 2.18 a
DKC 6799	Genuity Trecepta^{++}	0.91 ± 0.62 bcd	3.35 ± 0.93 a
P 2088R	None (RR2)	85.01 ± 59.23 a	6.44 ± 1.44 a
P 2089VYHR	Optimum Leptra^{++}	58.26 ± 37.91 ab	5.63 ± 1.64 a
P 1637/1870R	None (RR2)	0.29 ± 0.16 cd	4.27 ± 1.11 a
P 1637/1870YHR	Optimum Intrasect^{+}	53.14 ± 34.70 abc	3.50 ± 0.84 a
F > (*P*)			
Hybrid		0.0083	0.3438
Bt vs non-Bt		0.8471	0.8971
Cry Bt vs non-Bt		0.7326	0.9992
Vip Bt vs non-Bt		0.2200	0.8320
Cry Bt vs Vip Bt		0.2979	0.8154

LS means ± SEM within columns followed by the same letter are not significantly different (PROC MIXED, pair-wise t-tests of LSM, α = 0.05).
Hybrid: (df = 11, 66); Contrasting Statements: (df = 1, 66).
$^{+}$ Bt-hybrid expressing only pyramided *Cry* proteins.
$^{++}$ Bt-hybrid expressing pyramided *Cry* proteins with Vip3Aa20.

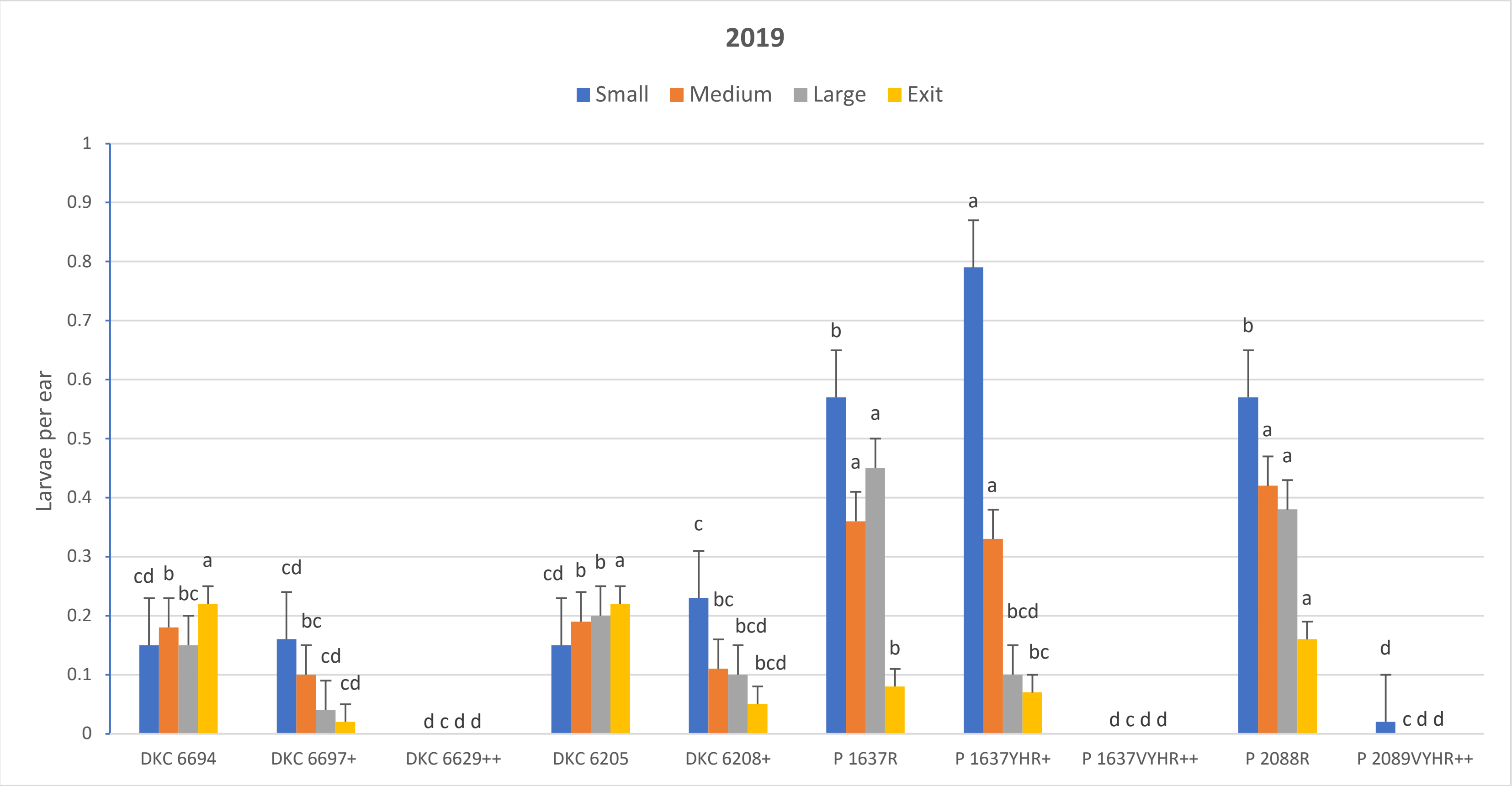

Figure 2.1. Effect of Bt traits on LS means ± SEM of number of corn earworm larvae per ear by size and exit holes in R3 growth stage 2019 field corn.

LS Means within larval size category with the same letter are not significantly different (pair-wise t-tests of LSM, α = 0.05). Bt hybrids marked with (+) express pyramided *Cry* proteins while hybrids marked with (++) express pyramided *Cry* proteins with Vip3Aa20. Unmarked hybrids are non-Bt hybrids. See Table 2.1 for specific proteins expressed for each entry and product type.

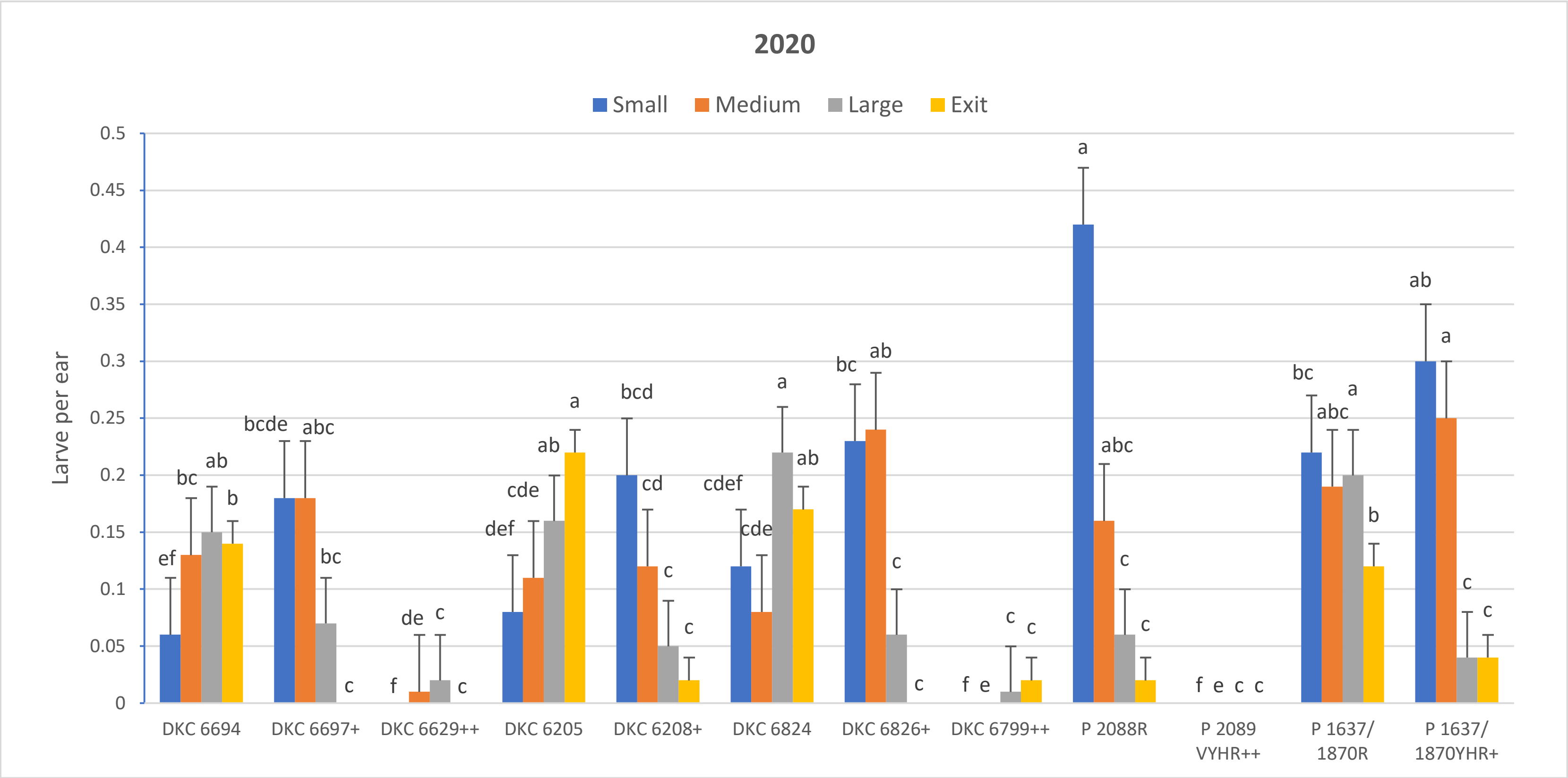

Figure 2.2. Effect of Bt traits on LS means ± SEM of number of corn earworm larvae per ear by size and exit holes in R3 growth stage 2020 field corn.

LS Means within larval size category with the same letter are not significantly different (pair-wise t-tests of LSM, α = 0.05). Bt hybrids marked with (+) express pyramided *Cry* proteins while hybrids marked with (++) express pyramided *Cry* proteins with Vip3Aa20. Unmarked hybrids are non-Bt hybrids. See Table 2.1 for specific proteins expressed for each entry and product type.

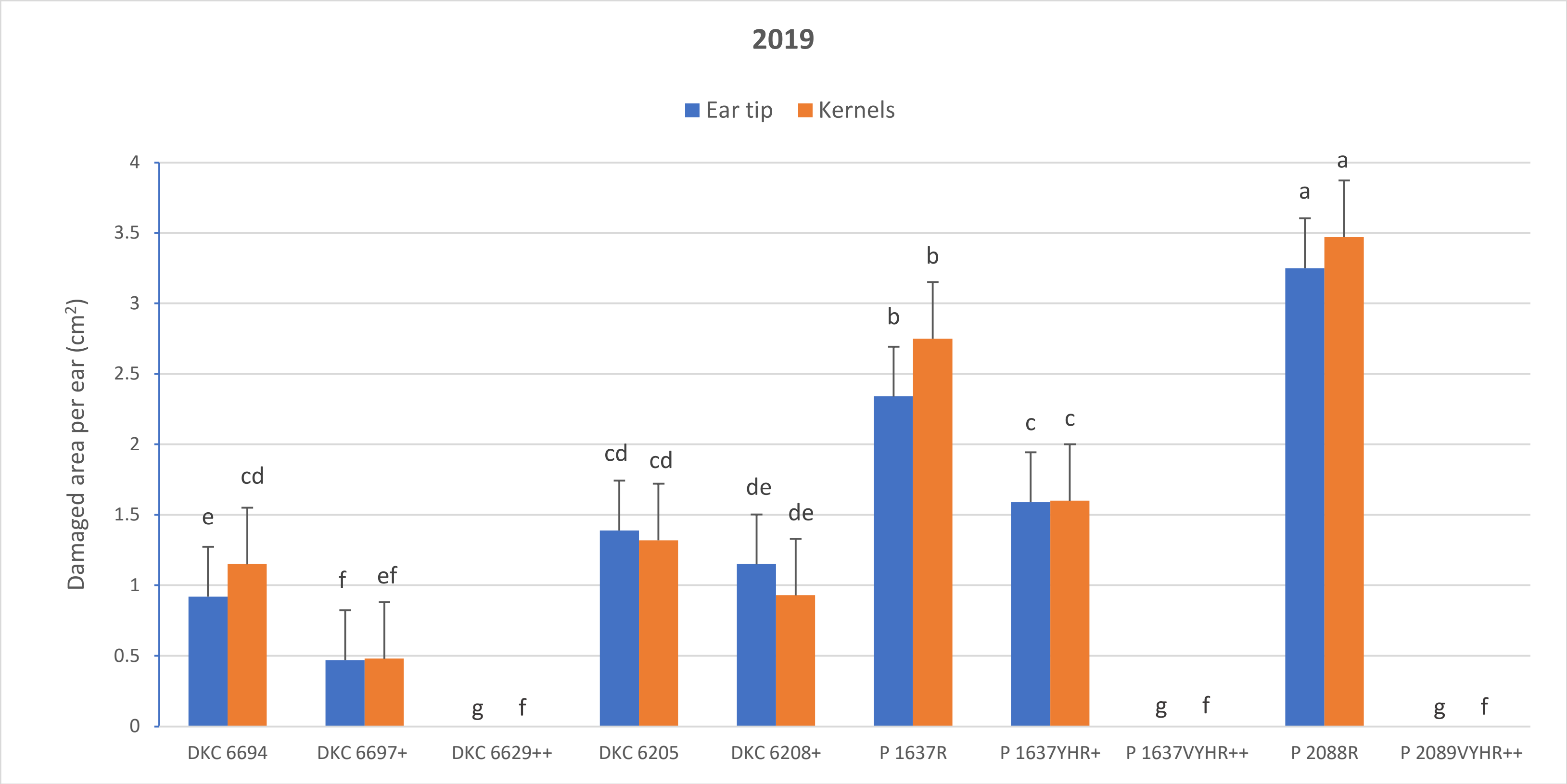

Figure 2.3. Effect of Bt traits on LS means ± SEM of damaged area by ear region per ear in R6 growth stage 2019 field corn.

LS Means within category with the same letter are not significantly different (pair-wise t-tests of LSM, $\alpha = 0.05$). Bt hybrids marked with (+) express pyramided *Cry* proteins while hybrids marked with (++) express pyramided *Cry* proteins with Vip3Aa20. Unmarked hybrids are non-Bt hybrids. See Table 2.1 for specific proteins expressed for each entry and product type.

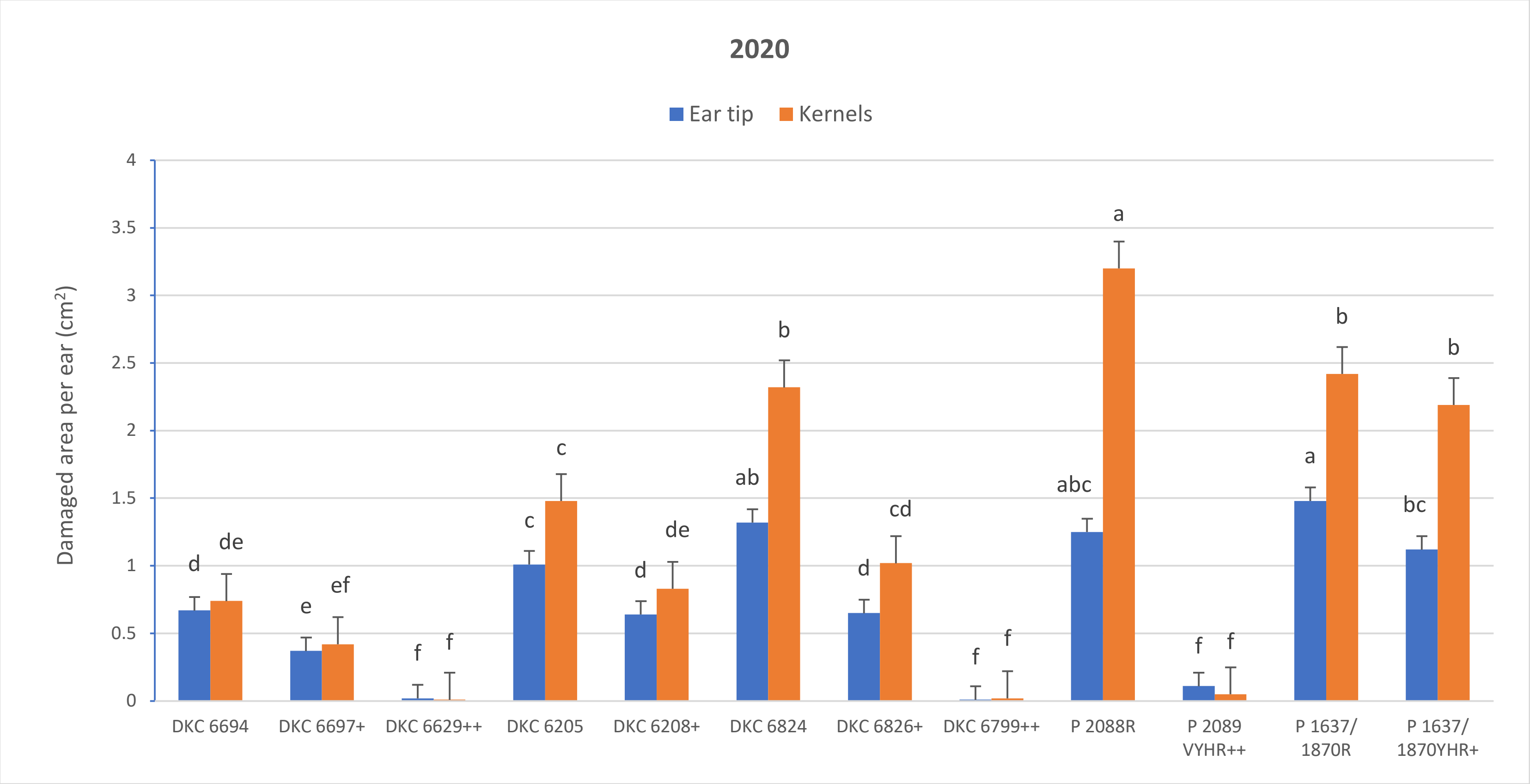

Figure 2.4. Effect of Bt traits on LS means ± SEM of damaged area by ear region per ear in R6 growth stage 2020 field corn.

LS Means within category with the same letter are not significantly different (pair-wise t-tests of LSM, α = 0.05). Bt hybrids marked with (+) express pyramided *Cry* proteins while hybrids marked with (++) express pyramided *Cry* proteins with Vip3Aa20. Unmarked hybrids are non-Bt hybrids. See Table 2.1 for specific proteins expressed for each entry and product type.

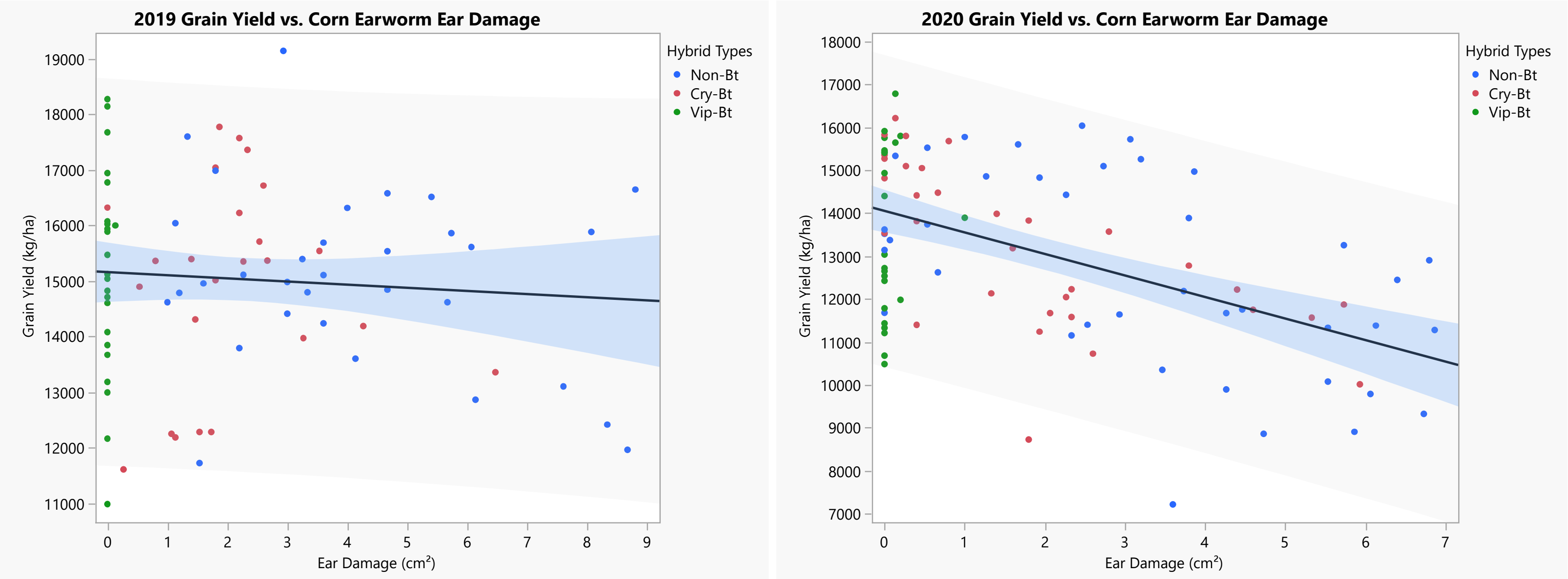

Figure 2.5. Linear regression analysis depicting relationship between corn earworm ear damage and grain yield of field corn by year.

2019: ($R^2 = 0.0061$; $F = 0.4791$; $P = 0.4909$)

2020: ($R^2 = 0.2638$; $F = 33.690$; $P < 0.0001$)

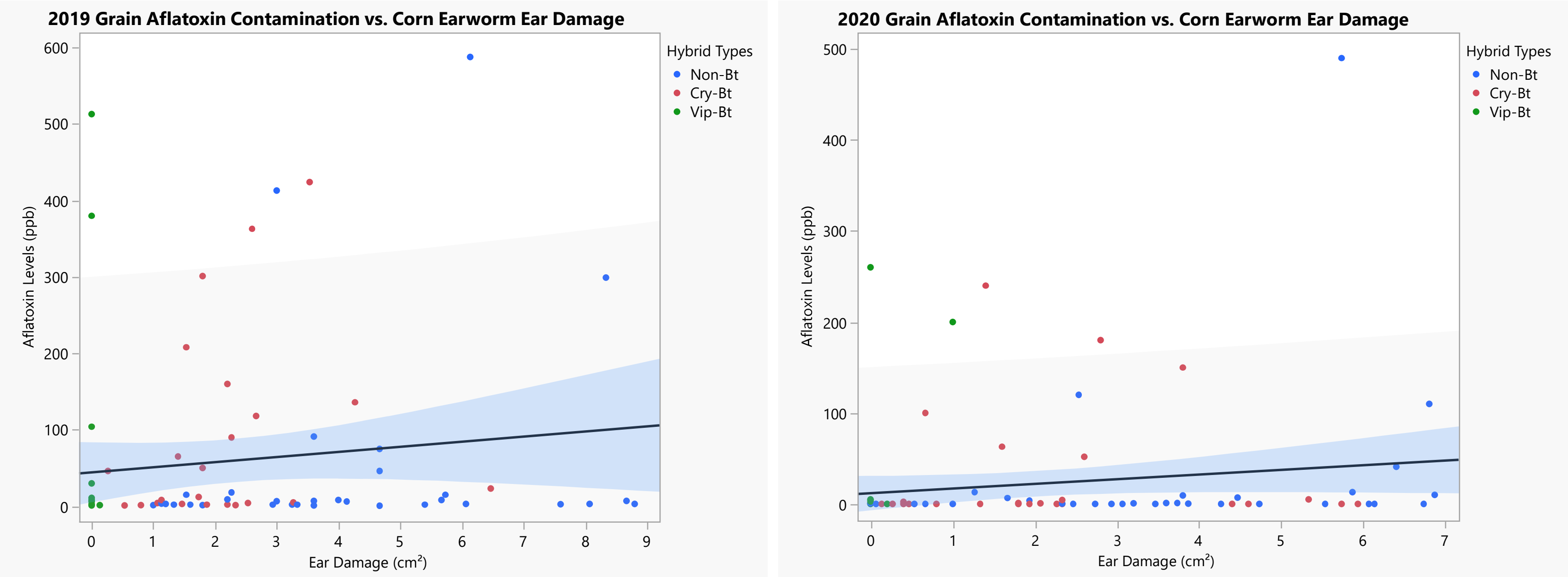

Figure 2.6. Linear regression analysis depicting relationship between corn earworm ear damage and grain aflatoxin contamination levels of field corn by year.

2019: ($R^2 = 0.0158$; $F = 1.2517$; $P = 0.2667$)

2020: ($R^2 = 0.0249$; $F = 2.4050$; $P = 0.1243$)

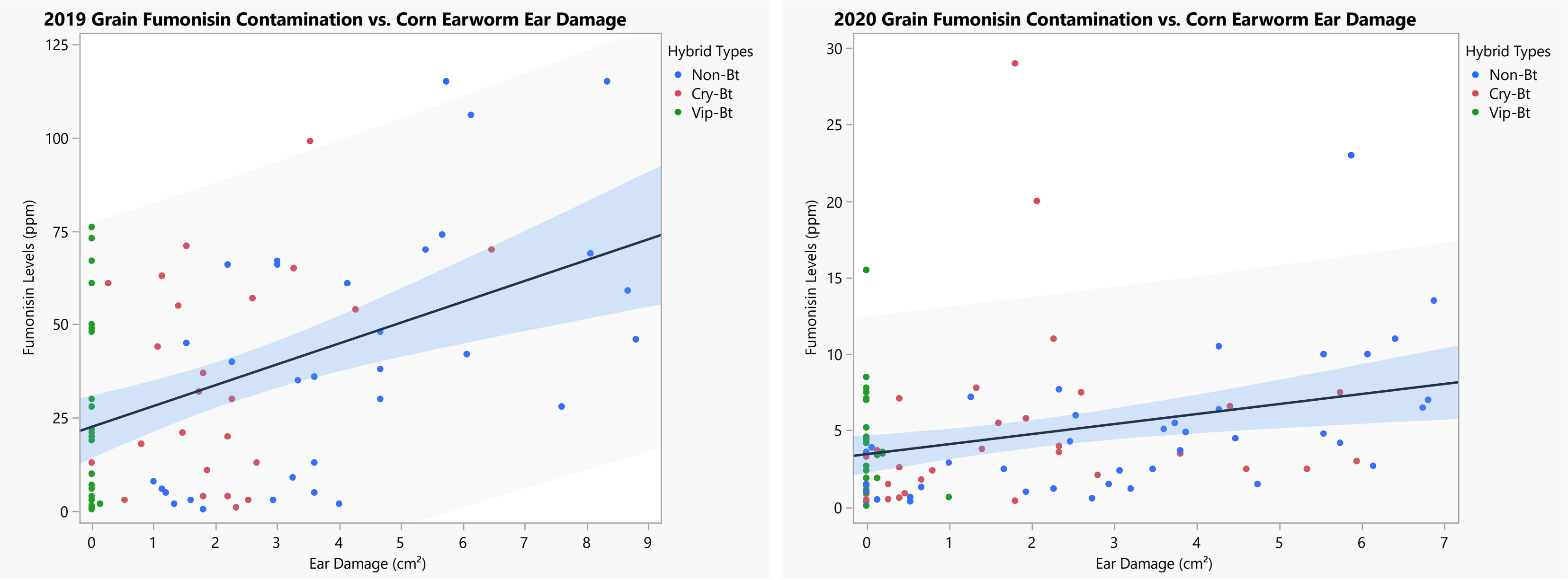

Figure 2.7. Linear regression analysis depicting relationship between corn earworm ear damage and grain fumonisin contamination levels of field corn by year.

2019: ($R^2 = 0.1970$; $F = 19.141$; $P < 0.0001$)

2020: ($R^2 = 0.0909$; $F = 9.3961$; $P = 0.0028$)

CHAPTER 3

IMPACT OF FALL ARMYWORM (*SPODOPTERA FRUGIPERDA*) WHORL DEFOLIATION ON GRAIN YIELD OF LATE-PLANTED BT AND NON-BT FIELD CORN

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Abstract

Fall armyworm, *Spodoptera frugiperda* (J. E. Smith), cause substantial whorl damage to field corn in the United States that can result in reduced grain yield production. Corn hybrids expressing Bt proteins have provided significant control, yet its technology remains threatened by increased cases of Bt resistance among local pest populations. A late planting of Bt field corn hybrids in Georgia had low infestation rates where most fall armyworm activity occurred in untreated plots. All Bt hybrids effectively prevented fall armyworm infestations. Hybrids expressing the Vip3Aa20 protein additionally prevented corn earworm infestations, but hybrids expressing only *Cry* proteins significantly produced the most grain yield. In paired micro-plots, whorl damage to non-Bt corn caused significant reductions in viable grain and ear development compared to their undamaged counterparts regardless of the extent of infestation. Fall armyworm infestations can potentially reduce yield production in corn, and implementing Bt technology can prevent this loss.

Keywords: *Bacillus thuringiensis*, *Helicoverpa zea*, Corn earworm, *Zea mays*, IPM, plant-insect interactions

Introduction

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), causes foliar damage through larval feeding on a variety of crops grown across the southeastern United States region. Larvae especially feed on the whorl and furl leaves of corn, *Zea mays* (L.), which can stunt plant growth or ear development if not kill the crop entirely. Outbreaks occur in both North and South America but have been primarily recorded within the United States in very irregular intervals and sometimes causing significant monetary loss (Sparks 1979). In 2016, the fall armyworm would become recognized as an invasive species after it was confirmed infesting corn fields across Africa (Goergen et al. 2016) before spreading into Asia (Ganiger et al. 2018, IPPC 2019, USDA-FAS 2019) and Australia (Spafford 2021).

Corn is more likely to be exposed to significant fall armyworm infestations when planted after the recommended planting dates of their respective regions (Ayala et al. 2013, Buntin 2008, Buntin et al. 2001). The inability of fall armyworm to diapause in cold weather makes them only active in warm climates, thus they die out where it freezes and moths must migrate from subtropical areas to temperate regions during the summer months (Luginbill 1928, Sparks 1979). Planting corn during the spring season when the soil temperature remains between 10° and 30° Celsius (Sassenrath et al. 2019) is highly recommended and possibly the best natural pest control strategy. However, intentions of an early or optimal planting date can easily be delayed due to unsuitable crop conditions including severe weather and poor soil quality. Scenarios where corn is planted in a double cropping practice may also be unfavorable if not timed properly (Buntin et al. 2008, Buntin et al. 2004a-b).

Modifying corn grain seeds with proteins from the *Bacillus thuringiensis* (Bt) bacterium has been a successful pest control tactic against lepidopteran pest species including the fall armyworm. The first transgenic Bt hybrids commercially introduced to the southeastern United States region in 1998 included YieldGard® products which immediately provided partial control against fall armyworm and reduced grain yield loss, even in severe outbreaks (Buntin et al. 2001, 2004a-b, DeLamar et al. 1999a-c). These initial products contained the Bt events Bt11 and MON810 that express Cry1Ab, a specific crystal-like (*Cry*) protein with a mode of action that destroys the cell lining within the target pest's midgut where it activates after ingested (Ostlie et al. 1997). Bt hybrids were initially used against the European corn borer, *Ostrinia nubilalis* (Hübner) (Koziel et al. 1993), and now are available for insecticidal use against a wider variety of lepidopteran pest species in corn (Buntin and Flanders 2019).

Additional Bt strains have also been discovered and commercially available since these first introductions. The Cry1Fa2 protein expressed from the TC1507 event has frequently been used as a fall armyworm control tactic for its better performance compared to Cry1Ab (Buntin 2008, Hardke et al. 2011). The linked proteins Cry1A.105 and Cry2Ab2 expressed from the MON89034 event has provided a greater level of efficacy towards a wider range of lepidopteran pests as a second-generation Bt product (Drury et al. 2008, Rule et al. 2014). Vip3Aa20, a vegetative insecticide protein (*Vip*) expressed from the MIR162 event, was introduced shortly after and currently is considered one of the most effective Bt toxins against fall armyworm as well as corn earworm, *Helicoverpa zea* (Boddie), infestations (Buntin and Flanders 2019, Burkness et al. 2010, Burtet et al. 2017, Moscardini et al. 2020). Multiple specific Bt toxins would often be expressed in single crop hybrids, a concept known as gene pyramiding. This tactic provides simultaneous crop protection against multiple lepidopteran pest species and enhanced insecticidal potency, yet these products generally were not any more effective than single-gene Bt hybrids towards fall armyworm control alone (Buntin et al. 2004b, Moscardini et al. 2020, Rule et al. 2014, Siebert et al. 2012).

The use of Bt traits as an insecticidal source in corn has grown significantly in the past couple decades due its continuing success, increasing from 19% of all corn acreage in 2000 to 82% in 2020 (USDA-ERS 2020). However, repeated use of Bt technology eventually resulted in resistance development to occur across local fall armyworm populations allowing them to survive exposure. First documented cases of resistance development occurred in Puerto Rico in 2006 against specific Cry1F proteins (Storer et al. 2010) and then appeared in the southeastern United States region several years later (Huang et al. 2014). Increased resistance development in *Cry* proteins began to emerge and spread across North and South America since this discovery with cross-resistance in pyramided Bt hybrids from single-gene products as a contributing factor (Farias et al. 2014, Huang et al. 2014, Niu et al. 2013, 2016a, Omoto et al. 2015, Vélez et al. 2013). Few studies reported the presence of Bt toxin resistant alleles for at least Cry2Ab2 and Vip3Aa51 at low frequencies in local fall armyworm samples through F_2 screenings (Niu et al. 2016b, Yang et al. 2019). A list of transgenic Bt hybrids still remain effective against fall armyworm infestations in the southeastern United States region (Buntin and Flanders 2019), but insecticide resistance management (IRM) requires additional protocol towards maintaining the efficacy of Bt technology. Planting non-Bt refuge corn is a commonly implemented IRM tactic to slow resistance development among moths (Buntin and Flanders 2019, Carrière et al. 2016), and recent research publications bring attention to additional methods that could greatly assist including careful selections of Bt pyramids and using alternate Bt strains to eradicate resistant populations (Dively et al. 2016, Niu et al. 2013, 2016a, Reisig and Kurtz 2018). Other research topics of interest such as further analysis of sublethal Bt exposure on surviving pest populations

(Guedes et al. 2017) are encouraged alongside the regular field monitoring of lepidopteran pest activity in Bt corn hybrids.

While fall armyworm infestations remain a constant issue on an annual basis, there has been a lack of reports focusing on the pest species' impact on corn yield and grain quality. The few relevant reports found even minor whorl infestations can significantly reduce overall corn production, especially infestations during the mid to late vegetative growth stages prior to tasseling (Cruz and Turpin 1983, Marenco et al. 1992). Yield reduction will also occur when corn plants are under stressful conditions such as drought or excessive heat while having significant whorl damage (Pruter et al. 2020). Studies have been implemented in countries where fall armyworm infestations are relatively new including India (Balla et al. 2019) and Zimbabwe (Baudron et al. 2019), but this focus has received little attention in the United States, specifically in the southeastern region where the pest is very active. Cruz and Turpin (1983) reported extensive yield loss in manually infested whorl-stage corn with fall armyworm eggs that created large infestations. Bilbo et al. (2020) had significant grain yield loss in late-planted non-Bt field corn in South Carolina that was only prevented by select Bt hybrids. Reporting such data is essential for establishing up-to-date economic thresholds for fall armyworm in field corn and monitoring their feeding behavior on different corn varieties. A number of states in the southeastern United States region developed different economic thresholds that range from 15% to 75% infested plants during the whorl growth stages (Bessin 2019, Boyd and Bailey 2004, Buntin 2021, Crow et al. 2021, NC State Extension 2015, Nuessly and Webb 2002, Reay-Jones 2019, Stewart and McClure 2015).

We evaluated the overall yield and grain quality response of untreated field corn to fall armyworm whorl defoliation during the vegetative growth stages. Examining for any potential yield loss between damaged and undamaged plants involved comparing ear samples based on the number of consecutive plants the ears were harvested from in any given row with notable foliar damage from larval feeding. Additionally, a selection of transgenic Bt corn hybrids were evaluated for their overall performance towards fall armyworm control based on the pyramided Bt toxins they express. Plantings were made late in the season in order to promote natural infestations of the lepidopteran pest species. We hypothesize all Bt corn hybrids tested will significantly reduce the presence of fall armyworm and retain grain yield production. The occurrence of whorl defoliation in untreated plots will cause a significant reduction in yield that will increase in magnitude with an increased number of infested plants.

Materials and Methods

Field Experiments: Field experiments were conducted at the Southwest Georgia Research and Education Center near Plains, Georgia (N 33.175964 W -84.409210) in 2019 and 2020. Soil was a Greenville sandy loam. Weed control and fertility practices followed the Georgia Extension Service recommendations for this location. Tillage was conventional with chisel plowing followed by disk harrowing. Before disking, 440 kg/ha of a 5-10-5 (N-P-K) granular fertilizer was applied and an additional 112 kg of nitrogen as ammonium nitrate was applied beside the rows and incorporated about 20 days after planting. Atrazine (Aatrex 4L, Syngenta Crop protection, Greensboro, North Carolina) with acetochlor (Warrant, Bayer CropScience LP, St. Louis, Missouri) were applied at planting. Plots were sprayed with glyphosate (Roundup WeatherMax, Monsanto Company, St. Louis, Missouri, or Glyphos brands) about 20 days after planting for weed control. All corn seed was received from the seed companies and pretreated with two or three fungicides and either clothianidin at 0.5 mg per kernel (Poncho 500, Bayer CropSciences RTP, Greensboro, North Carolina) or thiamethoxam at 0.5 mg per kernel (Cruiser 500, Syngenta Crop Protection, Greensboro, North Carolina). No other insecticides were applied. Natural rainfall was supplement by irrigation weekly with 6 cm of water as needed.

Transgenic Bt hybrid efficacy: Corn seed was planted at a rate of 79,040 seed per ha in 91 cm wide rows using a two-row Monosem® pneumatic planter. Plots were six rows wide and 9.1 m long. A selection of available hybrids with various pyramided Bt proteins for above-ground pests were evaluated and compared with comparable near isogenic non-Bt hybrids (Table 3.1). Hybrids were provided by DeKalb Seeds (Bayer CropSciences, St. Louis, Missouri) and DuPont Pioneer Hi-bred International Inc. (Corteva AgriScience, Johnston, Iowa). Planting occurred on July 2, 2019. Several rows of a non-Bt corn hybrid, DeKalb DKC 6694, were planted on both sides of the experiment acting as borders.

Corn observations for whorl damage caused by fall armyworm defoliation began three weeks after planting during the mid-vegetative growth stages (V3-10). Stand counts were performed on the middle two rows per plot in addition to inspection for whorl damage. Plant defoliation was rated using the Davis et al. (1992) 0-9 scale, where 0 represents no damage and 9 represents near total destruction of the whorl. Damage ratings of the middle two rows for every plot continued in two-week intervals to observe the sequence of foliar damage and how it affected the overall growth of individual plants. This protocol continued until the crops reached the milk stage (R3) (Adendroth et al. 2011) which then they were evaluated for corn earworm damage on the ears.

Twenty random ears from each plot were opened from their husks and inspected for the presence of corn earworm larvae. The total number of larvae was counted per plot with each larva categorized as small, medium, or large in size. Small larvae were identified as no larger than 7 mm, whereas large larvae were at least 24 mm in length. Medium-sized larvae were considered between 7 and 24 mm in length. Exit holes also counted towards corn earworm presence, indicating the larvae had burrowed out of the ear to pupate in the soil. Plots were evaluated for total corn earworm damage at the late dent stage (R5) to early physiological maturity stage (R6) (Adendroth et al. 2011). Another twenty random ears from each plot were inspected for total area damage (cm^2) caused by larval feeding. Damage measurements per ear were divided between the tip portion with unpollinated kernels and the rest of the ear containing viable kernels. The same two center rows where the fall armyworm damage ratings occurred had all their ears hand harvested upon full maturity. Kernels were extracted from their ears through a combination of an electric sheller and by hand. Grain weight from each plot was measured in kg, and a cup-sized sample from each plot was placed into a DICKEY-john Corp. Grain Analysis Computer (GAC 2100) machine to measure their moisture content percentage and test weight (kg/hL), a measure for grain quality. Grain yields were calculated and adjusted to a standard 15.5% moisture content.

Non-Bt corn yield response to foliar damage: Plantings took place in an adjacent block of field corn next to the transgenic Bt hybrid efficacy experiment in 2019 and in a separate planting in 2020. The 2019 experiment occurred within eighteen continuous rows by eight sections consisting of the non-Bt hybrid DeKalb DKC 6694 planted July 2. The 2020 experiment occurred in a 24-row plot 64 meters in length. The non-Bt hybrids Pioneer 1637R, DeKalb DKC 6205, and DeKalb DKC 6694 were each planted on July 16 in eight-row stripes covering the entire length and respectively labelled as the west, middle, and east plots. Alleyways were later added 2.5 m in width to result in making fifteen subplots 12.2 m in length.

Observations in field corn began shortly after tasseling during its silking stage (R1) (Adendroth et al. 2011). Plants in every row were examined for foliar damage due to fall armyworm larvae feeding and rated using the Davis et al. (1992) 0-9 scale, where 0 represents no damage and 9 presents the plant is completely destroyed. Damaged plants rated at least a 4 or 5 on the scale with notable foliar damage were tagged with a paper slip near the base of the stalk. The paper tags were color coded based on whether foliar damage occurred on individual plants (yellow), small clusters of two or three plants (orange), or large clusters of four or more plants (red). Small and large damaged plant clusters had the opposite ends of their respective clusters tagged with an appropriately colored paper slip. Ears from these tagged damage plants were hand harvested upon full maturity and bagged by their respective general cluster size. These samples were additionally paired up with hand-picked ears of the same respective cluster size from adjacent undamaged plants of the same non-Bt hybrid.

Viable kernels were extracted from the cobs using an electric sheller in 2019 and by hand in 2020. Additional measurement were made in 2020 prior to grain extraction including ear length (cm) and direct ear damage (cm^2) from a combination of sources including lepidopteran pest feeding, sap beetles (Coleoptera: Nitidulidae), and fungal development. Subsamples of specific number of kernels from each sample were also weighed. Sample sizes were either 100, 50, 20, or *n* kernel grains depending on the number of available viable kernels from each damaged or undamaged set, where *n* represents the total number of viable kernels in a given set. Average grain weight per ear, number of kernels per ear, and seed weight multiplied by a factor of 1000 were calculated using this yield data.

Data Analyses: The statistical software SAS version 9.4 and JMP Pro version 15.0.0 were used for data analysis (SAS Institute 2013, 2019). Results from the first study were statistically analyzed through an analysis of variance (ANOVA) mixed model with PROC MIXED and appropriate statistical procedures for a randomized completed block design. Hybrids were treated as a fixed effect and replicates were treated as a random effect for this mixed model. Single degree-of-freedom contrasts of selecting hybrids compared the effects of expressed Bt proteins among non-Bt hybrids, Bt hybrids expressing only *Cry* proteins, and Bt hybrids expressing Vip3Aa20. Degrees of freedom were estimated using the Kenward-Roger method. Treatment means were separated using pairwise T-test groupings in PROC PLM when a significant difference was indicated among *F*-values ($\alpha = 0.05$). Linear regression through a bivariate fit of two continuous data type variables using PROC CORR (SAS Institute 2013) examined the associations of fall armyworm whorl defoliation and corn earworm ear damage with grain yield.

Results from the second study were analyzed by year through ANOVA with factorial arrangements of damage type, cluster size and their interaction using a completely randomized design of a general linear mixed model with PROC MIXED (SAS Institute 2013). Damage type, cluster size, and their interaction were treated as fixed effects but there was no random effect. The SLICE function was used to compare means of damage types within cluster size ($\alpha = 0.05$). Degrees of freedom were estimated using the Kenward-Roger method.

Results

Transgenic Bt hybrid efficacy

Larval infestations: Fall armyworm infestation rates were extremely low in 2019. Recorded number of infested plants and foliar damage ratings were highest at the V5 growth stage which diminished quickly for the three remaining damage rating sessions (Table A.7). The presence of fall armyworm frequently occurred in only non-Bt hybrids (Table 3.2, Figure 3.1), especially DeKalb DKC 6205 that initially had an average plot infestation rate of 11.79 ± 3.63% and foliar damage rating of 4.82 ± 0.18. Infestation rates and damage ratings for all tested non-Bt hybrids decreased upon the second damage rating session, averaging altogether for an infestation rate around four percent and a foliar damage rating around four (Tables 3.2 and A.7). A combined total of only seven Bt hybrid plots from all four damage rating sessions had some level of fall armyworm infestation, all of which were hybrids expressing only *Cry* proteins. Percent infested plants of this hybrid type was only apparent in Pioneer 1637YHR and DeKalb DKC 6697, but fall armyworm infestations occurred more frequently in Pioneer 1637YHR, an Optimum Intrasect product, which also had the highest foliar damage ratings of all Bt hybrids (Tables 3.2 and A.7). DeKalb DKC 6697 and DKC 6208 each had visible defoliation in only one damage rating session. All Bt hybrids expressing Vip3Aa20 had no fall armyworm whorl defoliation.

Corn earworm ear infestations were below average in southern Georgia in 2019 but were more frequent among Bt and non-Bt plots than fall armyworm infestations that year. Bt hybrids expressing Vip3Aa20 had no corn earworm larvae or ear damage in addition to no fall armyworm whorl defoliation. Average ear infestation rates for both R3 and R6 growth stage corn were over 90% for non-Bt hybrids and Bt hybrids expressing only *Cry* proteins. Some of these hybrids including DeKalb DKC 6694 even had a 100% infestation rate at one or both growth stages (Tables 3.3 and 3.4). Despite similar infestation rates, Bt hybrids expressing only *Cry* proteins had significantly higher rates of total larval presence compared to non-Bt hybrids. The majority of these larvae were small followed by medium in size, unlike the presence of larvae in non-Bt hybrids that mostly were large in size or had already exited the ear to pupate in the soil (Table 3.3, Figure 3.2). Recorded ear damage at the R6 growth stage was highest in the Pioneer 1637R and Pioneer 1637YHR, followed by all other hybrids that did not express the Vip3Aa20 protein (Table 3.4). Most ear damage was located in the viable kernels (Figure 3.3). Ear damage by ear region and total amount was not significantly different when comparing non-Bt hybrids and Bt hybrids expressing only *Cry* proteins.

Overall Yield: Harvested grain yield was notably lower than average due to how late in the growing season corn seed was planted in order to assist in promoting natural infestations. Grain yields were significantly different among hybrids but were not completely associated with levels of foliar damage from fall armyworm and ear damage by corn earworm. Bt hybrids expressing Vip3Aa20 had the lowest of grain yields despite never having been infested by either lepidopteran pest species. DeKalb DKC 6629, a Genuity Trecepta product, especially underperformed having the lowest grain yield and test weight averages among all other Bt and non-Bt hybrids (Table 3.5). Bt hybrids that expressed only *Cry* proteins, however, significantly produced higher grain yields compared to non-Bt hybrids. Linear regression analysis confirmed there was no association between grain yield and fall armyworm whorl defoliation or corn earworm ear damage (Figure 3.4). Test weights were significantly different among hybrids but only due to hybrid agronomics rather than the presence of Bt traits (Table 3.5).

Non-Bt corn yield response to foliar damage

In 2019, non-Bt hybrid plots had moderate levels of fall armyworm infestation despite overall rates were well below average. Infestation rates rebounded in 2020 with fall armyworm whorl defoliation occurring more frequently among untreated plots. Measurements were not made, but infestation rates were estimated to have been no more than 20% in 2019 and no less than 50% in 2020. Five hundred fifty-five damaged cluster samples of varying cluster sizes were tagged and harvested for a combined total of 1,314 damaged plants across both years. Of the 555 damaged cluster samples, 346 were individually damaged, 93 were small clusters (average cluster size: 2.37 ± 0.05), and 116 were large clusters (average cluster size: 6.41 ± 0.27). The additional harvesting of ears from adjacent undamaged non-Bt corn paired with every damaged cluster based on cluster group resulted in a total of 2,628 ears collected altogether across both years.

The combined data from 2019 and 2020 found ears harvested from damaged plants had about 27.6% less average grain weight per ear and about 26.4% less average kernels per ear compared to ears from undamaged plants (Table 3.6, Figure 3.5). Some ears collected from damaged plants were significantly underdeveloped and did not produce any viable kernels, most occurring in 2020 when fall armyworm infestations were highest (Figure 3.6). No significant difference was found between 1000 seed weights of damaged and undamaged plants (Table 3.6, Figure 3.7). Cluster size did not significantly affect grain weight per ear, kernels per ear, and 1000 seed weight (Tables 3.6 and 3.7). Yield results were significantly different by year due to the magnitude of yield difference being greater in 2020 than it was in 2019 (Table 3.7). There were no significant interactions for any combined yield results except the interaction of year and cluster size for 1000 seed weight for which large clusters had 4.1% higher seed weights than the other cluster sizes in 2019 but 5.1% lower weights in 2020 (Tables 3.6 and 3.7). However, no significant interaction was detected when these results by year were combined. The interaction between damage type and cluster size was not significant for any variable by individual year except for average kernels per ear in 2020 (Table 3.7).

All ears across both plantings exhibited direct kernel damage caused by corn earworm feeding (Figure 3.6). In 2020, ears from plants with no fall armyworm whorl defoliation had significantly more direct ear damage than ears from plants with some level of fall armyworm whorl defoliation, though they were not significant by cluster size or the interaction between damage type and cluster size (Tables 3.7 and 3.8). Furthermore, ears from these damaged plants were about 17.1% shorter in ear length compared to their undamaged counterparts (Damaged: 8.94 ± 0.13 cm; Undamaged: 10.78 ± 0.13 cm), which was also consistent throughout each cluster size and non-Bt hybrid planted. No significant interaction was detected between damage type and cluster size for these measurements (Tables 3.7 and 3.8).

Yield results in 2020 had some variation but generally were similar across all three non-Bt hybrids with the presence of significant outliers. Most notably, DeKalb DKC 6694 produced on average the least amount of grain weight per ear (Pioneer 1637R: 13.24 ± 0.61 g; DeKalb DKC 6205: 12.68 ± 0.63; DeKalb DKC 6694: 9.69 ± 0.55 g) and kernels per ear (Pioneer 1637R: 96.14 ± 4.29 kernels; DeKalb DKC 6205: 87.86 ± 4.41 kernels; DeKalb DKC 6694: 68.14 ± 3.86 kernels). However, collected samples of this particular hybrid were mostly individual ears which were more likely to produce zero yield if harvested from plants with significant whorl defoliation.

Discussion

Fall armyworm infestations can be economically damaging across a number of agricultural commodities, including field corn production in the southeastern United States region where lepidopteran pest activity is significant. The use of corn hybrids expressing Bt traits has proven effective for reducing fall armyworm infestations across the region as well as other countries where the pest flourishes. Bt products expressing pyramided proteins have rarely performed any better than single-trait hybrids towards fall armyworm control alone (Moscardini et al. 2020, Rule et al. 2014, Siebert et al. 2012), yet continuous use of these single toxins have resulted in resistance development to occur among local pest populations as well as cause cross resistance in pyramided Bt products (Huang et al. 2014, Niu et al. 2013, 2016a, Vélez et al. 2013). Failure to control fall armyworm infestations can result in higher rates of whorl damage similar to that of untreated hybrids and may require reverting back to insecticidal sprays (Burtet et al. 2017). Greater whorl damage can be linked with significant grain yield loss with larval densities and vulnerable plant growth stages as key factors, yet this entire issue has received very little attention as an independent concept aside from older studies by Cruz and Turpin (1983) and Marenco et al. (1992). We evaluated in this study the efficacy of pyramided Bt field corn hybrids commercially available for fall armyworm control in a late planting. Additional observations were made in non-Bt hybrids to determine their current yield responses to significant fall armyworm whorl defoliation and if the size of localized damage clusters is associated with the amount of yield reduction present.

All Bt hybrids used in the 2019 planting significantly reduced fall armyworm infestations in field corn. However, infestation rates that year were low before rebounding in 2020, likely coinciding the species' irregular infesting behavior noted by Sparks (1979). The minimal fall armyworm presence in 2019 primarily occurred in only non-Bt hybrid plots. Bilbo et al. (2020) had greater levels of fall armyworm infestation in late-planted field corn in South Carolina in 2016 and 2017 but also observed all Bt hybrids had little infestation that did not reach the minimum threshold to require additional insecticidal treatment. The differences between Bt and non-Bt hybrids in our experiment were displayed the same way for foliar damage ratings. Non-Bt hybrids had an average damage rating score of around 4 on the 0-9 scale (Davis et al. 1992), indicating moderate foliar damage, while Bt-hybrids had an average rating of less than 1, indicating very little larval impact on the plants.

Of these Bt hybrids tested, Pioneer 1637YHR, an Optimum Intrasect product that expresses Cry1Ab and Cry1Fa2, was the least effective against fall armyworm based on percent infested plants and damage ratings. Cry1Ab never provided more than partial control of the pest, whereas Cry1Fa2 was much more effective until resistance development began to emerge and spread across local populations in North and South America (Farias et al. 2014, Huang et al. 2014, Storer et al. 2010, Vélez et al. 2013). In contrast, DeKalb DKC 6697, a Genuity VT Double PRO product that expresses Cry1A.105 and Cry2Ab2, had a similar infested plant percentage but with reduced foliar damage, and DeKalb DKC 6208, a SmartStax product that expresses the same linked proteins with Cry1Fa2, had minimal fall armyworm infestations (Tables 3.2, A.7). Bt hybrids that express Vip3Aa20 in addition to pyramided *Cry* proteins were the most effective in preventing any fall armyworm whorl defoliation and were the only hybrid to also prevent any corn earworm ear damage. However, the total yields of Bt hybrids expressing Vip3Aa20 were among the lowest recorded due to hybrid agronomics. Only those Bt hybrids expressing only *Cry* proteins produced significantly higher yields than non-Bt hybrids, similar to what Bilbo et al. (2020) reported (Table 3.5). Attempts to replicate this experimental protocol should consider multiple planting dates for better observations on fall armyworm infestation rates and how it affects grain production by plant timing among Bt hybrids.

On the other hand, the whorl damage received in non-Bt hybrids over 2019 and 2020 as a result of fall armyworm defoliation during the mid-vegetative growth stages resulted in a notable reduction in viable grain yield. Tagged damaged plants had an average 0-9 damage rating between 4 and 5 (Davis et al. 1992), enough to cause a very noticeable loss of leaf tissue and leaf area, which may affect ear development. Marenco et al. (1992) found foliar damage was highest and affected the number of U.S. No. 1 ears in sweet corn production the most during the late whorl stages (V9-R1), whereas damage during the earlier stages was not as severe. Our observations only found fall armyworm infestations to occur at the start of the mid-whorl growth stages (V3-V10) in 2019. The presence of fall armyworm in the 2020 late-planted field corn was greater and likely continued into the later whorl stages prior to tasseling.

Our combined yield results from 2019 and 2020 found fall armyworm whorl defoliation resulted in a significant loss of grain through reduced viable kernel availability in field corn production. The intensity of yield loss was not associated with how widespread fall armyworm infestations were in any given row. Our results reflect what was also concluded by Cruz and Turpin (1983) in their report that additionally states grain yield was inversely associated with foliar damage based on manual egg infestations. We can conclude the same results for natural infestations based on a late-planting scenario. Based on the lowest estimate of the near 20% yield loss in 2019, we estimate that 25% of plants infested with fall armyworm, which represents the current economic threshold in Georgia (Buntin 2021), would cause about a 7% yield loss in grain production. In 2020, greater levels of whorl damage additionally contributed to stunted ear development to the point some ears were incapable of producing viable kernels or even a fully developed cob (Figure 3.6). Ears from plants with no whorl defoliation were more likely to sustain feeding damage directly on the ear due to their increased vulnerability of having more fully developed ears and greater availability of viable kernels. Some fall armyworm may have fed on parts of the ear during the plant's reproductive growth stages, but corn earworm are speculated to have caused most of this observed damage based on similar feeding patterns.

Planting field corn at early or optimal times of the growing season helps in avoiding severe fall armyworm infestations. Situations including double cropping practices or natural phenomenon that may cause the crop to be planted at a much later date would need growers to consider using Bt technology or timely treatment applications during plant stages when foliar damage would affect yield production the most. The use of Bt hybrids expressing at least Cry1A.105 and Cry2Ab2 or Vip3Aa20 currently is enough to significantly reduce fall armyworm infestations and potentially any yield losses associated with the pest while taking account for local Bt resistance development.

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Table 3.1. Characteristics of Bt and non-Bt corn hybrids used in the 2019 late planting

Brand & Hybrid	**Product Name**	**Bt Traits**
Pioneer 1637R	Non-Bt	None
Pioneer 1637YHR	Optimum Intrasect	Cry1Ab, Cry1Fa2
Pioneer 1637VYHR	Optimum Leptra	Cry1Ab, Cry1Fa2, Vip3Aa20
DeKalb DKC 6694	Non-Bt	None
DeKalb DKC 6697	Genuity VT Double PRO	Cry1A.105, Cry2Ab2
DeKalb DKC 6629	Genuity Trecepta	Cry1A.105, Cry2Ab2
DeKalb DKC 6205	Non-Bt	None
DeKalb DKC 6208	SmartStax	Cry1A.105, Cry2Ab2, Cry1Fa2

Table 3.2. Effect of Bt traits on LS means ± SEM of percentage fall armyworm infested plants and whorl damage ratings averaged across four inspections from growth stages V5 to R3 in late-planted 2019 field corn with contrasting statements.

Brand & Hybrid	**Bt traits**	**Infested Plants (%)**	**Damage Rating^x per Infested Plant**	**Damage Rating^x per Plant**
P 1637R	None (RR2)	3.74 ± 1.11 a	3.87 ± 1.20 a	0.029 ± 0.011 a
P 1637YHR	Optimum Intrasect$^+$	0.32 ± 0.20 b	1.08 ± 0.49 b	0.008 ± 0.003 b
P 1637VYHR	Optimum Leptra^{++}	0 b	0 b	0 b
DKC 6694	None (RR2)	4.04 ± 1.76 a	3.94 ± 0.62 a	0.032 ± 0.005 a
DKC 6697	Genuity VT Double PRO$^+$	0.33 ± 0.27 b	0.26 ± 0.15 b	0.002 ± 0.001 b
DKC 6629	Genuity Trecepta^{++}	0 b	0 b	0 b
DKC 6205	None (RR2)	4.77 ± 1.42 a	4.31 ± 0.35 a	0.032 ± 0.005 a
DKC 6208	SmartStax$^+$	0.05 ± 0.05 b	0.19 ± 0.19 b	0.002 ± 0.002 b
F > (*P*)				
Hybrid		0.0008	<0.0001	<0.0001
Bt vs non-Bt		<0.0001	<0.0001	<0.0001
Cry Bt vs non-Bt		<0.0001	<0.0001	<0.0001
Vip Bt vs non-Bt		0.0003	<0.0001	<0.0001
Cry Bt vs Vip Bt		0.7160	0.0849	0.1098

LS means ± SEM within columns followed by same letters are not significantly different (PROC MIXED, pair-wise t-tests of LSM, α = 0.05).
Hybrid: (df = 7, 21); Contrasting Statements: (df = 1, 21).

[x] Davis et al. (1992) rating scale.

[+] Bt-hybrid expressing only pyramided *Cry* proteins.

[++] Bt-hybrid expressing pyramided *Cry* proteins with Vip3Aa20.

Table 3.3. Effect of Bt traits on LS means ± SEM of percentage corn earworm infested ears and number of corn earworm larvae per ear by size and exit holes in R3 growth stage late-planted 2019 field corn with contrasting statements.

Brand & Hybrid	**Bt Traits**	**Infested R3 ears (%)**	**Live larvae per R3 growth stage ear**				
			Small	**Medium**	**Large**	**Exit**	**Total**
P 1637R	None (RR2)	90.0 ± 4.1 b	0.82 ± 0.09 bc	0.49 ± 0.07 bc	0.19 ± 0.04 c	0 b	1.50 ± 0.05 bc
P 1637YHR	Optimum Intrasect[+]	87.5 ± 3.2 b	1.19 ± 0.07 a	0.34 ± 0.04 cd	0.06 ± 0.02 cd	0 b	1.59 ± 0.03 b
P 1637VYHR	Optimum Leptra[++]	0 c	0 d	0 f	0 d	0 b	0 e
DKC 6694	None (RR2)	100 a	0.12 ± 0.04 d	0.29 ± 0.07 de	0.84 ± 0.07 a	0.05 ± 0.03 b	1.30 ± 0.09 cd
DKC 6697	Genuity VT Double PRO[+]	97.5 ± 2.5 a	1.07 ± 0.22 ab	0.67 ± 0.10 a	0.13 ± 0.08 cd	0 b	1.89 ± 0.13 a
DKC 6629	Genuity Trecepta[++]	0 c	0 d	0 f	0 d	0 b	0 e
DKC 6205	None (RR2)	100 a	0.02 ± 0.01 d	0.12 ± 0.05 ef	0.70 ± 0.10 a	0.37 ± 0.11 a	1.22 ± 0.05 d
DKC 6208	SmartStax[+]	97.5 ± 1.4 a	0.54 ± 0.19 c	0.52 ± 0.05 ab	0.35 ± 0.07 b	0.02 ± 0.02 b	1.44 ± 0.16 bcd
F > (*P*)							
Hybrid		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Bt vs non-Bt		<0.0001	0.0371	0.5189	<0.0001	0.0073	<0.0001
Cry Bt vs non-Bt		0.1018	<0.0001	0.0001	<0.0001	0.0006	0.0002
Vip Bt vs non-Bt		<0.0001	0.0002	<0.0001	<0.0001	0.5433	<0.0001
Cry Bt vs Vip Bt		<0.0001	<0.0001	<0.0001	0.0837	1.0000	<0.0001

LS means ± SEM within columns followed by small letters are not significantly different (PROC MIXED, pair-wise t-tests of LSM, α = 0.05). Hybrid: (df = 7, 21); Contrasting Statements: (df = 1, 21).

[+] Bt-hybrid expressing only pyramided *Cry* proteins.

[++] Bt-hybrid expressing pyramided *Cry* proteins with Vip3Aa20.

Table 3.4. Effect of Bt traits on LS means ± SEM of percentage corn earworm infested ears and area damage by ear region per ear in R6 growth stage late-planted 2019 field corn with contrasting statements.

Brand & Hybrid	**Bt Traits**	**Infested R6 ears (%)**	**Damage (cm^2) per R6 growth stage ear**		
			Ear tip	**Kernels**	**Total**
P 1637R	None (RR2)	98.33 ± 1.67 a	2.27 ± 0.10 a	6.72 ± 0.30 a	8.98 ± 0.39 a
P 1637YHR	Optimum Intrasect^{+}	100 a	2.15 ± 0.10 a	6.38 ± 0.23 a	8.53 ± 0.22 a
P 1637VYHR	Optimum Leptra^{++}	0 b	0 c	0 c	0 c
DKC 6694	None (RR2)	100 a	1.50 ± 0.06 b	3.92 ± 0.36 b	5.42 ± 0.40 b
DKC 6697	Genuity VT Double PRO^{+}	100 a	1.47 ± 0.05 b	4.02 ± 0.38 b	5.48 ± 0.39 b
DKC 6629	Genuity Trecepta^{++}	0 b	0 c	0 c	0 c
DKC 6205	None (RR2)	96.25 ± 2.39 a	1.40 ± 0.12 b	3.59 ± 0.41 b	4.99 ± 0.52 b
DKC 6208	SmartStax^{+}	96.25 ± 2.39 a	1.46 ± 0.04 b	3.37 ± 0.33 b	4.84 ± 0.36 b
F > (P)					
Hybrid		<0.0001	<0.0001	<0.0001	<0.0001
Bt vs non-Bt		<0.0001	<0.0001	<0.0001	<0.0001
Cry Bt vs non-Bt		0.6147	0.6191	0.5416	0.7149
Vip Bt vs non-Bt		<0.0001	<0.0001	<0.0001	<0.0001
Cry Bt vs Vip Bt		<0.0001	<0.0001	<0.0001	<0.0001

LS means ± SEM within columns followed by small letters are not significantly different (PROC MIXED, pair-wise t-tests of LSM, $\alpha = 0.05$).
Hybrid: (df = 7, 21); Contrasting Statements: (df = 1, 21).

$^{+}$ Bt-hybrid expressing only pyramided *Cry* proteins.

$^{++}$ Bt-hybrid expressing pyramided *Cry* proteins with Vip3Aa20.

Table 3.5. Effect of Bt traits on LS means ± SEM of corn yield based on grain and test weights from harvested late-planted 2019 field corn with contrasting statements.

Brand & Hybrid	**Bt Traits**	**Grain Yield (kg/ha)**	**Test Weight (kg/hL)**
P 1637R	None (RR2)	3084 ± 239 ef	70.28 ± 0.48 ab
P 1637YHR	Optimum Intrasect^{+}	3822 ± 165 bc	71.11 ± 0.59 a
P 1637VYHR	Optimum Leptra^{++}	3223 ± 111 de	70.19 ± 0.47 ab
DKC 6694	None (RR2)	3418 ± 300 cde	69.68 ± 0.58 abc
DKC 6697	Genuity VT Double PRO^{+}	3674 ± 86 bcd	68.20 ± 0.59 cd
DKC 6629	Genuity Trecepta^{++}	2566 ± 165 f	67.87 ± 0.97 d
DKC 6205	None (RR2)	3977 ± 198 b	68.98 ± 0.55 bcd
DKC 6208	SmartStax^{+}	4996 ± 161 a	70.44 ± 0.49 ab
F > (P)			
Hybrid		<0.0001	0.0095
Bt vs non-Bt		0.0712	0.6379
Cry Bt vs non-Bt		0.0002	0.5908
Vip Bt vs non-Bt		0.0639	0.1320
Cry Bt vs Vip Bt		0.0001	0.3155

LS means ± SEM within columns followed by small letters are not significantly different (PROC MIXED, pair-wise t-tests of LSM, α = 0.05).
Hybrid: (df = 7, 21); Contrasting Statements: (df = 1, 21).

$^{+}$ Bt-hybrid expressing only pyramided *Cry* proteins.

$^{++}$ Bt-hybrid expressing pyramided *Cry* proteins with Vip3Aa20.

Table 3.6. Effect of fall armyworm whorl defoliation by damage cluster size during the vegetative growth stages on LS means ± SEM of grain weight and quality of 2019 and 2020 late planted non-Bt field corn.

Cluster Group*	Damage Type**	Ear Weight per Plant (g)						Number of Kernels per Ear						1000 Seed Weight (g)					
		2019		2020		Average		2019		2020		Average		2019		2020		Average	
1	-	38.67 ± 0.73 a	A	14.79 ± 0.73 a	A	26.80 ± 0.82 a	A	231.52 ± 4.69 a	A	101.40 ± 5.18 a	A	166.78 ± 4.89 a	A	169.20 ± 1.31 a	B	158.57 ± 5.14 a	A	164.41 ± 2.01 a	A
	+	31.01 ± 0.83 b		8.51 ± 0.66 b		19.69 ± 0.83 b		190.61 ± 5.28 b		66.74 ± 5.21 b		126.51 ± 5.22 b		166.31 ± 1.77 a		163.78 ± 5.70 a		163.79 ± 2.25 a	
2	-	37.59 ± 1.44 a	A	15.13 ± 0.95 a	A	26.38 ± 1.44 a	A	219.9 ± 9.31 a	A	99.22 ± 5.81 a	A	159.78 ± 8.20 a	A	173.52 ± 2.85 a	B	153.42 ± 4.06 a	AB	163.40 ± 2.74 a	A
	+	31.85 ± 1.60 b		7.96 ± 0.78 b		19.49 ± 1.55 b		193.38 ± 9.95 a		55.82 ± 5.17 b		122.41 ± 9.53 b		165.63 ± 2.25 a		154.85 ± 4.77 a		159.02 ± 3.16 a	
3	-	39.22 ± 1.09 a	A	16.67 ± 0.68 a	A	27.98 ± 1.22 a	A	222.09 ± 6.71 a	A	117.35 ± 5.30 a	A	170.18 ± 6.42 a	A	178.19 ± 2.24 a	A	145.83 ± 2.65 a	B	162.01 ± 2.31 a	A
	+	30.81 ± 1.02 b		7.78 ± 0.53 b		19.55 ± 1.17 b		177.31 ± 6.23 b		54.20 ± 3.74 b		117.16 ± 6.62 b		175.17 ± 1.85 a		150.36 ± 3.40 a		162.43 ± 2.12 a	
Average	-	37.73 ± 1.25 a		15.53 ± 0.47 a		27.05 ± 0.47 a		221.14 ± 7.29 a		105.99 ± 3.32 a		165.56 ± 3.15 a		172.98 ± 1.94 a		145.83 ± 3.19 a		163.06 ± 1.76 a	
	+	30.46 ± 1.25 b		8.08 ± 0.47 b		19.58 ± 0.48 b		183.73 ± 7.29 b		58.92 ± 3.41 b		122.03 ± 3.24 b		168.38 ± 1.94 b		150.36 ± 3.26 a		161.95 ± 1.69 a	

LS means ± SEM within columns followed by the same capital letter are not significantly different among cluster groups. LS means ± SEM within columns and cluster groups followed by the same lowercase letter are not significantly different between damage types (PROC MIXED, SLICE, $\alpha = 0.05$)

*Cluster groups include individuals (1), small clusters of two or three (2), or larger clusters of four or more (3).

**Indicates which set from a cluster group is undamaged (-) or damaged (+).

Table 3.7. Analysis of variance comparing grain weight and quality, ear development, and direct ear damage results by year, damage cluster size, damage type, and their interactions for potential influence on 2019, 2020, and combined late-planted non-Bt field corn.

Measurements	**Source**	**2019**		**2020**		**Combined**	
		F	***P***	***F***	***P***	***F***	***P***
Ear weight per plant	Year	-	-	-	-	1152.40	<0.0001
	Cluster Size	0.01	0.9971	0.39	0.6799	0.43	0.6490
	Damage Type	44.57	<0.0001	125.53	<0.0001	124.08	<0.0001
	Cluster Size x Damage Type	0.40	0.6721	1.57	0.2094	0.51	0.6031
	Year x Cluster Size	-	-	-	-	0.03	0.9694
	Year x Damage Type	-	-	-	-	0.17	0.6824
Kernels per plant	Year	-	-	-	-	755.49	<0.0001
	Cluster Size	1.51	0.2707	0.92	0.3997	0.59	0.5544
	Damage Type	29.04	<0.0001	97.47	<0.0001	93.68	<0.0001
	Cluster Size x Damage Type	0.49	0.6118	3.63	0.0273	1.08	0.3395
	Year x Cluster Size	-	-	-	-	0.90	0.4064
	Year x Damage Type	-	-	-	-	0.54	0.4645
1000 seed weight	Year	-	-	-	-	45.75	<0.0001
	Cluster Size	8.30	0.0003	3.45	0.0327	0.60	0.5473
	Damage Type	4.59	0.0326	0.67	0.4143	0.21	0.6474
	Cluster Size x Damage Type	0.52	0.5956	0.06	0.9411	0.37	0.6898
	Year x Cluster Size	-	-	-	-	8.35	0.0003
	Year x Damage Type	-	-	-	-	3.48	0.0625
Direct ear damage	Cluster Size	-	-	0.57	0.5668	-	-
	Damage Type	-	-	4.29	0.0388	-	-
	Cluster Size x Damage Type	-	-	0.21	0.8111	-	-
Ear length	Cluster Size	-	-	0.75	0.4735	-	-
	Damage Type	-	-	88.14	<0.0001	-	-
	Cluster Size x Damage Type	-	-	0.53	0.5910	-	-

Table 3.8. Effect of fall armyworm whorl defoliation by damage cluster size during the vegetative growth stages on LS means ± SEM of ear length with additional notes on direct area ear damage of 2020 late-planted non-Bt field corn.

Cluster Group*	Damage Type**	Direct Ear Damage (cm^2)		Ear Length (cm)	
1	-	11.87 ± 0.65 a	A	10.56 ± 0.21 a	A
	+	10.57 ± 0.63 a		8.91 ± 0.23 b	
2	-	12.69 ± 0.75 a	A	10.73 ± 0.21 a	A
	+	11.10 ± 0.60 a		8.94 ± 0.21 b	
3	-	11.49 ± 0.36 a	A	11.05 ± 0.16 a	A
	+	10.84 ± 0.40 a		8.96 ± 0.14 b	
Average	-	12.02 ± 0.40 a		10.78 ± 0.14 a	
	+	10.84 ± 0.41 b		8.94 ± 0.14 b	

LS means ± SEM within columns followed by the same capital letter are not significantly different among cluster groups. LS means ± SEM within columns and cluster groups followed by the same lowercase letter are not significantly different between damage types (PROC MIXED, SLICE, pair-wise t-tests of LSM, α = 0.05)
*Cluster groups include individuals (1), small clusters of two or three (2), or larger clusters of four or more (3).
**Indicates which set from a cluster group is undamaged (-) or damaged (+).

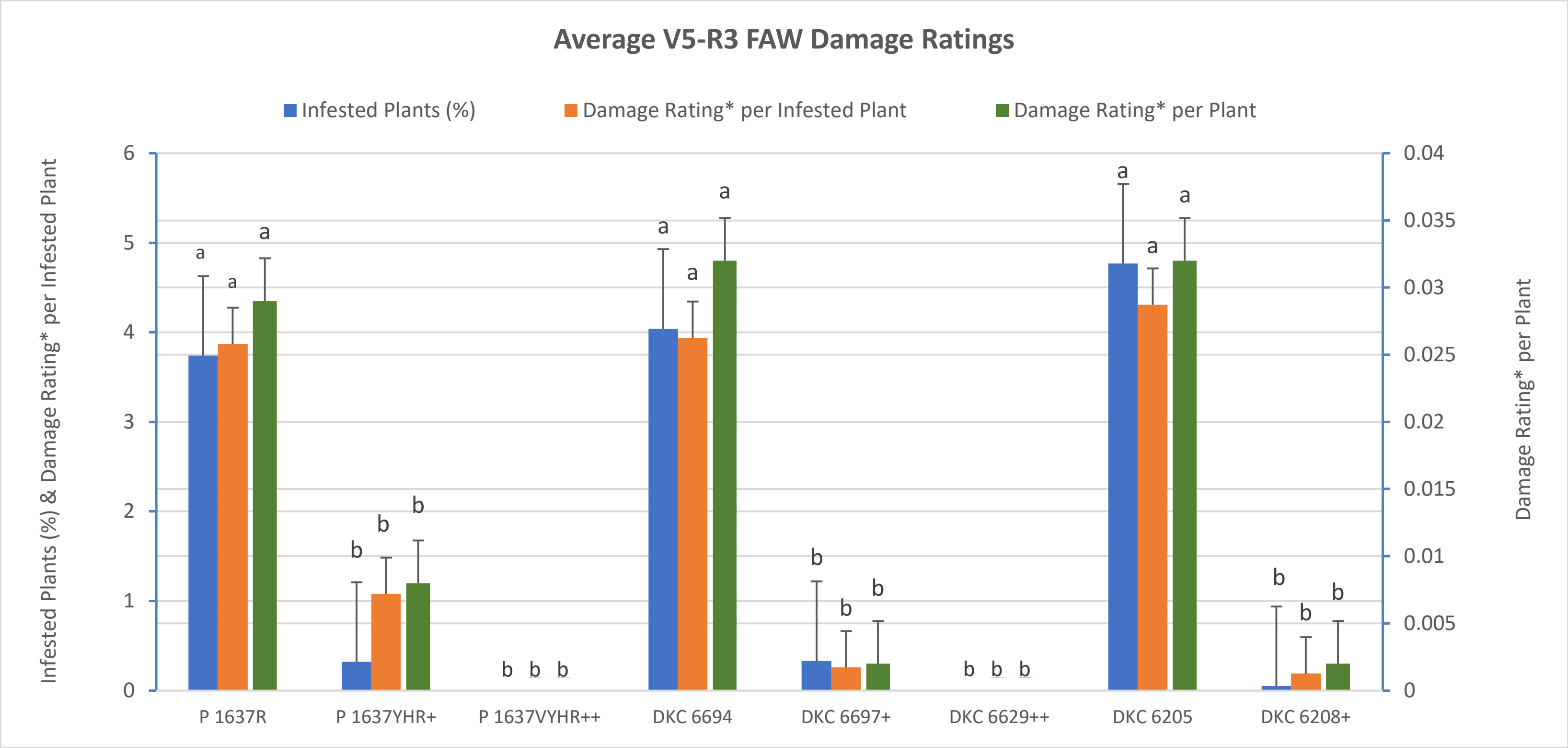

Figure 3.1. Effect of Bt traits on LS means ± SEM of percentage fall armyworm infested plants and whorl damage ratings averaged across four inspections from growth stages V5 to R3 in late-planted 2019 field corn.

Damage rated using Davis et al. (1992) 0-9 plant defoliation scale. LS Means with the same letter are not significantly different (pair-wise t-tests of LSM, $\alpha = 0.05$). Bt hybrids marked with (+) express pyramided *Cry* proteins while hybrids marked with (++) express pyramided *Cry* proteins with Vip3Aa20. Unmarked hybrids are non-Bt hybrids.

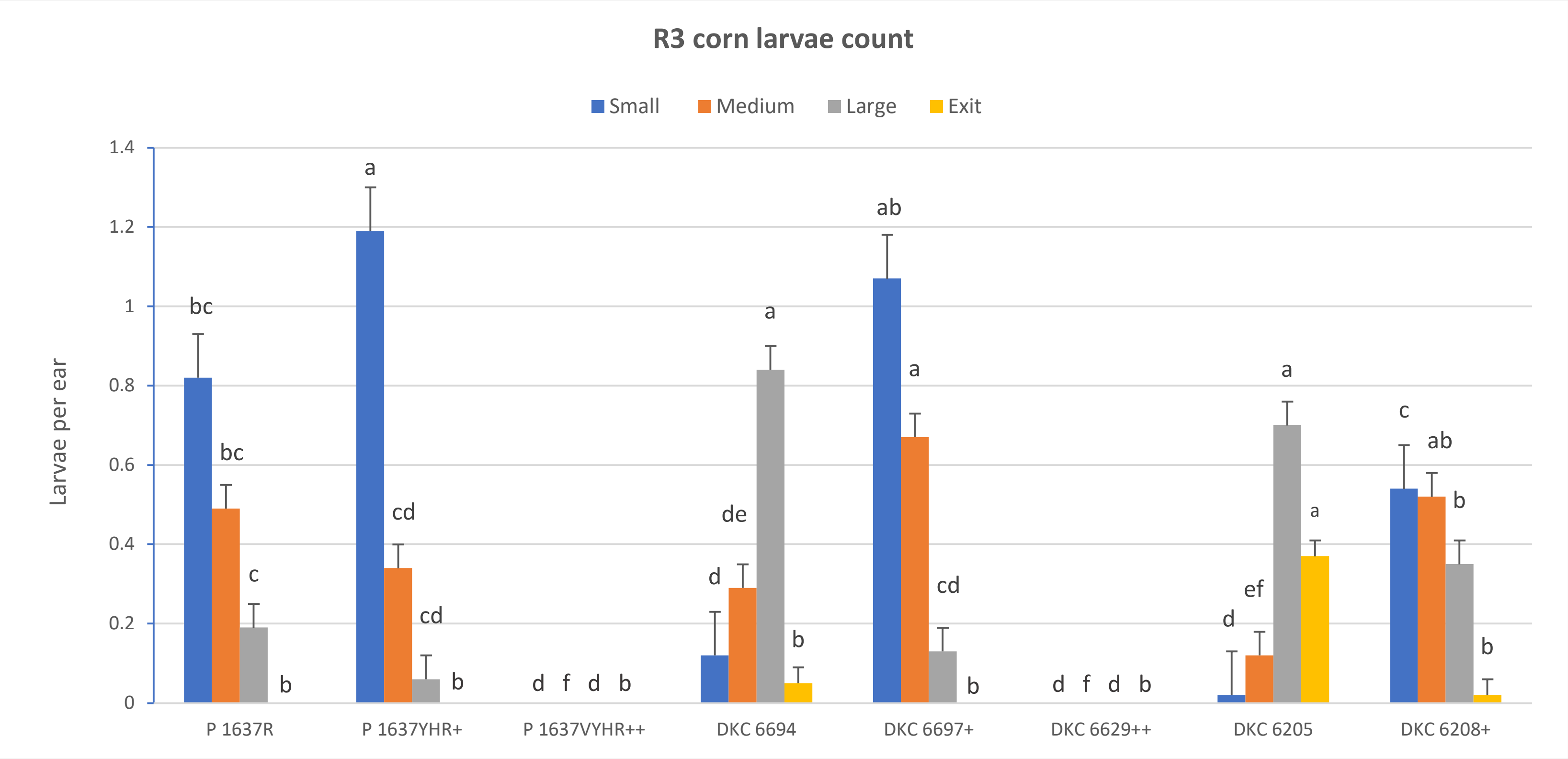

Figure 3.2. Effect of Bt traits on LS means ± SEM of number of corn earworm larvae per ear by size and exit holes in R3 growth stage late-planted 2019 field corn.

LS Means within larval size category with the same letter are not significantly different (pair-wise t-tests of LSM, $\alpha = 0.05$). Bt hybrids marked with (+) express pyramided *Cry* proteins while hybrids marked with (++) express pyramided *Cry* proteins with Vip3Aa20. Unmarked hybrids are non-Bt hybrids. See Table 3.1 for specific proteins expressed for each entry and product type.

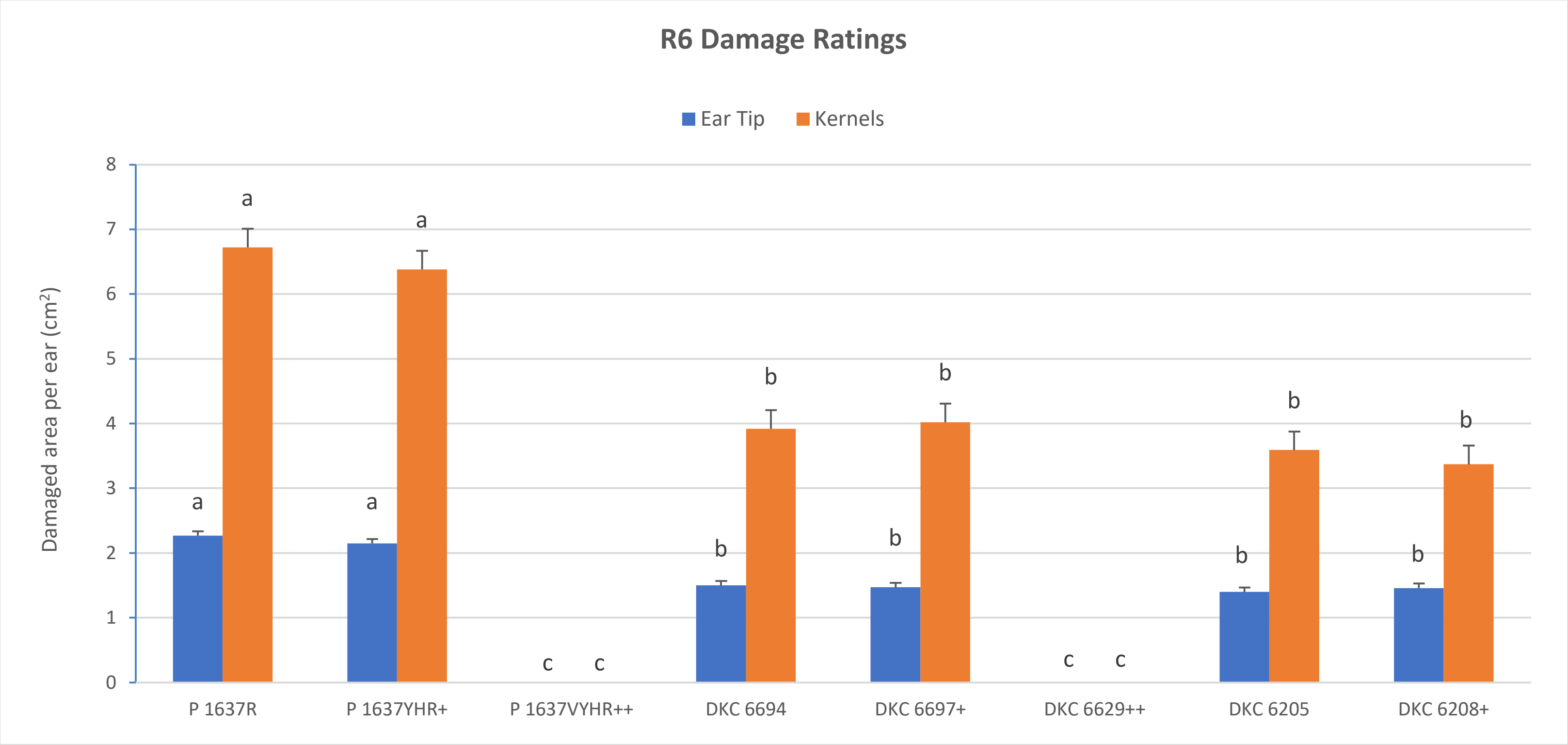

Figure 3.3. Effect of Bt traits on LS means ± SEM of damaged area by ear region per ear in R6 growth stage late-planted 2019 field corn.

LS Means within category with the same letter are not significantly different (pair-wise t-tests of LSM, $\alpha = 0.05$). Bt hybrids marked with (+) express pyramided *Cry* proteins while hybrids marked with (++) express pyramided *Cry* proteins with Vip3Aa20. Unmarked hybrids are non-Bt hybrids. See Table 3.1 for specific proteins expressed for each entry and product type.

Figure 3.4. Linear regression analysis depicting relationship between grain yield of 2019 late-planted field corn and fall armyworm whorl defoliation/corn earworm ear damage.

Fall Armyworm Whorl Defoliation: ($R^2 = 0.0028$; $F = 0.0848$; $P = 0.7730$)

Corn Earworm Ear Damage: ($R^2 = 0.0865$; $F = 2.8421$; $P = 0.1022$)

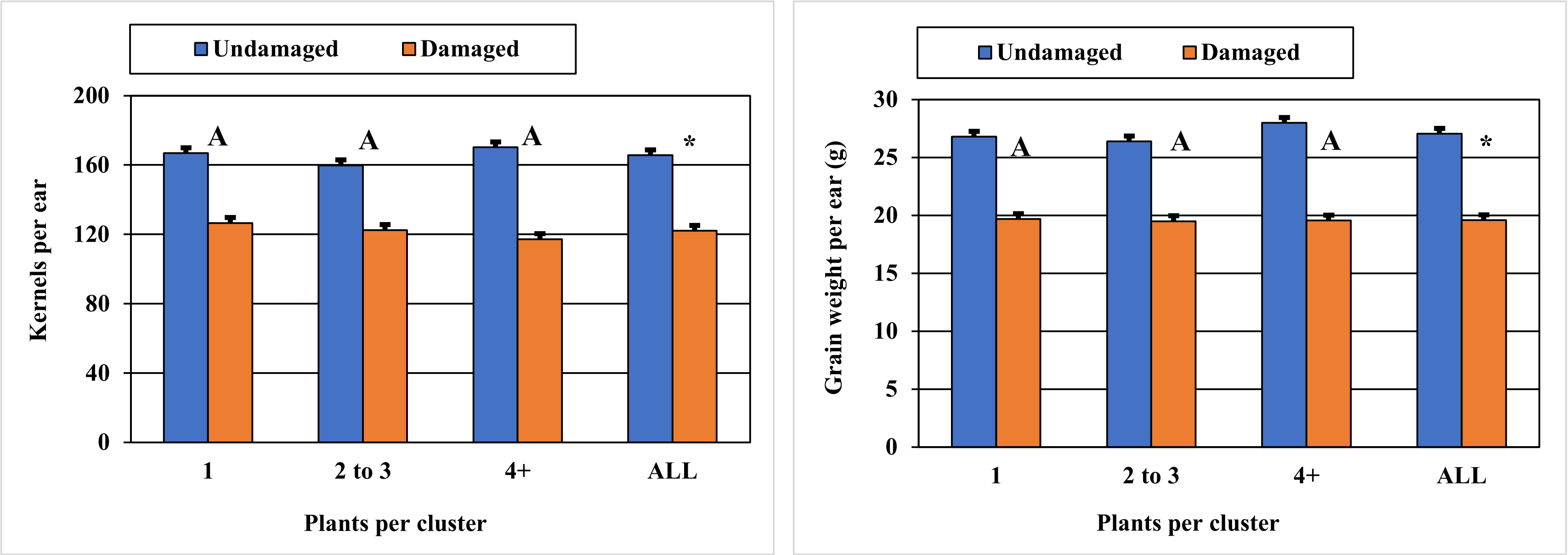

Figure 3.5. Effect of fall armyworm whorl defoliation by damage cluster size during the mid-vegetative growth stages on LS means ± SEM of grain weight and kernels per ear of late-planted field corn combined across 2019 and 2020.

Clusters with same capital letter are not significantly different, α = 0.05.
(*) or (ns) respectively indicate a significant or insignificant effect of fall armyworm whorl defoliation within cluster type or average of all clusters, α = 0.05.

Figure 3.6. Visual comparisons of harvested ears from 2020 late-planted non-Bt field corn from undamaged plants or plants with significant defoliation caused by fall armyworm.

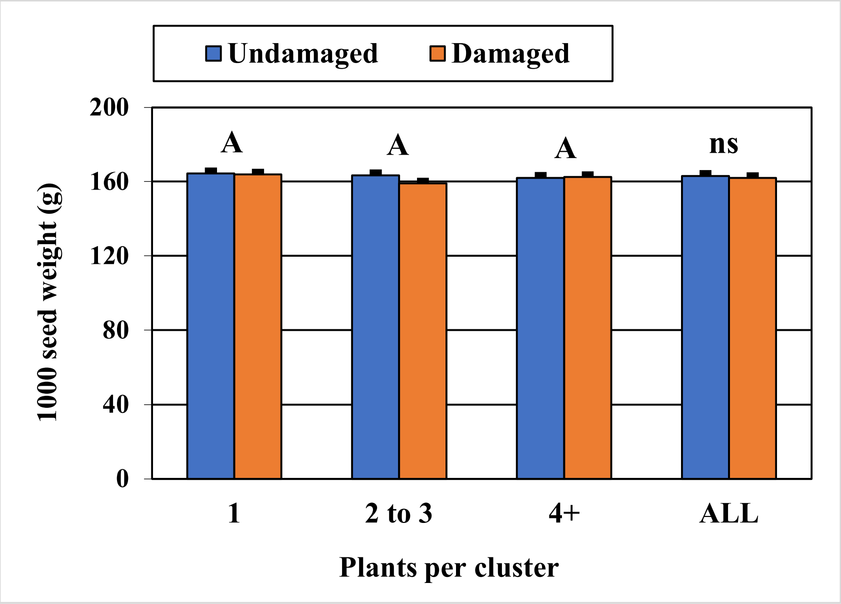

Figure 3.7. Effect of fall armyworm whorl defoliation by damage cluster size during the mid-vegetative growth stages on LS means ± SEM of 1000 seed weight of late-planted field corn combined across 2019 and 2020.

Clusters with same capital letter are not significantly different, α = 0.05.
(*) or (ns) respectively indicate a significant or insignificant effect of fall armyworm whorl defoliation within cluster type or average of all clusters, α = 0.05.

CHAPTER 4

CONCLUSION

Bt resistance development has been an ongoing concern among local populations of lepidopteran pest species in the southeastern United States region. Continuous field monitoring, therefore, remains essential for staying up to date on the efficacy of Bt technology towards lepidopteran pest control and locating potentially new cases of resistance emergence. The results from our 2019 and 2020 studies provide additional knowledge about the current efficacy of a variety of field corn hybrids expressing pyramided Bt proteins. We tested for control of fall armyworm, *Spodoptera frugiperda* (J. E. Smith), and corn earworm, *Helicoverpa zea* (Boddie), in the state of Georgia and how it affected overall grain yield and quality.

Only those transgenic Bt hybrids that expressed Vip3Aa20 in addition to pyramided *Cry* proteins provided the best control against fall armyworm whorl defoliation and corn earworm ear damage. Observations during the vegetative and reproductive growth stages of field corn found the expression of this *Vip* protein in hybrids resulted in little to no presence of larvae and damage on the foliage and ears. Results were especially consistent throughout optimal and late plantings.

Hybrids that expressed only pyramided *Cry* proteins displayed significant success against fall armyworm but only partial success in preventing corn earworm infestations. The variety of these specific hybrids produced inconsistent performances against corn earworm and were as ineffective in late plantings as non-Bt hybrids. This result was especially the case for those Bt hybrids that expressed only Cry1Ab and Cry1Fa2 which failed to reduce corn earworm infestations and ear damage in all plantings. Both proteins were of the first used against lepidopteran pests since Bt technology became commercially available in the southeastern United States region (Buntin 2008, Buntin et al. 2001). However, their potency has dropped from increased field resistance as well as cross-resistance development between single-toxin and pyramided hybrids (Huang et al. 2014, Niu et al. 2016a-b, 2021, Reay-Jones et al. 2020, Welch et al. 2015). Hybrids expressing Cry1A.105 and Cry2Ab2 provided adequate reductions in corn earworm infestations but were not as effective as hybrids expressing Vip3Aa20. Resistance development to these linked *Cry* proteins remain at a high risk from the significant number of surviving larval populations in treated plots and concerns of what potential effects sublethal Bt exposure may have on their susceptibility (Guedes et al. 2017). The use of *Cry* proteins still resulted in reduced larval development and ear damage despite their high infestation rates. Exposure of larvae to *Cry* proteins may also reduce the fecundity of surviving adult moths (Bilbo et al. 2018).

Grain yield loss caused by extensive foliar or ear damage as a result of larval feeding occurred less frequently in select Bt hybrids. Hybrids expressing Vip3Aa20 displayed trends of significantly reducing losses in both grain weight and quality once over the two-year study. Despite having the largest presence within our studies compared to fall armyworm, the corn earworm is not normally responsible for causing significant yield loss of field corn except under extremely large infestations (Olivi et al. 2019). The differences in yield between non-Bt hybrids and Bt hybrids expressing Vip3Aa20 when planted at the recommended time, therefore, was minimal yet consistent enough to be significant. Planting Bt hybrids expressing Vip3Aa20 at a later date, however, resulted in them producing less grain yield than non-Bt hybrids despite never having any recorded fall armyworm or corn earworm infestations in their plots. In the same late planting, Bt hybrids expressing only *Cry* proteins significantly produced the most grain yield compared to non-Bt hybrids and Bt hybrids expressing Vip3Aa20, similar to recent findings by Bilbo et al. (2020). In the optimal plantings, all Bt hybrids performed equally in grain production while yielding significantly more than non-Bt hybrids in 2020. Hybrids that express Vip3Aa20, while the most effective at preventing fall armyworm and corn earworm infestations, may not be a suitable source of grain for late plantings due to hybrid agronomics.

Grain aflatoxin contamination was highly variable among hybrids and was not associated with ear damage caused by corn earworm feeding nor was it significantly reduced with the presence of Bt toxins either year. Grain fumonisin contamination was significantly associated with corn earworm ear damage both years with trends of Bt hybrids expressing Vip3Aa20 having less contamination in 2019. However, fumonisin contamination results in 2019 exceeded the federal standard limit for grain intended for human consumption (FDA 2001).

In addition to evaluating Bt trait efficacy against fall armyworm and corn earworm infestations, we examined the amount of grain yield loss associated with fall armyworm whorl defoliation during the mid-vegetative growth stages of late-planted non-Bt field corn. This topic has received little attention aside from earlier studies by Cruz and Turpin (1983) and Marenco et al. (1992) and one recent study by Bilbo et al. (2020). Our combined results from 2019 and 2020 found the effect of whorl defoliation during the mid-vegetative growth stages of field corn significantly affected grain yield through reduced viable kernel availability. Additional data analysis in 2020 also found whorl damage significantly affected ear development and direct ear damage caused by other sources tends to occur more frequently on undamaged plants. The intensity of fall armyworm infestations based on the size of damaged plant clusters in a row did not affect average grain production. We conclude, in Georgia agriculture, a fall armyworm infestation threshold of 25% results in about a 7% yield loss in field corn production. Our results demonstrate the protection Bt technology provides against minimal-to-moderate fall armyworm infestations and its ability to prevent grain loss.

Our studies underline the value implementing Vip3A proteins as part of Bt technology provides towards lepidopteran pest control in Georgia field corn production. Planting Bt hybrids expressing this type of protein, especially Vip3Aa20, is highly recommended for control of both fall armyworm and corn earworm, including populations with some form of *Cry*-protein resistance as part of a high-dose treatment (Burkness et al. 2010, Niu et al. 2016a, 2021, Reisig and Kurtz 2018, Yang et al. 2019a). Significant control against fall armyworm would help in reducing grain yield loss as a result of decreased viable kernel availability. On the other hand, significant control against corn earworm could assist in reducing grain fumonisin contamination and potentially grain yield loss if field corn is planted at the recommended time. However, the yield potential of hybrids with Vip3A proteins could be problematic in late plantings despite consistent control against these lepidopteran pests (Bilbo et al. 2020). This yield production issue can be resolved by planting Bt hybrids expressing Cry1A.105 and Cry2Ab2 in late plantings, but susceptibility to corn earworm ear damage in these hybrids would be prevalent.

Treatments that do not provide significant-enough control of lepidopteran pests are vulnerable to field resistance development in larvae. Such an issue has yet to be reported for Vip3A proteins aside from few cases of resistant alleles found in larval populations in Texas (Yang et al. 2019b, 2020). However, Yang et al. (2021) noted a decrease in Vip3A susceptibility in corn earworm populations across the southeastern United States region since 2016, recognizing these as early warning signs for emerging field resistance to the protein. Most cases of Bt resistance development in fall armyworm and corn earworm populations are associated with Cry1Ab and Cry1Fa2 in both North and South America due to extensive use of these proteins as single-toxin products (Ayala et al. 2013, Farias et al. 2014, Huang et al. 2014, Niu et al. 2021, Omoto et al. 2015, Reay-Jones et al. 2020, Storer et al. 2010, Yang et al. 2019a). Hybrids expressing at least Cry1A.105 and Cry2Ab2 can still provide suitable lepidopteran pest control in early and optimal plantings while maintaining insecticide resistance management (IRM) tactics for extending the lifespan of Bt hybrid products.

In conclusion, our data shows additional benefits in protecting field corn from direct and indirect insect damage that has not been previously well-documented. The impacts lepidopteran pest species, especially fall armyworm and corn earworm, cause towards grain production without treatment not only could reduce yield potentials but also may result in accumulated fumonisin contamination that is hazardous to humans and animal health. Bt technology has the capability of controlling these pest infestations that contribute to direct and indirect damage in corn, especially in temperate and tropical regions where insect activity is significant. Providing this type of treatment, therefore, requires considerable examination of plant-insect interactions of treated plots to assure the efficacy of Bt technology and detect for any signs of resistance development.

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APPENDIX

Table A.1. Effect of Bt traits on LS means ± SEM of percentage corn earworm infested R3 growth stage field corn by plant date per year with contrasting statements.

Brand & Hybrid	**Bt Traits**	**2019**		**2020**	
		PD1	**PD2**	**PD1**	**PD2**
DKC 6694	None (RR2)	58.3 ± 6.9 d	51.7 ± 8.3 b	6.7 ± 2.7 cd	78.3 ± 9.6 ab
DKC 6697	Genuity VT Double PRO^{+}	36.7 ± 4.3 e	20.0 ± 6.1 cd	3.3 ± 1.9 d	75.0 ± 3.2 ab
DKC 6629	Genuity Trecepta^{++}	0 f	0 e	5.0 ± 5.0 cd	0 c
DKC 6205	None (RR2)	76.7 ± 6.4 c	55.0 ± 7.4 b	33.3 ± 4.7 a	80.0 ± 12.8 a
DKC 6208	SmartStax^{+}	51.7 ± 6.9 d	31.7 ± 7.4 c	3.3 ± 1.9 d	70.0 ± 10.0 ab
DKC 6824	None (RR2)	---	---	30.0 ± 5.8 ab	80.0 ± 8.6 a
DKC 6826	Genuity VT Double PRO^{+}	---	---	13.3 ± 8.2 bcd	61.7 ± 9.9 b
DKC 6799	Genuity Trecepta^{++}	---	---	6.7 ± 6.7 cd	0 c
P 2088R	None (RR2)	100 a	75.0 ± 5.7 a	23.3 ± 8.8 abc	75.0 ± 7.9 ab
P 2089VYHR	Optimum Leptra^{++}	0 f	3.3 ± 3.3 de	0 d	0 c
P 1637/1870R	None (RR2)	95.0 ± 3.2 ab	73.3 ± 6.1 a	41.7 ± 15.5 a	85.0 ± 5.0 a
P 1637/1870YHR	Optimum Intrasect^{+}	86.7 ± 2.7 bc	75.0 ± 8.8 a	28.3 ± 9.9 ab	80.0 ± 2.7 a
P 1637VYHR	Optimum Leptra^{++}	0 f	0 e	---	---
F > (*P*)					
Hybrid		<0.0001	<0.0001	0.0003	<0.0001
Bt vs non-Bt		<0.0001	0.0012	<0.0001	<0.0001
Cry Bt vs non-Bt		<0.0001	<0.0001	0.0022	0.0479
Vip Bt vs non-Bt		<0.0001	<0.0001	0.0006	<0.0001
Cry Bt vs Vip Bt		<0.0001	<0.0001	0.1182	<0.0001

LS means ± SEM within columns followed by the same letter are not significantly different (PROC MIXED, pair-wise t-tests of LSM, $\alpha = 0.05$).
2019: Hybrid: (df = 9, 54); Contrasting Statements: (df = 1, 54).
2020: Hybrid: (df = 11, 66); Contrasting Statements: (df = 1, 66).
$^{+}$ Bt-hybrid expressing only pyramided *Cry* proteins.
$^{++}$ Bt-hybrid expressing pyramided *Cry* proteins with Vip3Aa20.

Table A.2. Effect of Bt traits on LS means ± SEM of total corn earworm larvae per ear in R3 growth stage field corn by plant date per year with contrasting statements.

Brand & Hybrid	**Bt Traits**	**2019**		**2020**	
		PD1	**PD2**	**PD1**	**PD2**
DKC 6694	None (RR2)	0.62 ± 0.08 d	0.78 ± 0.20 bc	0.07 ± 0.03 cde	0.90 ± 0.16 a
DKC 6697	Genuity VT Double PRO^{+}	0.38 ± 0.04 d	0.25 ± 0.07 de	0.03 ± 0.02 de	0.85 ± 0.07 a
DKC 6629	Genuity Trecepta^{++}	0 e	0 e	0.05 ± 0.05 de	0 b
DKC 6205	None (RR2)	0.87 ± 0.06 c	0.65 ± 0.07 cd	0.33 ± 0.05 ab	0.82 ± 0.14 a
DKC 6208	SmartStax^{+}	0.55 ± 0.08 d	0.43 ± 0.10 cde	0.03 ± 0.02 de	0.75 ± 0.13 a
DKC 6824	None (RR2)	---	---	0.30 ± 0.06 ab	0.90 ± 0.10 a
DKC 6826	Genuity VT Double PRO^{+}	---	---	0.18 ± 0.11 bcde	0.88 ± 0.23 a
DKC 6799	Genuity Trecepta^{++}	---	---	0.07 ± 0.07 cde	0 b
P 2088R	None (RR2)	1.70 ± 0.14 a	1.35 ± 0.31 a	0.25 ± 0.09 bcd	1.05 ± 0.17 a
P 2089VYHR	Optimum Leptra^{++}	0 e	0.03 ± 0.03 e	0 e	0 b
P 1637/1870R	None (RR2)	1.70 ± 0.10 a	1.22 ± 0.23 ab	0.48 ± 0.21 a	0.98 ± 0.04 a
P 1637/1870YHR	Optimum Intrasect^{+}	1.40 ± 0.12 b	1.20 ± 0.20 ab	0.28 ± 0.10 abc	0.98 ± 0.06 a
P 1637VYHR	Optimum Leptra^{++}	0 e	0 e	---	---
F > (*P*)					
Hybrid		<0.0001	<0.0001	0.0017	<0.0001
Bt vs non-Bt		<0.0001	<0.0001	0.0002	<0.0001
Cry Bt vs non-Bt		0.0002	0.0498	0.0075	0.3347
Vip Bt vs non-Bt		<0.0001	<0.0001	0.0027	<0.0001
Cry Bt vs Vip Bt		<0.0001	<0.0001	0.1330	<0.0001

LS means ± SEM within columns followed by the same letter are not significantly different (PROC MIXED, pair-wise t-tests of LSM, $\alpha = 0.05$).

2019: Hybrid: (df = 9, 54); Contrasting Statements: (df = 1, 54).

2020: Hybrid: (df = 11, 66); Contrasting Statements: (df = 1, 66).

$^{+}$ Bt-hybrid expressing only pyramided *Cry* proteins.

$^{++}$ Bt-hybrid expressing pyramided *Cry* proteins with Vip3Aa20.

Table A.3. Effect of Bt traits on LS means ± SEM of total area damage (cm^2) per ear in R6 growth stage field corn by plant date per year with contrasting statements.

Brand & Hybrid	**Bt Traits**	**2019**		**2020**	
		PD1	**PD2**	**PD1**	**PD2**
DKC 6694	None (RR2)	2.90 ± 0.45 c	1.23 ± 0.13 de	0.02 ± 0.02 b	2.82 ± 0.25 c
DKC 6697	Genuity VT Double PRO^{+}	1.10 ± 0.29 de	0.82 ± 0.36 ef	0.15 ± 0.11 b	1.42 ± 0.40 d
DKC 6629	Genuity Trecepta^{++}	0 e	0 f	0.05 ± 0.05 b	0 e
DKC 6205	None (RR2)	3.02 ± 0.50 bc	2.42 ± 0.52 c	0.45 ± 0.23 b	4.53 ± 0.46 b
DKC 6208	SmartStax^{+}	1.93 ± 0.35 cd	2.23 ± 0.14 cd	0.33 ± 0.17 b	2.62 ± 0.12 cd
DKC 6824	None (RR2)	---	---	1.87 ± 0.47 a	5.42 ± 0.35 ab
DKC 6826	Genuity VT Double PRO^{+}	---	---	0.27 ± 0.16 b	3.07 ± 0.96 c
DKC 6799	Genuity Trecepta^{++}	---	---	0 b	0.05 ± 0.05 e
P 2088R	None (RR2)	7.22 ± 0.75 a	6.23 ± 0.90 a	2.45 ± 0.59 a	6.45 ± 0.26 a
P 2089VYHR	Optimum Leptra^{++}	0 e	0 f	0.32 ± 0.23b	0 e
P 1637/1870R	None (RR2)	6.50 ± 0.80 a	3.73 ± 0.38 b	2.52 ± 0.72 a	5.28 ± 0.70 ab
P 1637/1870YHR	Optimum Intrasect^{+}	4.38 ± 0.73 b	2.00 ± 0.26 cd	1.60 ± 0.50 a	5.02 ± 0.31 b
P 1637VYHR	Optimum Leptra^{++}	0 e	0 f	---	---
F > (*P*)					
Hybrid		<0.0001	<0.0001	<0.0001	<0.0001
Bt vs non-Bt		<0.0001	<0.0001	<0.0001	<0.0001
Cry Bt vs non-Bt		0.0002	0.0179	0.0004	<0.0001
Vip Bt vs non-Bt		<0.0001	<0.0001	0.0002	<0.0001
Cry Bt vs Vip Bt		<0.0001	<0.0001	0.0737	<0.0001

LS means ± SEM within columns followed by the same letter are not significantly different (PROC MIXED, pair-wise t-tests of LSM, $\alpha = 0.05$).
2019: Hybrid: (df = 9, 54); Contrasting Statements: (df = 1, 54).
2020: Hybrid: (df = 11, 66); Contrasting Statements: (df = 1, 66).
$^{+}$ Bt-hybrid expressing only pyramided *Cry* proteins.
$^{++}$ Bt-hybrid expressing pyramided *Cry* proteins with Vip3Aa20.

Table A.4. Effect of Bt traits on LS means ± SEM of grain yield from harvested field corn (kg/ha) by plant date per year with contrasting statements.

Brand & Hybrid	**Bt Traits**	**2019**		**2020**	
		PD1	**PD2**	**PD1**	**PD2**
DKC 6694	None (RR2)	14367 ± 393 a	15097 ± 321 c	12925 ± 423 e	11133 ± 280 ab
DKC 6697	Genuity VT Double PRO[+]	12591 ± 590 a	15316 ± 248 bc	15472 ± 328 ab	12069 ± 401 ab
DKC 6629	Genuity Trecepta[++]	14120 ± 779 a	16506 ± 651 ab	15135 ± 304 abc	11820 ± 470 ab
DKC 6205	None (RR2)	13962 ± 769 a	17353 ± 717 a	14334 ± 738 cd	10662 ± 765 b
DKC 6208	SmartStax[+]	13808 ± 893 a	17104 ± 473 a	15478 ± 159 ab	11066 ± 888 ab
DKC 6824	None (RR2)	---	---	14965 ± 231 abc	11133 ± 366 ab
DKC 6826	Genuity VT Double PRO[+]	---	---	14420 ± 356 bcd	11287 ± 470 ab
DKC 6799	Genuity Trecepta[++]	---	---	15746 ± 121 a	11166 ± 417 ab
P 2088R	None (RR2)	13270 ± 882 a	16074 ± 293 abc	15013 ± 423 abc	12466 ± 430 a
P 2089VYHR	Optimum Leptra[++]	14358 ± 485 a	17302 ± 426 a	15442 ± 594 abc	12069 ± 469 a
P 1637/1870R	None (RR2)	14608 ± 574 a	15671 ± 492 bc	14997 ± 478 abc	8796 ± 560 c
P 1637/1870YHR	Optimum Intrasect[+]	14262 ± 460 a	16342 ± 359 abc	13795 ± 87 de	11849 ± 138 ab
P 1637VYHR	Optimum Leptra[++]	12929 ± 688 a	15474 ± 298 bc	---	---
F > (*P*)					
Hybrid		0.4530	0.0054	0.0005	0.0013
Bt vs non-Bt		0.3962	0.3262	0.0107	0.0053
Cry Bt vs non-Bt		0.1775	0.5682	0.2002	0.0323
Vip Bt vs non-Bt		0.6147	0.0358	0.0006	0.1852
Cry Bt vs Vip Bt		0.6541	0.6427	0.0378	0.4501

LS means ± SEM within columns followed by the same letter are not significantly different (PROC MIXED, pair-wise t-tests of LSM, α = 0.05).

2019: Hybrid: (df = 9, 54); Contrasting Statements: (df = 1, 54).

2020: Hybrid: (df = 11, 66); Contrasting Statements: (df = 1, 66).

[+] Bt-hybrid expressing only pyramided *Cry* proteins.

[++] Bt-hybrid expressing pyramided *Cry* proteins with Vip3Aa20.

Table A.5. Effect of Bt traits on LS means ± SEM of grain aflatoxin contamination (ppb) from harvested field corn by plant date per year with contrasting statements.

Brand & Hybrid	**Bt Traits**	**2019**		**2020**	
		PD1	**PD2**	**PD1**	**PD2**
DKC 6694	None (RR2)	111.50 ± 100.53 ab	2.50 ± 0.46 b	0.07 ± 0.07 a	30.12 ± 29.96 bc
DKC 6697	Genuity VT Double PRO^{+}	66.25 ± 48.21 ab	13.50 ± 12.17 b	0.05 ± 0.05 a	17.40 ± 15.22 bc
DKC 6629	Genuity Trecepta^{++}	5.13 ± 2.22 b	1.50 ± 0.50 b	0 a	0.01 ± 0.01 c
DKC 6205	None (RR2)	6.25 ± 3.20 b	24.13 ± 22.29 b	0.26 ± 0.22 a	3.55 ± 3.16 bc
DKC 6208	SmartStax^{+}	56.00 ± 28.34 ab	2.38 ± 0.55 b	0.17 ± 0.17 a	50.75 ± 35.24 ab
DKC 6824	None (RR2)	---	---	1.00 ± 0.97 a	1.75 ± 1.75 bc
DKC 6826	Genuity VT Double PRO^{+}	---	---	25.00 ± 25.00 a	0.19 ± 0.19 c
DKC 6799	Genuity Trecepta^{++}	---	---	1.12 ± 1.12 a	0.70 ± 0.70 bc
P 2088R	None (RR2)	227.25 ± 138.10 a	13.50 ± 10.84 b	7.30 ± 2.76 a	162.75 ± 111.07 a
P 2089VYHR	Optimum Leptra^{++}	137.58 ± 125.30 ab	96.38 ± 94.58 b	51.30 ± 49.58 a	65.22 ± 64.93 bc
P 1637/1870R	None (RR2)	22.13 ± 17.67 ab	4.25 ± 1.78 b	0.27 ± 0.17 a	0.30 ± 0.30 c
P 1637/1870YHR	Optimum Intrasect^{+}	147.00 ± 96.78 ab	222.25 ± 67.46 a	105.00 ± 61.85 a	1.27 ± 1.27 bc
P 1637VYHR	Optimum Leptra^{++}	30.00 ± 24.68 ab	3.13 ± 1.66 b	---	---
F > (*P*)					
Hybrid		0.3653	0.0022	0.1030	0.0166
Bt vs non-Bt		0.8705	0.3004	0.3677	0.3002
Cry Bt vs non-Bt		0.1197	0.0308	0.3014	0.6538
Vip Bt vs non-Bt		0.2140	0.8284	0.8034	0.0692
Cry Bt vs Vip Bt		0.1161	0.0195	0.6080	0.3467

LS means ± SEM within columns followed by the same letter are not significantly different (PROC MIXED, pair-wise t-tests of LSM, $\alpha = 0.05$).

2019: Hybrid: (df = 9, 54); Contrasting Statements: (df = 1, 54).

2020: Hybrid: (df = 11, 66); Contrasting Statements: (df = 1, 66).

$^{+}$ Bt-hybrid expressing only pyramided *Cry* proteins.

$^{++}$ Bt-hybrid expressing pyramided *Cry* proteins with Vip3Aa20.

Table A.6. Effect of Bt traits on LS means ± SEM of grain fumonisin contamination (ppm) from harvested field corn by plant date per year with contrasting statements.

Brand & Hybrid	Bt Traits	2019		2020	
		PD1	PD2	PD1	PD2
DKC 6694	None (RR2)	58.50 ± 6.30 abc	5.50 ± 1.04 def	2.35 ± 0.84 a	4.43 ± 1.46 a
DKC 6697	Genuity VT Double PRO⁺	54.00 ± 11.21 abc	6.75 ± 3.77 def	2.19 ± 0.76 a	6.10 ± 0.85 a
DKC 6629	Genuity Trecepta⁺⁺	27.00 ± 8.50 cd	2.75 ± 1.20 f	2.65 ± 0.48 a	6.07 ± 0.07 a
DKC 6205	None (RR2)	32.25 ± 6.80 bcd	2.63 ± 0.94 f	1.45 ± 0.51 a	11.35 ± 4.03 a
DKC 6208	SmartStax⁺	29.75 ± 6.38 bcd	4.75 ± 2.17 ef	1.23 ± 0.46 a	12.75 ± 5.63 a
DKC 6824	None (RR2)	---	---	0.79 ± 0.19 a	5.50 ± 1.57 a
DKC 6826	Genuity VT Double PRO⁺	---	---	2.28 ± 0.67 a	8.10 ± 4.01 a
DKC 6799	Genuity Trecepta⁺⁺	---	---	2.49 ± 0.73 a	4.20 ± 1.73 a
P 2088R	None (RR2)	98.75 ± 13.42 a	51.50 ± 6.29 a	3.95 ± 1.12 a	8.93 ± 2.07 a
P 2089VYHR	Optimum Leptra⁺⁺	66.25 ± 6.05 ab	21.00 ± 3.54 abc	2.64 ± 0.86 a	8.63 ± 2.42 a
P 1637/1870R	None (RR2)	52.25 ± 11.35 abc	26.75 ± 14.37 bcd	2.76 ± 1.07 a	5.78 ± 1.76 a
P 1637/1870YHR	Optimum Intrasect⁺	72.00 ± 9.60 ab	42.25 ± 8.67 ab	2.23 ± 0.70 a	4.78 ± 1.33 a
P 1637VYHR	Optimum Leptra⁺⁺	29.25 ± 14.87 d	21.63 ± 13.87 cde	---	---
F > (P)					
Hybrid		0.0101	<0.0001	0.3080	0.2819
Bt vs non-Bt		0.0786	0.4570	0.7096	0.8845
Cry Bt vs non-Bt		0.8607	0.1663	0.7919	0.8270
Vip Bt vs non-Bt		0.0048	0.0284	0.2444	0.4802
Cry Bt vs Vip Bt		0.0832	0.5907	0.2322	0.4806

LS means ± SEM within columns followed by the same letter are not significantly different (PROC MIXED, pair-wise t-tests of LSM, α = 0.05).
2019: Hybrid: (df = 9, 54); Contrasting Statements: (df = 1, 54).
2020: Hybrid: (df = 11, 66); Contrasting Statements: (df = 1, 66).
⁺ Bt-hybrid expressing only pyramided *Cry* proteins.
⁺⁺ Bt-hybrid expressing pyramided *Cry* proteins with Vip3Aa20.

Table A.7. Effect of Bt traits on LS means ± SEM of percentage fall armyworm infested plants and whorl damage ratings by inspection date from growth stages V5 to R3 in late-planted 2019 field corn with contrasting statements.

Brand & Hybrid	**Bt traits**	**V5 (July 25th)**		**V7 (August 7th)**		**R1 (August 21st)**		**R3 (September 4th)**	
		Infested Plants (%)	**Damage Rating**[x]	**Infested Plants (%)**	**Damage Rating**[x]	**Infested Plants (%)**	**Damage Rating**[x]	**Infested Plants (%)**	**Damage Rating**[x]
P 1637R	None (RR2)	6.50 ± 3.71 a	3.75 ± 0.77 a	3.57 ± 1.32 a	3.97 ± 1.41 a	3.03 ± 0.67 ab	4.38 ± 0.65 a	1.86 ± 0.90 abc	3.37 ± 1.14 a
P 1637YHR	Optimum Intrasect[+]	0.36 ± 0.36 c	0.75 ± 0.75 a	0.74 ± 0.52 bc	2.33 ± 1.51 ab	0 b	0 b	0.18 ± 0.18 bc	1.25 ± 1.25 b
P 1637VYHR	Optimum Leptra[++]	0 c	0 b	0 c	0 b	0 b	0 b	0 c	0 b
DKC 6694	None (RR2)	5.02 ± 2.44 bc	3.63 ± 0.65 a	3.61 ± 1.86 a	3.92 ± 1.38 a	3.87 ± 1.31 a	4.70 ± 0.46 a	3.67 ± 1.86 a	3.50 ± 1.17 a
DKC 6697	Genuity VT Double PRO[+]	1.32 ± 1.09 bc	1.04 ± 0.60 b	0 c	0 b	0 b	0 b	0 c	0 b
DKC 6629	Genuity Trecepta[++]	0 c	0 b	0 c	0 b	0 b	0 b	0 c	0 b
DKC 6205	None (RR2)	11.79 ± 3.63 a	4.82 ± 0.18 a	2.80 ± 0.95 ab	4.17 ± 0.93 a	2.06 ± 0.77 b	4.31 ± 0.51 a	2.43 ± 0.78 ab	3.96 ± 0.26 a
DKC 6208	SmartStax[+]	0 c	0 b	0.20 ± 0.20 c	0.75 ± 0.75 b	0 b	0 b	0 c	0 b
F > (*P*)									
Hybrid		0.0031	<0.0001	0.0085	0.0053	<0.0001	<0.0001	0.0138	0.0002
Bt vs non-Bt		0.0003	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	0.0002	<0.0001
Cry Bt vs non-Bt		0.0003	<0.0001	0.0003	0.0009	<0.0001	<0.0001	0.0006	<0.0001
Vip Bt vs non-Bt		0.0103	<0.0001	0.0004	0.0004	<0.0001	<0.0001	0.0020	<0.0001
Cry Bt vs Vip Bt		0.6872	0.0753	0.6687	0.2315	1.0000	1.0000	0.9090	0.3637

LS means ± SEM within columns followed by small letters are not significantly different (PROC MIXED, pair-wise t-tests of LSM, α = 0.05). Hybrid: (df = 7, 21); Contrasting Statements: (df = 1, 21).

[x] Davis et al. (1992) rating scale.

[+] Bt-hybrid expressing only pyramided *Cry* proteins.

[++] Bt-hybrid expressing pyramided *Cry* proteins with Vip3Aa20.